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Anaerobic digestion: biodegradability and biogas production of model wastes

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Extract:
Anaerobic digestion is a desirable treatment practice in terms of minimizing volume, treating of pollutants and biogas production. In this thesis model wastes have been investigated with respect to biogas and methane production in order to find out what wastes are suitable for anaerobic digestion, and discussing ways to further the research to optimize the production of renewable energy.

Key words:

1. Anaerobic digestion
2. Biogas production
3. Methane
4. Biodegradability

Preface

As part of this thesis the author has been staying at the University of Minnesota (UMN), conducting experiments on anaerobic digestion in the Gortner lab at the St. Paul campus. The author's involvement in this particular research is a result of a growing collaboration between the departments of environmental engineering at the Norwegian University of Science and Technology (NTNU) and UMN. I was encouraged by my supervisor to grab this opportunity to experience something different by studying in a new country and be part of a different academic environment.

Being part of UMN was interesting and enjoyable as I got to experience life on campus in the US, and work with people in water and wastewater engineering in a different country.

I would like to thank my supervisors, Professor Stein Wold Østerhus and Associate Professor Cynthia Hallé at the Department of Hydraulic and Environmental Engineering, NTNU, for useful and constructive guidance. My gratitude also goes to my supervisor in Minnesota, Professor Paige Novak at the department of Civil Engineering, UMN. In addition to letting me use her lab, helping me start this project, and the useful Wednesday discussions, I appreciate that she showed care in my social well-being while staying in Minnesota, inviting me to her house for a delicious meal with the other students in the lab.

Special thanks go to Amy Prok, whose project is the reason my project came into being. She has helped me getting to know the lab, helping with the procedures and being a great discussion partner. In addition to that we had two amazing Thanksgiving dinners.

Furthermore, I would like to thank Professor Timothy LaPara for being part of the Wednesday discussions and Professor Raymond Hozalski for taking care of me socially with a Christmas dinner as well as conversations about sports and other things.

Last, but not least, I would like to thank Nils Darre Seip for being my partner in crime as we went to Minnesota together, staying in the same hotel room, and for those crazy long days in the office as the deadline was approaching.

Erlend Lausund,

Trondheim, 28.03.2014

Abstract

Anaerobic digestion is a desirable treatment practice of waste as pollution control and energy recovery both can be achieved. In this project the model wastes starch, tween 80, gelatin, ethylene glycol (EG), propylene glycol (PG), and dimethylformamide (DMF) have been tested in anaerobic digesters in terms of biogas and methane production. Experiments were conducted using 160 mL serum bottles as reactors and each waste was investigated using a COD concentration of 4 g/L in each reactor at mesophilic temperatures (37°C). In two instances, gelatin and DMF, other COD concentrations were tested, 57 and 0.4 g /L respectively. The reactors with inoculum were first fed with a control substrate, consisting of 50% TWAS and 50% TPS as used in the Empire Wastewater Treatment Plant, in a start-up period, before being spiked with the model wastes at hour 70 and hour140. Gas and methane productions were recorded at between and after each spike until termination of each run. They were then plotted in graphs showing accumulated production volumes and production rates. The results show that methane production is lagging compared to gas production for all wastes investigated, including the control substrate. Starch and gelatin wastes produce more gas and methane than the control, while tween, EG, and PG produce 50% of the amounts recorded with the control. Observation with DMF at the same COD concentration show that this is inhibitory, but with lower concentration, an acclimatization of the inoculum was observed as the gas and methane yield increased. With high concentration gelatin wastes, ammonia inhibition was observed, as the gas and methane yield dropped very low. These experiments have covered anaerobic digestion of single wastes. Further research should include co-digestion of wastes, using the proper wastes these model wastes simulate, as well as testing different retention times to see if acclimatization is effective and what concentrations would be viable after acclimatization.

Sammendrag

Ved anaerob nedbrytning av avløpsvann vil det kunne oppnås både forurensningskontroll og energigjenvinning. I dette prosjektet har forskjellige testavløpsvann basert på stoffenee stivelse, Tween 80, gelatin, etylenglykol (EG), propylenglykol (PG) og dimethylformamid (DMF) blitt testet med hensyn til biogass- og metanproduksjon. Eksperimentene ble utført med 160 mL serumflasker som reaktorer, og hvert avløpsvann ble undersøkt med en COD-konsentrasjon i hver på 4 g/L ved 37^0C. Ved to tilfeller ble andre COD-konsentrajoner undersøkt; gelatin og DMF med respektive konsentrasjoner på 57 og 0.4 g/L. Reaktorene inneholdt inokulum fra anaerobe nedbrytningstanker på Empire Wastewater Treatment Plant, Minnesota, og bestod av 50% TWAS og 50% TPS. Reaktorene ble matet tre ganger; først med et kontrollsubstrat med samme innhold som inokulumet, deretter to ganger med modellavfall. Matingen foregikk ved starten, etter 70 timer og etter 140 timer. Gass- og metanproduksjon ble loggført med varierende mellomrom fra start til slutt og akkumulert volum ble plottet. Resultatene viser at metanproduksjonen er tregere enn gassproduksjonen for alle undersøkte avløpsvannn, inkludert kontrollsubstrat. Stivelse- og gelatinvannene produserer mer gass og metan enn kontrollen, mens tween, EG og PG produserer om lag 50% mindre. DMF-vannet med en en reaktorkonsentrasjon på 4 g COD/L førte til inhibering av mikroorganismene, men med lav COD-konsentrasjon (0.4 g COD/L) vises tegn til akklimatisering med økt gass- og metanproduksjon. Med høy konsentrasjon av gelatin, ble ammonia-inhibering observert da produksjonsutbyttet sank til nær null. Disse eksperimentene har tatt for seg anaerob nedbrytning av enhetlig avfall og en videreføring bør omfatte studier av avløpsvann med forskjellige blandinger av forurensningstypene samt testing av det egentlige avløpsvannet som har blitt simulerert.

Contents

List of Figures

List of Tables

List of Acronyms

ADF	Aircraft deicing fluid
BOD5	Biological oxygen demand
C:N	Carbon:Nitrogen
COD	Chemical oxygen demand
DMF	Dimethylformamide
EG	Ethylene glycol
FOG	Fat, oil and grease
GC	Gas chromatograph
LCFA	Long-chain fatty acid
PBS	Phosphate buffered saline
PG	Propylene glycol
TPS	Thickened primary sludge
TWAS	Thickened waste activated sludge
UASB	Up-flow anaerobic sludge blanket
VFA	Volatile fatty acid

1. Introduction

With increasing regulations within waste management combined with increasing amounts of waste generated due to expansions in industrial sectors, the need for safe and effective methods of waste disposal is becoming more and more imminent. Wastewater effluent from industrial plants may often contain hazardous materials that are a risk to the environment or may even pose health risks for humans that get in contact with it. It is therefore of high importance that this wastewater is treated according to proper standards to ensure that treatment plants do not discharge hazardous materials to rivers, lakes and streams. In this project a possible way of making this type of waste disposal more effective, especially in terms of reducing volumes of wastewater in activated sludge processes, have been looked at.

In 1995 a U.S. National Research Committee (NRC) stated:

"*We are convinced . . . that socially compatible and environmentally sound economic development is possible only by charting a course that makes full use of environmentally advantageous technologies. By this, we mean technologies that utilize resources as efficiently as possible and minimize environmental harm while increasing industrial productivity and improving quality of life*."(McCarty, 2001)

When it comes to wastewater treatment, a technology that would fulfill these goals is anaerobic digestion. It is a natural process that eliminates objectionable organic matter and reduces pathogenic microorganisms. The effluents from anaerobic reactors are biosolids that are stabilized and ready to use as soil conditioners and, on top of that, this process produces biogas. Today, with the ever more clear evidence of man-induced climate change, renewable and sustainable sources of energy are increasing in demand. The potential of biogas as a source of energy is enormous and utilization of this energy can be increased by a ten-fold in a developed country such as Sweden (Lantz et al., 2007).

This report intends to address the possibilities of anaerobic digestion as part of an ongoing project at University of Minnesota in cooperation with the Metropolitan Council in the Twin City Area, Minnesota investigating model wastes simulating different industrial wastes in lab-scale reactors and looking at various ways of optimizing biogas and methane yields.

Many studies have been conducted on anaerobic digestion with various industrial wastes, municipal wastes, agricultural wastes and food wastes (Long et al., 2012b, Elbeshbishy and Nakhla, 2012, Cabbai et al., 2013, Bouallagui et al., 2005). However, some chemicals are not so common in anaerobic digestion,

but are present in industrial wastewaters in the Twin City area. An example is a plastic film company that release dimethylformamide in their wastewater, or aircraft deicers from local airports such as the Minneapolis Saint Paul International Airport. The current situation for the treatment of these wastewaters is that it goes through the whole process of aeration and biodegradation in activated sludge tanks. This demands a lot of volume in the pools and extra energy needed to degrade the wastes as mixing is important. A great aspect of anaerobic treatment by methane fermentation is that no oxygen or nitrate is needed as an electron acceptor, the organic matter itself or the carbon dioxide acts an acceptor. This means that organic loads in anaerobic reactors can be much higher, and the reactors can then be a lot smaller. With industries like the plastic film company wanting to expand, a way of treating the increasing waste needs to be found. With the treatment plants not able to expand in size, alternative methods of degrading these industrial wastes need to be found, and one way might be to lead this waste directly into anaerobic digesters. In Figure 1 below the current situation is shown as well as a proposed situation with anaerobic digesters, Figure 2.

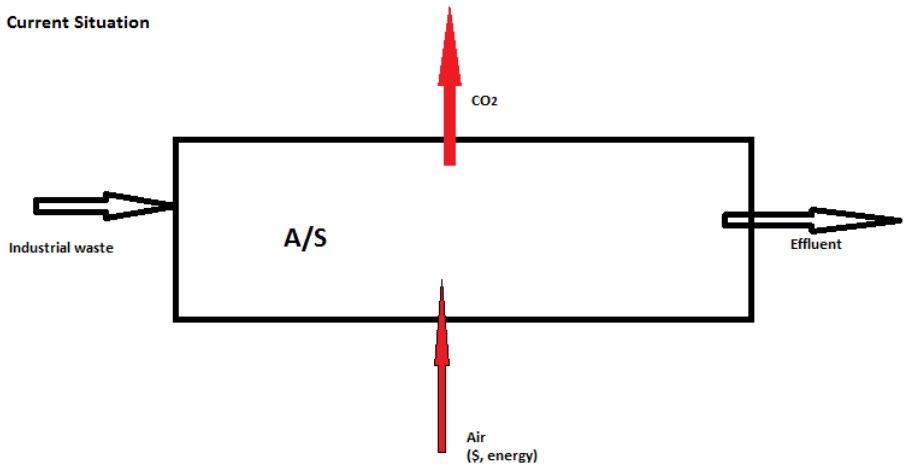

Figure 1 - Current situation at treatment plant. All waste passes through activated sludge tanks. Large volumes of waste requiring large tanks and substantial input in form of energy.

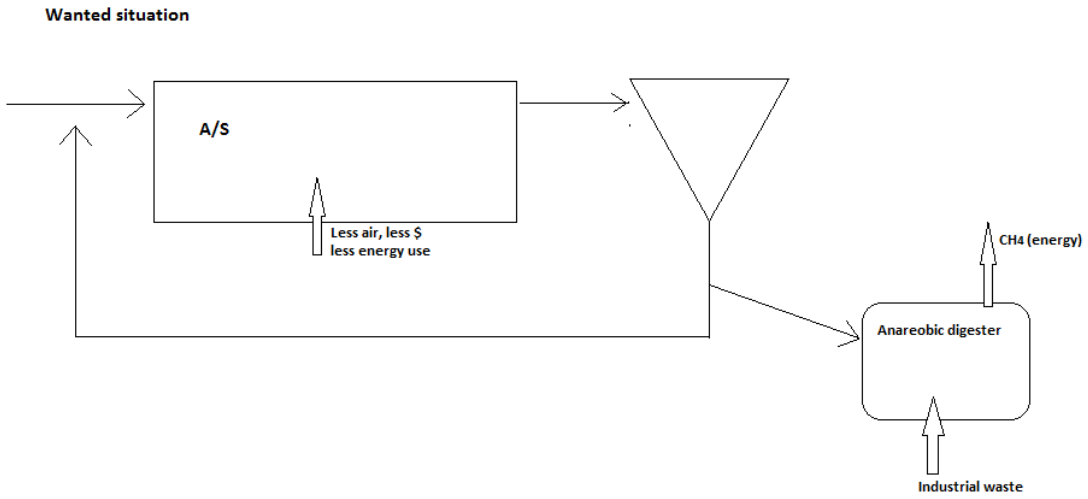

Figure 2 - Desired situation at treatment plant. Re-direction of certain industrial wastes to anaerobic digesters, resulting in energy production and smaller volumes in activated sludge tanks.

In order for this to work, one needs to find the best way to add the industrial wastes to the digesters; what concentrations of wastes are optimal, if co-digestion of different wastes is desirable in order to reach the highest possible methane yield and best possible biodegradation.

1.1 Background and objective

The project this report is part of uses lab-scale reactors with inoculum collected from Empire Wastewater Treatment Plant to check different combinations of wastes and comparing the gas production, and the main aim of this report is to see whether dimethylformamide (DMF), glycols and other model wastes at a given concentration are degradable under anaerobic conditions, whether biogas will be produced and thereby find out if it is possible to run the desired industrial wastes from the plastic film company and from MSP International Airport without having to perform an impossible expansion of existing treatment plants in the area and to, ultimately see if, with this situation, an expansion of these industries is possible.

Figure 3 - Bench-scale reactor with sludge volume of 1.5 L

Figure 4 - Columns used for gas volume measurements. Based on water displacement.

This part of the project's objective is to act as a preliminary investigation of how different model wastes behave in lab-scale digesters simulating the digesters at Empire Wastewater Treatment Plant; how is the gas and methane production of model wastes compared to the municipal waste substrate that is in use now? How do these wastes compare with other studies in the field of anaerobic digestion and what are possible ways to further the research with these wastes?

In the next chapter the basic principles of anaerobic digestion are explained as well as previous studies in the field focusing on wastes similar to the wastes used in these experiments.

2. Literature Review

In this chapter the theoretical background for the study is reviewed. All chemicals used in the experiments have been researched with respect to anaerobic digestion and gas production. Of the chemicals simulating industrial wastes from airport deicing and wastes containing dimethylformamide, the environmental hazards related to them have been shed a light upon, as well as alternative treatments available.

2.1 Biogas production and anaerobic digestion

Biogas is a renewable source of energy where the gas is produced through anaerobic digestion. Anaerobic digestion is a natural process in which biodegradable material is broken down by microorganisms without the presence of oxygen. It is used in the treatment of wastewater, but is also important in the stabilization of organic wastes. It has even been discovered that that chlorinated hazardous compounds are degraded within these processes.(McCarty, 2001)

It has been known for a long time that the anaerobic digestion of organic matter results in the production of methane. In fact, as early as 1776, Volta discovered that methane was formed in the sediments of lakes and ponds. About 80 years later, in 1856, Reiset realized that decomposing manure piles let out methane, and started a study to explain the decomposition of organic material.(McCarty, 2001). The development in the use of anaerobic processes then went on to include treatment of domestic wastewater by the end of the 19th century, and studies from that time concluded that these treatments resulted in considerably lower sludge volumes than aerobic treatment.

By the 1920's Arthur Bunswell applied this technology in industrial wastewater treatment. Up until this time the reactor design had consisted of single tanks, where all the processes took place simultaneously. This resulted in long retention times, but by 1950 it was discovered that one could separate the anaerobic bacteria from the effluent and thereby reducing the retention time as well as the reactor size. Later the field has advanced a lot, for example with the introduction of the up-flow anaerobic sludge blanket (UASB) reactor in the 1970's (McCarty, 2001). Today, anaerobic digestion is widely applied for energy recovery as methane from wastewaters, solid wastes, wastes from forestry as well as food wastes.

Because anaerobic digestion produces a combustible gas that can used as an energy source, the degradation of wastes through this is good for the environment in the ways that it biodegrades waste-products as well as producing a source of energy. A source of energy that is considered clean and help reduce greenhouse gas emissions.

In Figure 5 below Lantz et al show various types of wastes as well as multiple ways of utilizing the energy captured.

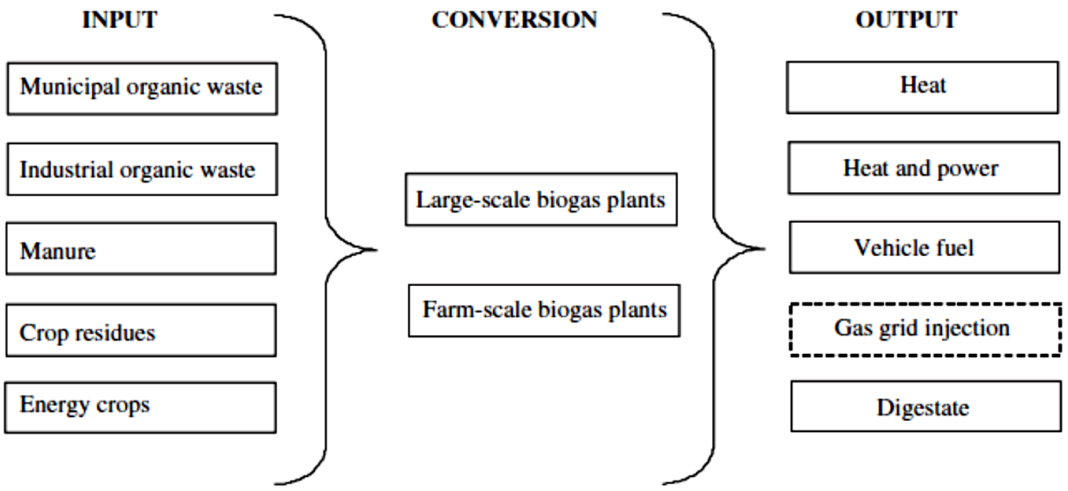

Figure 5 - Overview of biogas systems in Sweden showing sources, types of conversion plants and uses of biogas (Lantz et al., 2007)

As we can see from this, the possibilities are immense, with low-value energy for heating, be it within the treatment plants or elsewhere, generating electricity or refining the gas so that it can be used by vehicles. In their study Lantz et al pointed out that the possibilities of using this source of energy is immense, especially considering that only one tenth of the potential is being utilized. So by focusing attention on the use of anaerobic digestion and energy recovery from methane gas, a great improvement can be made, reducing the greenhouse gas emissions and a possibly contribute to the fight against global warming(Lantz et al., 2007).

2.1.1 Conversion processes

Anaerobic digestion of organic matter consists of four main processes; hydrolysis, acidogenesis, acetogenesis and methanogenesis. These are shown in the flow chart in Figure 6 on the next page and explained briefly.

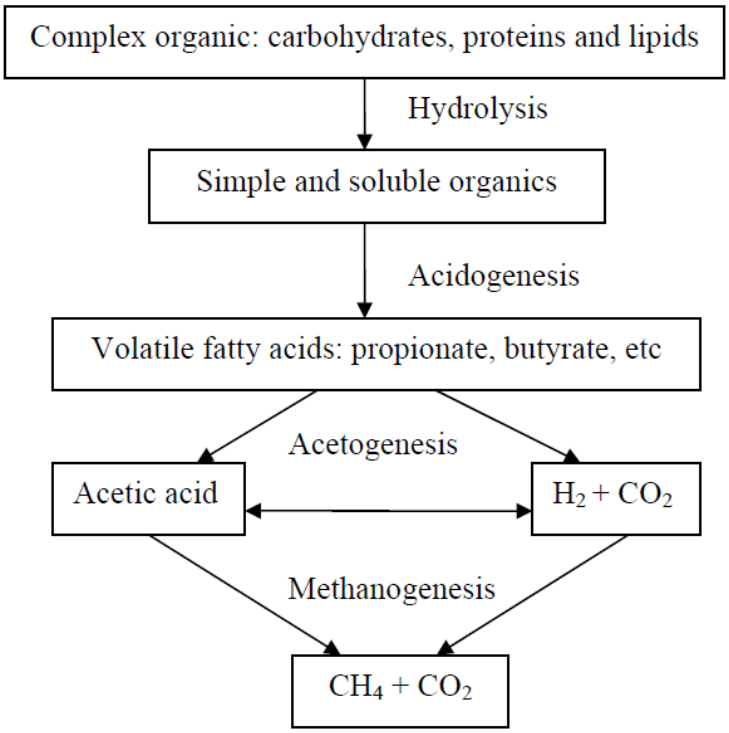

Figure 6 - Flow chart showing the processes within anaerobic digestion (Gujer and Zehnder, 1983)

2.1.1.1 Hydrolysis of biopolymers

The first step required in order for the microbial population to utilize complex biopolymers is the hydrolysis or liquefaction, because bacteria, generally, are unable to take up particulate organic matter; the material needs to be broken down into soluble polymers or monomers. During this stage, fermentative bacteria convert the soluble complex organic matter and compounds of high molecular weight such as proteins, lipids, nucleic acids and polysaccharides into amino acids, sugars and fatty acids. The end-products of the hydrolysis as well as the hydrolytic enzymes mediating process depend on what kind of compounds are being hydrolyzed; complex polymeric matter is converted into monomers by hydrolytic enzymes secreted by the bacteria such as lipases, proteases, cellulases or others. Also, during this stage, lipids, proteins, nucleic acids and polysaccharides are hydrolyzed to fatty acids, amino acids, purines and pyrimidines, and monosaccharaides, respectively. The enzymes involved are lipases, which degrade fats, proteases, which degrade proteins, and cellulases, which degrade cellulose.

There are many factors that can affect the rate of which the materials can be hydrolyzed. For example large particles with a low surface-to-volume ratio would have a slower rate than that of small particles. Starches, protein and cellulose would be hydrolyzed at different rates, and non-degradable matter like waxes or lignin would slow down the hydrolysis of particulates with which they are associated (Gujer and Zehnder, 1983). Therefore, the hydrolysis step of anaerobic digestion may, in some instances, be the limiting factor when it comes to the complete process. This happens for example when high solid organic waste is degraded. To overcome this problem and improve the hydrolysis there are various ways of pretreatment that could be applied; both mechanical and chemical pretreatment methods.

2.1.1.2 Acidogenesis

In acidogenesis the hydrolysis products (amino acids, fatty acids, and sugars) are the substrates for fermentation. These are converted by acidogenic bacteria into volatile fatty acids, alcohols, carbon dioxide, and hydrogen. Byproducts such as ammonia (NH_3) and hydrogen sulfide (H_2S), are also produced (Gujer and Zehnder, 1983). The main acids formed in this stage include acetic acid (CH_3COOH), propionic acid (CH_3CH_2COOH), butyric acid ($CH_3CH_2CH_2COOH$) and ethanol (CH_2H_5OH). The methane precursors hydrogen and acetate formed here are set for the final step, while the other products, the intermediary degradation products, have to undergo another step, the acetogenesis (Gujer and Zehnder, 1983, Henze, 2008).

2.1.1.3 Acetogenesis

The following step in the anaerobic digestion is the acetogenesis. Here, the simple end-products from the acidogenesis, apart from the acetic acid, are anaerobically oxidized further by acetogenic bacteria to form mainly acetic acid, as well as carbon dioxide and hydrogen gas (Henze, 2008).

2.1.1.4 Methanogenesis

The production of methane gas (CH_4) in anaerobic digestion happens when the materials have been completely broken down into two products; acetate and hydrogen, as well as other end-products that do not participate in the methanogenesis. There are two substrates from which methane is formed, and thus two types of methanogenesis and two groups of methanogenic bacteria. The acetoclastic methanogens degrade the acetic acid (electron donor) to form carbon dioxide and methane:

$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$ (Gujer and Zehnder, 1983)

The other precursor for methane, hydrogen, is degraded by the hydrogen-utilizing methanogens; consumers that use hydrogen (H_2) as electron donor and carbon dioxide (CO_2) as acceptor to produce methane:

$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ (Gujer and Zehnder, 1983)

70% of methane produced in a digester originates from acetic acid through this reaction. The reason for that is the limited concentration of hydrogen in the digester, meaning that acetate is the primary precursor for the production of methane.

The anaerobic digestion can occur in digesters based on systems with different number of stages. The simplest is a one-stage system where all the mentioned processes take place and occur simultaneously in a single reactor. The two- or multistage system will separate the processes so that hydrolysis, acidogenesis, acetogenesis and methanogenesis will take place in different reactors depending on the number of reactors (Bouallagui et al., 2005).

2.1.2 COD in relation to methane production

The chemical oxygen demand (COD) is an important aspect of anaerobic digestion in the way that it shows the theoretical potential of methane production from organic matter. It takes place in the absence of O_2:

Organic matter + heat $\rightarrow CH_4 + CO_2 + H_2O$ + energy

The COD can be used to determine the energy potential of the organic matter. Energy, and therefore, oxygen demand, on right hand side of the equation must equal the oxygen demand on the left hand side of the equation. Most of the energy produced in anaerobic digestion is stored as methane (CH_4)(Droste, 1997).

$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$ + heat

COD is the term used in the presentation of the graphs shown in this report as the experiments have been conducted with model wastes where the COD is easily controlled. However, when dealing with actual wastes, a term used more often is volatile suspended solids (VSS). The relationship between these two was found to range between 1.14 and 1.66 mgCOD/mgVSS by the determination of biomass COD as the difference between the total COD and the soluble COD from samples based on sludges obtained from a

laboratory-scale treatment plant with activated sludge (Contreras et al., 2002). It has also been shown that the relationship between COD and VSS was 1.48 mgCOD/mgVSS as shown in Figure 7 below. (Horan, 2003)

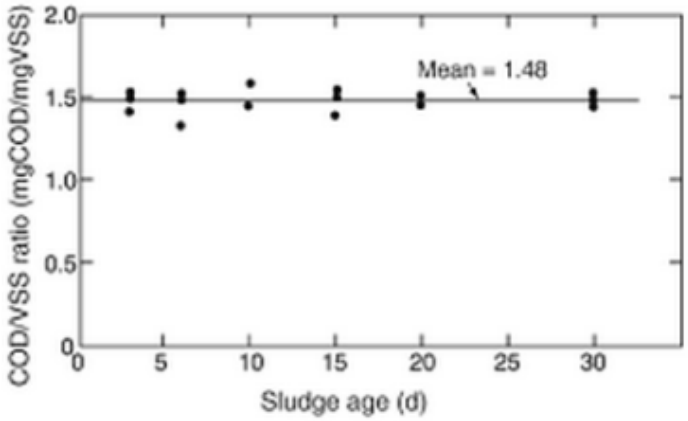

Figure 7 – Relationship of COD/VSS ratio of a mixed liquor with sludge age from laboratory scale aerobic completely mixed activated sludge systems. The constancy of the ratio indicates that the ratio of the constituent fraction of the mixed liquor are the same and equal to 1.48 mg COD/mgVSS(Horan, 2003)

2.1.3 Former studies related to biogas and methane production.

Most of former studies related to biomethane production investigate the methane production of agricultural wastes, municipal wastes, and food wastes such as fruit and vegetable wastes. In these studies both mono- and co-digestion have been studied, and there has also been studies comparing the conditions under which the anaerobic digestion has taken place. Anaerobic digestion usually take place under one of two temperature ranges; the thermophilic range or the mesophilic range, although it has been proven that it is also possible at psychrophilic temperatures, temperatures below 20 ^{0}C (Bouallagui et al., 2003). The temperature optima under thermophilic conditions and mesophilic conditions are 55 ^{0}C and 35 ^{0}C respectively (Ward et al., 2008) as one can also see in Figure 8 below

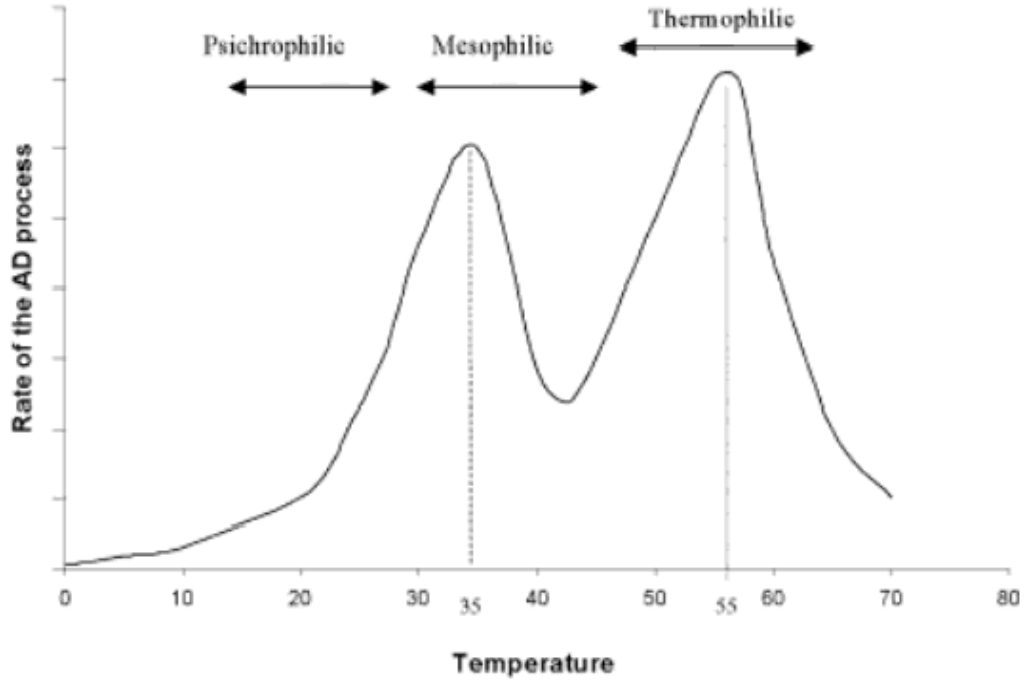

Figure 8 - Temperature ranges for anaerobic digestion. Peaks represent the temperatures at which the rate of the anaerobic digestion is most rapid (Henze 2008)

According to the review by Ward et al even small temperature changes, sometimes by only 1 ^{0}C, have shown to reduce the production rate of biogas considerably (Ward et al., 2008). Previous studies have shown conflicting results when it comes to which condition, thermophilic or mesophilic, is better with respect to COD removal, methane yield and energy recovery. Gannoun et al compared anaerobic digestion of olive mill and abattoir wastewater at 37 ^{0}C and 55^{0}C to find that the thermophilic digester had a higher COD removal and biogas yield than the mesophilic digester (Gannoun et al., 2007). In digestion of vegetable waste and wood chips, a methane yield of 95% was achieved after 11 days using thermophilic temperatures, while on the other hand, under mesophilic conditions the reactors needed 27 days to achieve the same level of biogas yield (Hegde and Pullammanappallil, 2007). However, it has

been proven that mesophilic digester have improved degradation rates; experiments comparing the degradation rates of proteinaceous wastewater under mesophilic (37 ^{0}C) and thermophilic (55^{0}C) conditions resulted in a COD removal of approximately 84% whereas the COD removal achieved in the thermophilic reactor amounted to a varying 69-83%(Fang and Chung, 1999). Also, In studies comparing two-stage reactors with various conditions (mesophilic-mesophilic, mesophilic-thermophilic and thermophilic-thermophilic set-up) to degrade potato waste, the results showed that mesophilic conditions in the second stage had a higher methane yield than the reactors with a second stage under thermophilic conditions, although in the thermophilic reactors the reaction would go faster, resulting in a shorter retention time(Parawira et al., 2007).

When comparing reactors using the different conditions, i.e. temperatures, one also has to take into account that a possible increased methane production at thermophilic conditions has to be balanced with the extra energy needed to keep the reaction going at a temperature of 55 ^{0}C which is considerably higher than the temperatures needed for mesophilic temperatures.

Table 1 - Optimal growth temperatures for some methanogenic bacteria (Ward et al., 2008)

Temperature range	Genus	Optimal temperature (°C)
Mesophilic	*Methanobacterium*	37–45
	Methanobrevibacter	37–40
	Methanosphaera	35–40
	Methanolobus	35–40
	Methanococcus	35–40
	Methanosarcina	30–40
	Methanocorpusculum	30–40
	Methanoculleus	35–40
	Methanogenium	20–40
	Methanoplanus	30–40
	Methanospirillum	35–40
	Methanococcoides	30–35
	Methanolobus	35–40
	Methanohalophilus	35–45
Thermophilic	*Methanohalobium*	50–55
	Methanosarcina	50–55

Table 1 shows that the optimal temperatures for the methanogenesis lie in the temperature ranges mentioned previously. However, these temperatures might not always be ideal for the other phases of anaerobic digestion, and this is why more complex set-ups of anaerobic digesters using two- or multi-stage digesters could be favorable when it comes to the methane yield potential and COD removal(Ward et al., 2008).

2.2 Substrates and inhibition

The composition of the substrate is important when it comes to the methane yield in anaerobic processes. As mentioned earlier the most common substrates used in this field are agricultural wastes and municipal wastes, but other possible sources of substrates are from the industrial sector; food industry, paper industry and textile industry among others. There are many factors which affect the gas production. This can result in high total ammonia nitrogen concentrations and lead to lower methane yields and inhibition. Shock loadings with stored feed slurry have proven to cause inhibition in digesters due to high ammonia concentrations (Hobson, 1984). Ammonia inhibition occurs because ammonia is produced by the biological degradation of nitrogenous matter, such as proteins and urea. Ammonia is, nevertheless, not only a disadvantage because nitrogen is also an essential nutrient for the microorganisms in anaerobic processes. As long as the ammonia concentration is below 200 mg/L it is believed that the concentration is actually beneficial, but this varies with the type of inoculum and substrate (Liu and Sung, 2002). In order to estimate the ammonia quantity produced one can use the following stoichiometric relationship:

$$C_aH_bO_cN_d + \frac{4a-b-2c+3d}{4}H_2O \rightarrow \frac{4a+b-2c-3d}{8}CH_4 + \frac{4a-b+2c+3d}{8}CO_2 + dNH_3 \quad (\mathbf{1}$$

(Chen et al., 2008)

With municipal wastes, other common possible inhibitors in addition to ammonia exist. As sludge production is an important part of the sewage treatment, heavy metals can be present in the sludge as they are resistant to biodegradation. This can accumulate to toxic concentrations, and inhibit the process. This is not a problem with the wastes used in these experiments, as they are clean model wastes containing only the pure chemicals diluted with PBS. In industrial wastes there are numerous possibilities for inhibition, depending on the type of industry, but causes of inhibition, other than ammonia and heavy metals, include long chain fatty acids (LCFAs), which decrease the pH in the digester to a level where the methanogens do not operate properly, organics that have been reported to have toxic effects such as halogenated benzenes and other benzenes, or hypersaline wastewaters that are generated from food processing industries(Chen et al., 2008). Many of the compounds causing inhibition are actually essential for the anaerobic process to proceed, but they become inhibitory at high concentrations. For many of these inhibitors, acclimation of the inoculum is possible. This is due to changes in the predominant species of methanogens or a changes in the methanogenic population (Zeeman et al., 1985). Acclimation has proven to be effective using low concentrations of inhibitory substrates, and once the inoculum has

adapted, much larger concentrations than the initial inhibitory concentration can be degraded (Chen et al., 2008) .

So, in general, a substrate that is heterogeneous with corresponding qualities with a good C:N ratio (the ideal ratio for anaerobic digestion is between 20:1 and 30:1(EPA, 2012)), and low contents of possibly inhibitory substances such as ammonia, sulfide, heavy metals, LCFAs and organics will generate higher yields of methane and thereby be more suitable for this process. A way to maximize these conditions is the possibility of mixing different substrates to ensure the best possible yield. Mixing substrates can also help degrade substances that on their own would be inhibitory; this will be addressed later in 2.3 Co-digestion after a presentation of the model wastes that have been used as substrates in these experiments.

2.2.1 Starch

Starch is a polysaccharide consisting of a chain of many glucose units. It is largely produced in plants as energy store, and is also the most common carbohydrate in foods. Rice, potatoes, and wheat all consist largely of starch.

Apart from the food industries, starch is also consumed to a great extent by the papermaking industry, as well as in textiles, cosmetics, pharmaceuticals and paints industries (Higson and Smith 2011).

In anaerobic digestion of carbohydrates, the substrate is first hydrolyzed to sugars that, in turn, are degraded by acidogens to volatile fatty acids (VFA) before the acetogens convert the VFAs to acetate, hydrogen and carbon dioxide ready for the methanogenesis. Nothing that can be utilized by the methanogenic bacteria are produced before the acetogenesis, meaning the accumulation of VFAs will not have an effect on the methanogenesis and biogas yield (Elbeshbishy and Nakhla, 2012).

Many studies have shown that wastewater rich on carbohydrates such as starch is an excellent source of energy with respect to its production of methane in anaerobic digesters. For example in the study of bioreactor performance in anaerobic digestion of fruit and vegetable waste (Bouallagui et al., 2005), the authors conclude that this technology represents a commercially viable process to convert fruit and vegetable wastes to methane gas, and thereby use it as a renewable source of energy. Another study assessed the biogas production from anaerobic digestion of wheat starch processing waste and found the economic profit to be £30000 annually, in 1989(Butcher, 1989). Other studies of food wastes (Cabbai et al., 2013) show a considerable methane yield compared to yields from normal sewage sludge, although the results varied from different sources.

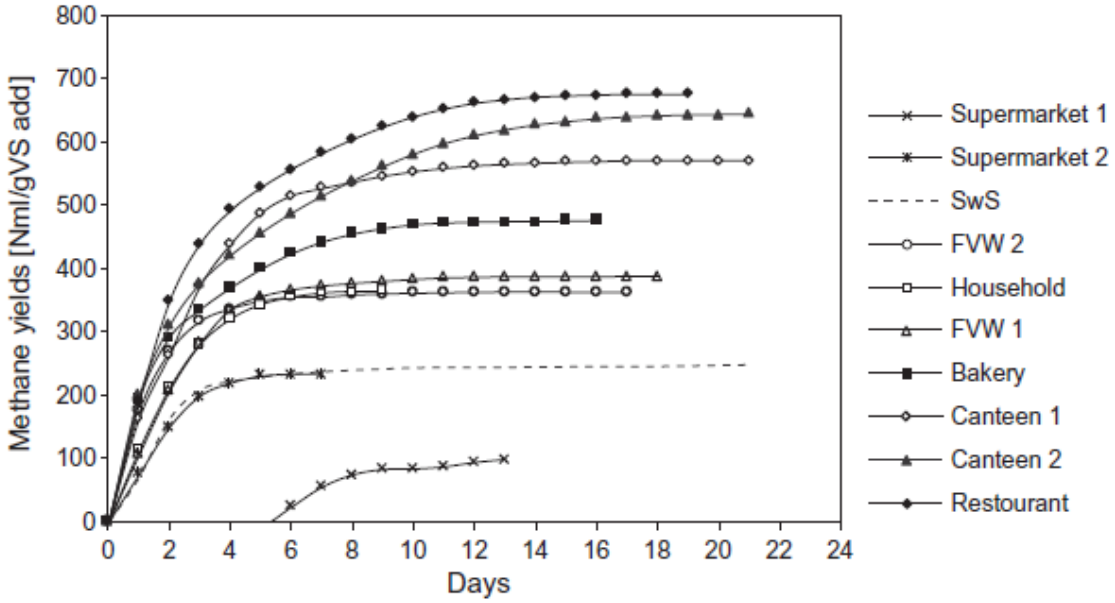

Figure 9 - Cumulative methane yield curves from organic fraction municipal solid wastes and sewage sludge (OFMSW) (Cabbai, Ballico et al. 2013)

2.2.2 Gelatin

Gelatin is a mixture of peptides and protein originating from partial hydrolysis of collagen. The collagen is often extracted from animal parts such as skin and bones from domesticated cattle, chicken, pigs and fish. When heated, gelatin melts into a liquid only to turn solid again when cooled. Mixed with water, gelatin forms a gel. Gelatin is as an additive in foods, like gelatin desserts, marshmallows and candy such as jelly beans. It is also used in clarification of juices, as a stabilizer in foods like cream cheese and yoghurt and also to simulate fat in non-fat food products to add volume without calories.

Because gelatin is a mixture of hydrocarbons containing proteins, one can look at earlier studies on anaerobic digestion of proteinaceous wastes such as authors Fang and Chung did in their study of anaerobic treatment of proteinaceous wastewater under mesophilic and thermophilic conditions. Their study showed among other things, that proteins are highly digestible anaerobically, with a 9 hour retention time being sufficient to achieve a COD removal of approximately 84% in an up-flow anaerobic sludge blanket reactor (UASB) (Fang and Chung, 1999).

The methane production in anaerobic digestion of proteins is slower than that of starch due to the fact that in the hydrolysis phase the proteins are converted into peptides and amino acids by proteolytic enzymes. These are in turn acidified into volatile fatty acids, hydrogen, ammonium and reduced sulfur. As mentioned before, the VFAs need to go through the acetogenesis in order to be utilized by the

methanogens. The step in which the VFAs are formed, the acidogenesis, happen faster which can in turn lead to accumulation of acids that affect the pH. A reduction in pH can, in turn, have a negative effect on the methanogenesis, resulting in lower methane yield (Elbeshbishy and Nakhla, 2012).

As one can see from their studies in the Table 2 below, the anaerobic digestion is more efficient when using co-digestion of different substances, M1 is 100% proteins, M5 is 100% starch, while M2-M4 are mixtures with different protein/starch contents. The advantages of anaerobic co-digestion are many and the reason of higher yields in terms of biogas production has to with the C:N-ratio and the different processes within the reactor.

Table 2 - Methanization and biodegradability of different mixtures containing starch and protein (Elbeshbishy and Nakhla 2012)

Mixture	Methanisation (% M)	Biodegradability (% BD)
	%	%
M1	62	76
M2	69	78
M3	75	79
M4	91	93
M5	80	84

2.2.3 Tween 80

Tween 80 is a polysorbate and belongs to a class of emulsifiers. Polysorbates are oily liquids derived from PEG-ylated sorbitan (a derivative of sorbitol) esterified with fatty acids (Partanen et al., 2001). The brand tween 80 is a polysorbate 80 which is commonly used in foods as an emulsifier; often in ice cream to make it smoother, easier to handle as well as resisting melting. It is also used in the medical industry and laboratories; notably in vaccines and estrogen regulating medicines, while it is also used in a mixture with phenol red to tests solutions for phenotypes. From the molecular formula, $C_{64}H_{124}O_{26}$, one can identify this chemical as a hydrocarbon derived from oleic acid and therefore has been used to simulate anaerobic digestion of fats oils and grease (FOG). Previous studies have shown that in anaerobic digestion of FOG wastes, the main component being degraded, LCFAs or lipids, are partly hydrolyzed to sugars and amino acids (≈10%) while the rest turns to fatty acids. These go through anaerobic oxidation where 1/3 turns into hydrogen and the rest to acetate (Gujer and Zehnder, 1983, Long et al., 2012b). In their review paper, Long, Aziz et al, conclude that anaerobic co-digestion of fats oils and grease with municipal biosolids increase the biogas production in the digester (Long et al., 2012b). Again, this shows the importance of co-digestion in order to optimize methane yield, as well as the degradability of lipids.

2.2.4 Ethylene glycol and propylene glycol

Propylene and ethylene glycols (PG and EG) are often used in aircraft deicing fluids in order to remove and prevent accumulation of ice and snow on aircraft surfaces. In the United States, glycol-based deicers are the only deicers certified by the United States Federal Aviation Administration to ensure passenger safety (Castro et al., 2005). And the extent to which it is used is large; an estimated 4700–5800 tons of ethylene glycol, diethylene glycol, and propylene glycol are used annually at Canadian federal airports(Tham and Kennedy, 2004). It is of high importance to deal with safe disposal of aircraft deicing fluids (ADFs) to protect both human health and the environment, because as much 80% of the liquid applied ends up on the pavement, in the deicing area, while the rest runs off the aircrafts during taxiing and take-off. Complete degradation of glycols is possible in natural conditions, but there are concerns related to the oxygen consumption during degradation. The BOD_5 (Biological oxygen demand over a 5-day period) concentrations are high with regard to ethylene and propylene glycol, and as a point of reference; 1 L of pure propylene glycol has the same COD as 6000 l of domestic wastewater (Switzenbaum et al., 2001). Because of this, in addition to the concerns voiced about additives in the ADFs negatively affecting the environment, it is important that airports and other industries using these chemicals are able to collect and treat the waste. There are several ways described by Switzenbaum, Veltman et al, but in this case the treatment of the chemicals is the most important.

Anaerobic digestion of ADFs is a viable alternative, along with aerobic digestion, constructed wetlands and even plant-assisted bioremediation (Castro et al., 2005). Previous studies have shown that in addition to the glycols, additives are present. At high concentrations these additives can have toxic effects on the micro organic population in the reactors, possibly leading to inhibition (Cancilla et al., 1998). Another aspect of anaerobic digestion of PG is the production of propionate and hydrogen that can possibly slow down the degradation or even halt the degradation completely (Zitomer et al., 2001). However, anaerobic digestion is a very good alternative other studies have shown. Using a bench-scale UASB reactor experiments using ADFs with a COD of up to 12000 mg/L were conducted, and results showed that the COD removal and methane production in the reactors were close to the theoretical values (Tham and Kennedy, 2004). Zitomer et al found in their study that 98% of the PG-based ADF that BOD_5 was removed in anaerobic digestion and that the co-digestion of ADFs and conventional wastewater could be profitable in terms of biogas production because the conventional wastewater holds nutrients, ammonia-nitrate and provides alkalinity needed to degrade the glycol while the glycol provides plenty of organics increasing the methane production (Zitomer et al., 2001).

2.2.5 Dimethylformamide

Dimethylformamide is commonly referred to as DMF, has a molecular formula of C_3H_7NO and does not occur naturally. DMF is a versatile organic solvent primarily used in the production of polyurethane products and acrylic fibers. It is used in a wide range by manufacturers; in pharmaceutical products, electronic components and textile coatings (Pellizzare, 1978).

DMF may be carcinogenic, though available studies have not been able to conclude as the studies performed on animals so far has been considered inadequate (Vidhya and Thatheyus, 2013).

In their attempt to isolate bacteria able to biodegrade DMF, Vidhya and Thatheyus were able to conclude that there are only a limited number of bacteria strains that can utilize DMF as a source of carbon and nitrogen. It is important to figure this out as biodegradation is considered the most environmentally sustainable way of degradation, resulting in the least amount of sludge (Vidhya and Thatheyus, 2013). However, no studies have been found on DMF in anaerobic digestion.

2.3 Co-digestion

As shown in most studies on anaerobic digestion, mixing different wastes and getting co-digestion is often a better alternative in order to have a stable and high biogas production. Some wastes, as the graph below shows, will have a very high yield on its own, but if mixed with other wastes, the total yield (that of the normal municipal sludge and the other waste) will be better.

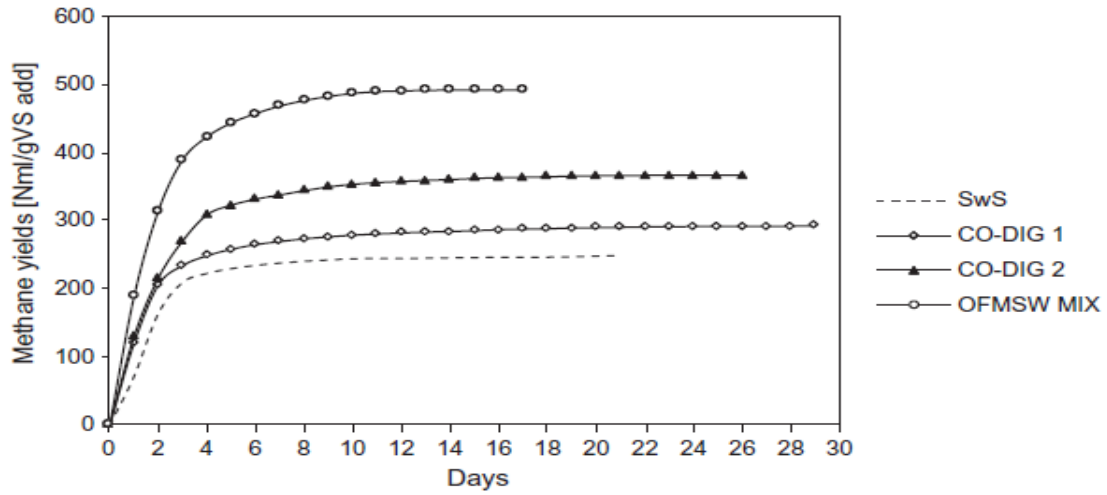

Figure 10 - Cumulative methane yields for mixtures of OFMSW, sewage sludge and co-digestion between the two (Cabbai, Ballico et al. 2013)

The reason why co-digestion can improve the yield of anaerobic digestion is the fact that co-substrates provide the processes with missing nutrients and thereby help to optimize the processes (Mata-Alvarez et al., 2000), for example by providing alkalinity or giving a better C:N-ratio. The C:N ratio is easy to adjust; for example if one has a carbon-rich waste without nitrogen-contents it would be advantageous to add wastes that contain nitrogen to some extent. With high protein content wastes the anaerobic process will yield ammonia, which at certain concentrations can cause inhibition, but the nitrogen content in the proteins are advantageous for biogas production as nitrogen is an essential nutrient. It is therefore important to balance the carbon and nitrogen contents to avoid low yields and possibly inhibition. This is shown in Table 2 taken from (Elbeshbishy and Nakhla, 2012), the substance M4 has the highest biogas yield, higher than that of M1 and M5 which are 100% protein and starch, respectively. The mixture M4 consisted of 80% starch and 20% protein, while the last two had higher contents of protein. From this one can draw the conclusion that it is important to investigate different mixes of different wastes in order to achieve the highest possible biogas yield. Another reason for co-digestion of different substrates is to degrade wastes that on their own have the potential to be toxic or not even degradable as the wastes do not contain the nutrients needed. With aircraft deicing fluids it was evident that the glycols were good sources of carbon, causing a higher methane yield mixed with sewage sludge than the sewage sludge yielded on its own (Zitomer et al., 2001).

3. Methods

In this chapter the methods used to conduct the experiments will be addressed including the procedures in the making of the standard curve for the methane detection, the preparation of the diluting solution as well as the procedures for measuring gas production and methane content.

3.1 Chemicals

The chemicals or wastes analyzed with respect to gas production in anaerobic digestion have already been introduced in chapter 2, showing previous studies involving these in the field of biodegradability and their roles in everyday life; for example where they are used primarily. These include Tween 80, starch, gelatin, EG, PG and DMF. Other substances used in the experiments will be introduced here, they include the inoculum used in the digestion, the control feed, and phosphate buffered saline (PBS) used to dilute the wastes to obtain the desired COD-content.

3.1.1 Inoculum

The inoculum is collected from the Empire Wastewater Treatment Plant in Minnesota and consists of 50% thickened waste activated sludge (TWAS) and 50% thickened primary sludge (TPS). This has undergone an anaerobic digestion process in lab-scale digesters, with a sludge volume of 1500 mL at a temperature of 37 °C with constant mixing. These reactors have been fed once a day with a mix similar to the control feed for a period of four weeks prior to the first extraction of inoculum for the serum bottles used in this study. In the start-up period these reactors were fed daily with substrate, starting with small amounts, 20 mL, increasing every week until a constant level of 80 mL per day was added. While adding substrate, excess sludge was removed from the reactors to keep the volume in the reactors steady.

All six lab-scale reactors contribute with inoculum used in the serum bottles. This is to ensure that the reactors undergo the same procedures, and also to keep the steady volume without having to add too much substrate.

The inoculum in each serum bottle is a mix of the effluent from each of the bench-scale reactors. This ensures each serum bottle have inoculum as similar as possible.

3.1.2 Control feed

The control feed used as substrate in each run is based on the same as the inoculum; 50% TWAS and 50% TPS. These substrates are collected once a month from Empire Wastewater Treatment Plant and stored in plastic containers (10 L) in a cooler at 4°C. They are used daily as substrate for the lab-scale digesters as well as for the control samples in these experiments. The TPS and the TWAS are stored separately and not mixed together until feeding the serum bottles in this case, or the larger reactors for their daily feed.

3.1.3 PBS

In the experiments, a PBS solution was used to dilute the model wastes to ensure that the right COD levels were used in the anaerobic digestion. PBS is often ordered in tablet form, where it is dissolved in water when it is needed. However, in this experiment the PBS-solution was made on site; mixing different salts with milli-Q water.

3.2 Experiment set-up and procedure

The main experiment was carried out in 4 runs with developments in the procedures, mainly from run 1 to run 2 with an expansion with respect to the number of serum bottles and number of measurement that were possible to make practically. Also the set-up for the preparation of the calibration curve and the PBS-solution is addressed.

3.2.1. Standard curve for gas chromatography

The calibration curve for the gas chromatograph (GC) is important to establish in order to monitor the methane content of the biogas produced. It is easy to detect gas production as a whole, but in order to find the composition, calibration curves have to be made using the same conditions and method in the GC as was done in the main experiment. This is done using known amounts of the gas one needs to find. In this study the methane content was considered the most important; hence a calibration curve for methane was established.

Equipment used for obtaining a calibration curve:

- Vial with lock
- Beaker
- Gas container
- Gas Chromatograph
- Microsyringe with lock (2000 µL)
- Fume hood

The vial is submerged in water and filled. The vial is then bubbled with methane gas (99%) from a tube from the gas container, until there is no water left in the vial. This is done in the fume hood to keep up the safety precautions. Now the vial is full of methane. The gas is then turned off and the vial is capped and taken out of the beaker. With the gas in the vial it is easy to extract desired volumes of gas using a syringe for collecting gas samples. Different amounts of methane are taken by the syringe from the vial to be analyzed for methane content in the GC. Whenever the total taken amount of methane from the vial is equal to 1000 µL the vial needs to be refilled with methane to ensure that the diminishing partial pressure has as little effect as possible. Different gas volumes are then analyzed in the gas chromatograph and the results are shown in a graph. The area under the curve at a given time interval specifies what gas is present and indicates the amount of that gas. Knowing the injected amounts and the corresponding areas will then help estimating the methane volume in future samples. The amounts injected in the GC with pure methane were 0 µL, 100 µL, 200 µL, 400 µL and 500 µL. These were carried out in triplicates and the curve is obtained is shown in the Figure 11 below.

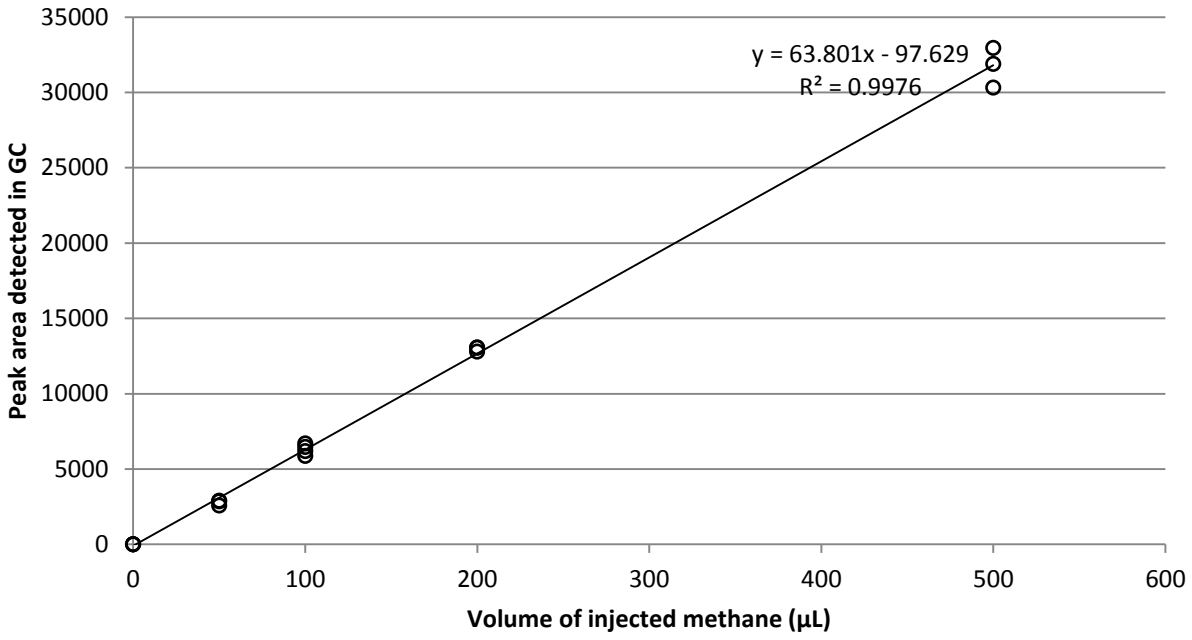

Figure 11 - Calibration curve for methane detection in the GC with the method used.

The equation given in the graph will be used to calculate the methane volume from each sample of biogas and thereby the percentage of methane and total accumulated methane production in each serum bottle.

3.2.2 PBS

Equipment used to make the phosphate buffered saline is as follows:

- Spatulas
- Balance
- Disposable plastic trays
- Glass bottle (1 L)
- pH-meter

The PBS used to dilute the wastes in the experiments contains a mixture of these salts:

- Sodium chloride (NaCl)
- Potassium chloride (KCl)
- Disodium phosphate ($Na_2HPO_4 \bullet 2\ H_2O$)
- Potassium dihydrogen phosphate (KH_2PO_4)

In Table 3 below the different concentrations of the salts in the solution needed are shown both in mmol/L and g/L.

Table 3 - Composition of PBS

Composition of PBS		
Salt	Concentration (mmol/L)	Concentration (g/L)
NaCl	137	8.01
KCl	2.7	0.20
$Na_2HPO_4 \bullet 2\ H_2O$	10	1.78
KH_2PO_4	2.0	0.27

The solution was made on two occasions, both times with 1 L of PBS. This means that the used amounts are shown in the last column. The procedure is to measure the desired masses of each salt on the balance, using disposable trays and spatulas, and mixing them with, in this instance, milli-Q water. The solution was stored at room temperature in a glass bottle. All the salts were easily dissolved in the water. The pH of the solution was checked with a calibrated pH-meter and the value was at a satisfactory 7.4 in both cases.

3.2.3 Set-up for measurements

The main experiment is complex and involves many steps in order to obtain the gas production from the reactors as well as the methane content. All the experiments were carried out in triplicates with feeding of the reactors at three times; first at the start (always with control feed as substrate), then after 70 hours and then at 140 hours. In the last two spikes triplicates of the control feed as well as two or three model wastes were carried out. The equipment used in this experiment is listed below:

- Serum bottles (160 mL)
- Graduated cylinders
- Funnels
- Beakers
- Pipettes
- Autoclaved plastic pipette caps
- Septa
- Metal caps
- Crimper
- Glass bottles
- Glove box
- Fume hood
- Syringe
- Needles
- Gas chromatograph
- Gas production measuring device

3.2.4 Procedure

First of all the substrates need to be prepared and ready. To do this the desired amounts need to be calculated in order to ensure that the COD in each reactor is the same for each bottle and all the different wastes. The calculations are shown Appendix A. The desired COD concentration in each reactor, or serum bottle, is 4000 mg/L which requires the added wastes to be diluted with the PBS solution. The wastes are in different states, so measuring the amounts involves different equipment and procedures. For the liquid wastes the desired volumes of the waste is taken with pipettes with autoclaved pipette caps and mixed with the PBS solution in a beaker or glass bottle. For the powder wastes, the desired mass is weighed on a balance and dissolved in the PBS solution in beakers or glass bottles. These are now ready

to be added to the reactors. The reason for not adding the wastes directly to the reactors, but adding PBS, is to be able to add the same volume of waste to each reactor even though the wastes are different.

The next step is to extract inoculum from the 6 larger reactors and dividing it between all the serum bottles. The serum bottles have a working volume of 160 mL, but due to projected gas production and the practicalities of gas sampling the headspace need to be substantial. Therefore an inoculum volume of 60 mL in each serum bottle is chosen. This is also convenient for the bench-scale reactors as they will not be depleted more than the normal daily extraction of effluent. The extraction is carried out by moving the larger reactors into a fume hood and with the help of gravity needed amounts of the inoculum can be extracted and put in a beaker. The same amount of effluent is taken from each reactor, the amount depends on how many serum bottles are needed in the run; with nine serum bottles the volume of effluent extracted from each of the 6 large reactors amounted to 100 mL and when increasing the number of serum bottles the effluent extracted was consequently increased. All the extracted inoculum is then mixed together in a large beaker (1L) so as to get as homogeneous inoculum as possible, to get all the serum bottles to have the same inoculum. Instead of measuring out 60 mL of inoculum for each bottle with a graduated cylinder, the level to which 60 mL would reach in the bottle was marked with a permanent marker. The inoculum was the poured into each serum bottle with the help of a funnel. Another option is to pour the inoculum into graduated cylinders to measure the exact volumes and then pour into the bottles.

Once the serum bottles are filled with inoculum and the substrates are diluted and ready, be it only control substrates or other model wastes, the small reactors are ready to be fed. This will start the reaction at once. In order to make sure this reaction happens in the absence of oxygen at all times, the feeding takes place in an oxygen free environment, for example a glove box, as seen in Figure 12. Here it is important to put all the needed equipment and substances inside at the same time in order to keep the time schedule for feeding. All the serum bottles with inoculum, all the substrates and the equipment and tools needed to add the substrates to the bottles need to go in the glove box. A glove box is used in order to work with materials in an oxygen free environment. It has two chambers, one for the working space where the atmosphere contains mainly nitrous gases and nitrogen, and one chamber to put the equipment and working elements in and out of the glove box. Here, air is pumped out and replaced with nitrogen and nitrous gases through 3 steps of pumping and refilling. When everything needed is in the working chamber of the glove box, adding the substrates can commence. 10 mL of the diluted wastes are added to each bottle, making the total volume of inoculum and substrate 70 mL. The wastes are measured in graduated cylinders and added to the bottles with the help of funnels; each waste has its own graduated cylinder and funnel. The wastes are added to the bottles at five minute intervals, and once added, a septum is placed on top with a metal cap and sealed with a crimper. The reason for the five minute

intervals is practical, as the time for the GC-analysis is approximately 5 minutes, and one wants the measurements to be carried out at the same times after the start of the reactions for all serum bottles. All the bottles stay in the glove box until the last bottle is fed, capped and sealed.

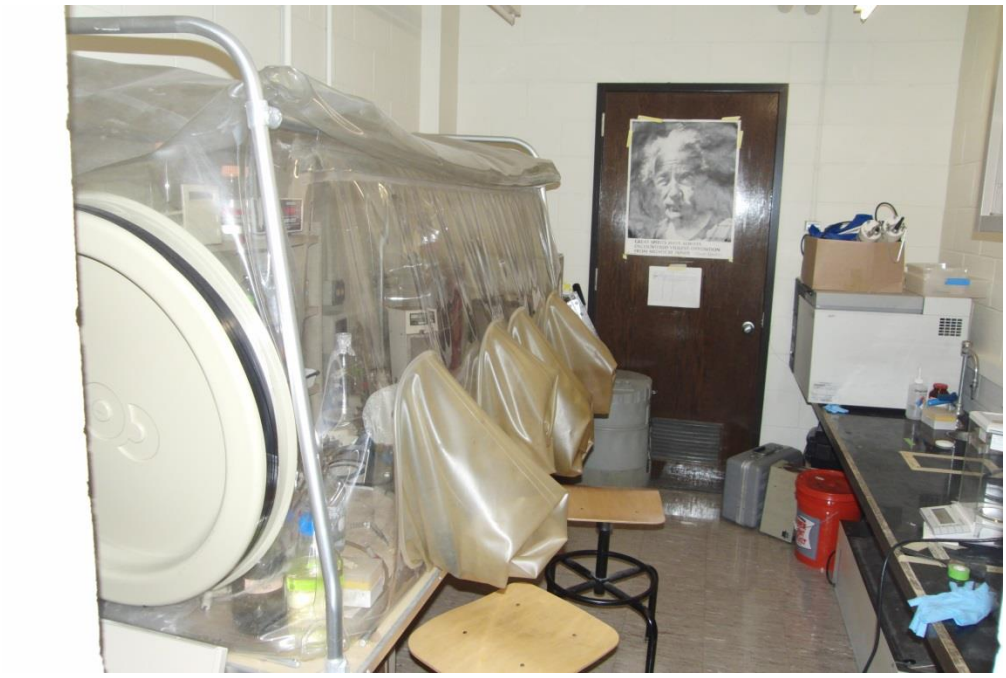

Figure 12 - Glove box used in the feeding and spiking of the reactors. Makes it possible to work in an oxygen free environment.

When the bottles are fed they should be placed in an environment suited for anaerobic digestion. During these experiments mesophilic conditions were tested, thus the bottles were place in a shaker table as shown below with a temperature of 37 ºC with 80 revolutions per minute. The bottles are placed in these conditions at the same time intervals and order as the way they were fed to keep each reaction as similar as possible.

Figure 13 - Shaker table at which the reactors were put. The temperature is set to 37º C; the table is set to 80 rpm.

Two hours after the first bottle is fed the measurement of gas production and methane content for the same bottle is taken. The bottle is then taken out of the shaker table, a gas sample of 200 µL is taken with a syringe puncturing the septum and transferring the gas for analysis to the gas chromatograph. The total gas production is also measured with a device based on displacement of liquid at atmospheric pressure, by putting a needle attached to a tube through the septum and achieving equal pressure in the tube and in the serum bottle. Then these measurements are carried out with five minute intervals until all the bottles have undergone a measurement. Once a measurement is taken, the bottle is placed back in its place on the shaker table to continue the anaerobic digestion under the proper conditions.

These measurements are taken at multiple times; at 2 hours, 3 hours, 5 hours and 8 hours the first day, and once every day after, giving the following timetable showing a proposed time for the measurement of each bottle at each measurement hour.

Table 4 - Measurement schedule showing possible times to perform measurements from each reactor with a spike at 10:00. B1 to B9 represent the different serum bottles

Hour	B1	B2	B3	B4	B5	B6	B7	B8	B9
2	12:00	12:05	12:10	12:15	12:20	12:25	12:30	12:35	12:40
3	13:00	13:05	13:10	13:15	13:20	13:25	13:30	13:35	13:40
5	15:00	15:05	15:10	15:15	15:20	15:25	15:30	15:35	15:40
8	18:00	18:05	18:10	18:15	18:20	18:25	18:30	18:35	18:40
24	12:00	12:05	12:10	12:15	12:20	12:25	12:30	12:35	12:40
48	12:00	12:05	12:10	12:15	12:20	12:25	12:30	12:35	12:40
72	12:00	12:05	12:10	12:15	12:20	12:25	12:30	12:35	12:40

The experiments are carried out in triplicates, meaning that with the timetable shown above, bottle 1 to 3 would contain the control feed while 4 to 6 and 7 to 9 would have model waste each. Given the time of measurements and the time needed for the GC-analysis, the maximum number of bottles one can use is 12; hence the maximum number of wastes analyzed in each run can be three plus the control substance.

When approaching 72 hours, the gas production seems to level off and it is time to spike the serum bottles with the same concentration of COD as was done in the previous feeding. Once the measurement is taken at 72 hours the bottles are not placed back on the shaker table, but the caps are taken off, samples of 1000 µL are taken in triplicates of each bottle for future DNA analysis to check the microbial community, especially methanogens. The samples are taken using pipettes with autoclaved caps with extra-large openings in order to be able to handle the thick liquids. The samples are stored in autoclaved plastic containers, marked and frozen. Once the samples are taken, another 7 mL of the sludge is extracted, leaving us again with 60 mL of inoculum. The substrates for the spike have been prepared prior to the measurement at 72 hours following the same procedure as for the first feeding, so once the samples are taken, the bottles are ready to be fed in the glove box and placed on the shaker table for the same procedure.

With the last spike the procedure is identical. The only thing differing from the first spike is the measurements go on for an extra 48 hours to see whether the gas production has levelled off completely or if the gas production will start to accelerate again.

After the final measurement, samples are taken as previously, and the rest of the sludge is disposed of safely.

3.3 Analytical methods

3.3.1 Biogas production

The biogas volume measuring device is based on displacement of liquid using a tube attached to needle in one end and a long glass cylinder in the other. The glass cylinder kind of resembles a burette and is attached to a tube in the other side which leads to a bottle with a hole. This apparatus is held up on a stand with two clamps, one for the bottle, and one for the 'burette'. The level of the liquid in the bottle is at the same height as the level of the liquid in the burette. Once the needle is pushed into an environment with higher pressure, the device will seek to equal the pressure letting the gas out of the high pressure environment into the tube thereby pushing the liquid down in the burette until the pressure is equal at all points. Once this is achieved one can read off the new liquid level in the burette and see how much liquid has been displaced. Below is both a sketch of the device as well as a photo.

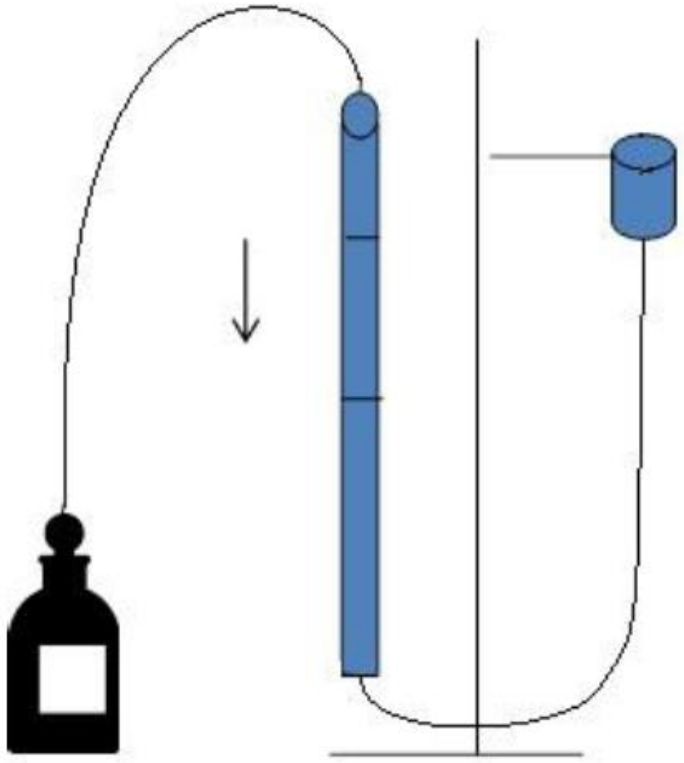

Figure 14 - Sketch of gas the gas measuring device

Figure 15 - Gas measuring device

The needle should be changed regularly in order to avoid clogging and to get as accurate results as possible. It is also important to make sure the surface of the liquid returns to equal levels in the burette and the bottle. Different liquids can be used. In many instances the displacement liquids have been water, but in this case, a solution of hydrochloric acid was used. This type is normally used when one needs to store the gas in the device for a pending GC-analysis. If that is the case one should also flush the device with nitrogen before each measurement.

3.3.2 Gas chromatography

Gas chromatography is a method of analyzing the contents of different compounds in liquids or gases. It is based on the principle that there is a difference in how different constituents of a sample pass through a column at different rates depending on the adsorption in the column filling and the carrier gas. Once they have passed through the column they are detected; the time of detection and the amounts are recorded. As the compounds have different retention times in the column, it is possible to identify the compounds and the amounts; given the retention time for each compound in the column is known.

In these experiments a HP 6890 series GC system was used to find the methane amounts of 200 µL samples of biogas. The samples were injected in the GC using a microsyringe and running the analysis for

5 minutes. The retention time of methane in the GC was 3.5 minutes, and the peak area was recorded and compared with the standard curve to find the methane amounts.

Table 5 - Specifications on the method and GC used in the methane content analysis

GC and method specs (HP 6890 series GC system)	
Oven temperature	75°C (constant)
Carrier gas	Nitrogen (N_2)
Column	10 ft. molecular sieve 13X – 45/60 mesh

3.3.3 Data processing

The measurements taken were recorded as gas production between each measurement, and the samples for the GC reflected the methane content of the gas produced between each measurement, and thereby the methane production between each measurement. As the samples for the GC were taken before the volume measurements, there was always a different amount of gas as the pressure in the serum bottles would be affected by the pressure. The actual volume of the gas analyzed by the GC was estimated using Boyle's law.

$$P_1V_1 = P_2V_2 \quad \textbf{(2)}$$

The pressure of each sample was found using the volume of the headspace in the serum bottles and the volume produced, found by the measurements. For example, if the volume recorded in the instrument is 90 mL and the volume of the headspace in the serum bottle is 90 mL, the atmospheric pressure in the bottle would be

$$P = \frac{Volume\ recorded\ +\ Volume\ headspace}{Volume\ headspace} \quad \textbf{(3)}$$

$$2\ atm = \frac{90\ mL + 90\ mL}{90\ mL} \quad \textbf{(4)}$$

The difference in pressure was taken into account when analyzing for the methane content and was found using the standard curve and the peak area detected by the GC.

Once having both the methane and gas production between each measurement from each model waste, the accumulated volumes were found by adding and subtracting the values found from the reactors where no COD was added to eliminate the gas produced by the inoculum alone. Another manipulation had to be done in order to compare the results to each other and other studies, as there was a difference in COD concentration. For most of the wastes the feeding and spikes contained the same COD amounts; 4 g/L, but to compare them to other concentrations, the COD amount had to be taken into account; therefore the graphs showing accumulated gas and methane production show a production based on the amount of COD added. For example if the first feeding had a COD concentration of 4 g/L the gas produced would be divided by 4 g COD, or if the concentration was as high as 57 g/L the volume measured would be divided by 57. Also, each reactor volume was 70 mL, this was also taken into account.

Gas production rates were also plotted, showing the production rates of every waste in terms of mL/h to show the relationship between gas production and methane production and to see if there is a difference in development.

3.4 Summary

The measurements were conducted in four different runs, in addition to a run with blanks. They were all conducted with the same temperatures and revolutions per minute in the shaker table as well with as the same routine on feeding with a first round of control substrate for all reactors, then dividing them on different wastes from spike 1 and onwards. Each run can be divided into three parts; part 1 between the initial feeding and spike 1, part 2 between spike 1 and spike 2, and part 3 between spike 2 and termination.

Each run had a triplicate containing the control substrate and two or three other model wastes.

Table 6 - Overview of all serum bottle reactors and which runs included which wastes

Run	**Serum bottle**	**Initial feed hour 0**		**Spike 1 hour 70**		**Spike 1 hour 140**	
		Type	COD (g/L)	Type	COD (g/L)	Type	COD (g/L)
1	1-3	Control	4	Control	4	Control	4
	4-6	Control	4	Tween 80	4	Tween 80	4
	7-9	Control	4	Starch	4	Starch	4
2	1-3	Control	4	Gelatin	57	Gelatin	57
	4-6	Control	4	Control	4	Control	4
	7-9	Control	4	EG	4	EG	4
3	1-3	Control	4	Gelatin	4	Gelatin	4
	4-6	Control	4	Control	4	Control	4
	7-9	Control	4	DMF	4	DMF	4
	10-12	Control	4	PG	4	PG	4
4	1-3	Control	4	Control	4	Control	4
	4-6	Control	4	DMF	0.4	DMF	0.4
	7-9	Control	4	EG	4	EG	4
	10-12	Control	4	PG	4	PG	4
Periods mentioned			Part 1		Part 2		Part3

What happens with the serum bottles in each run is the same, and the most important steps are shown in Figure 16 on the following page. A timetable for the measurements is given earlier in table 4.

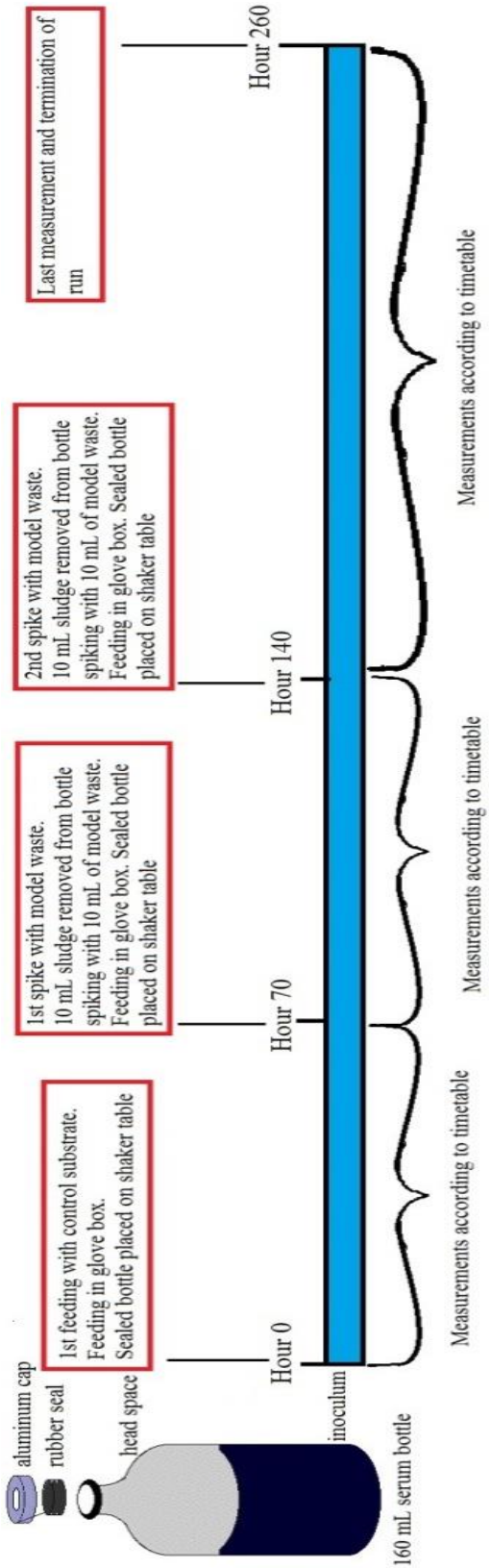

Figure 16 - Illustration showing the lifespan of a serum bottle reactor, important events are indicated and described in the time line

4. Results

The results recorded were in the form of gas and methane volumes. From this accumulated volumes have been derived in addition to production rates of gas and methane from each waste. The results are shown with the gas and methane productions first, followed by the rates.

4.1. Gas production

4.1.1 Control

Figure 17 shows that the gas productions from each run differ substantially, especially run 1 and run 2 from run 3 and run 4. The part that separates run 3 and 4 is the part before spike 1, from 0 hours until 70 hours. From spike 1 and onwards they follow the same pattern. In the time span before spike 1, runs 1 to 3 follow the same pattern, while the gas production is slightly higher for run 4. The reason for that is simply a miscalculation of the COD concentration in the control substrate feeding routine. The miscalculation was discovered between the first and second feeding in run 3. Prior to this discovery, the COD-concentration in all control substrate feedings were considerably lower, and this explains why the gas productions of the first 2 runs were so low (and also the start of run 3) compared to the accumulated gas production seen in run 4. Consequently, as run 4 is the only complete run with the desired COD concentration, all accumulated gas productions, and methane productions are compared with the results from the control substrates in run 4. Also, graphs showing this will include graphs with hour 0 starting from spike 1 to show relations where there are equal COD concentrations and only COD in the form of the desired model waste.

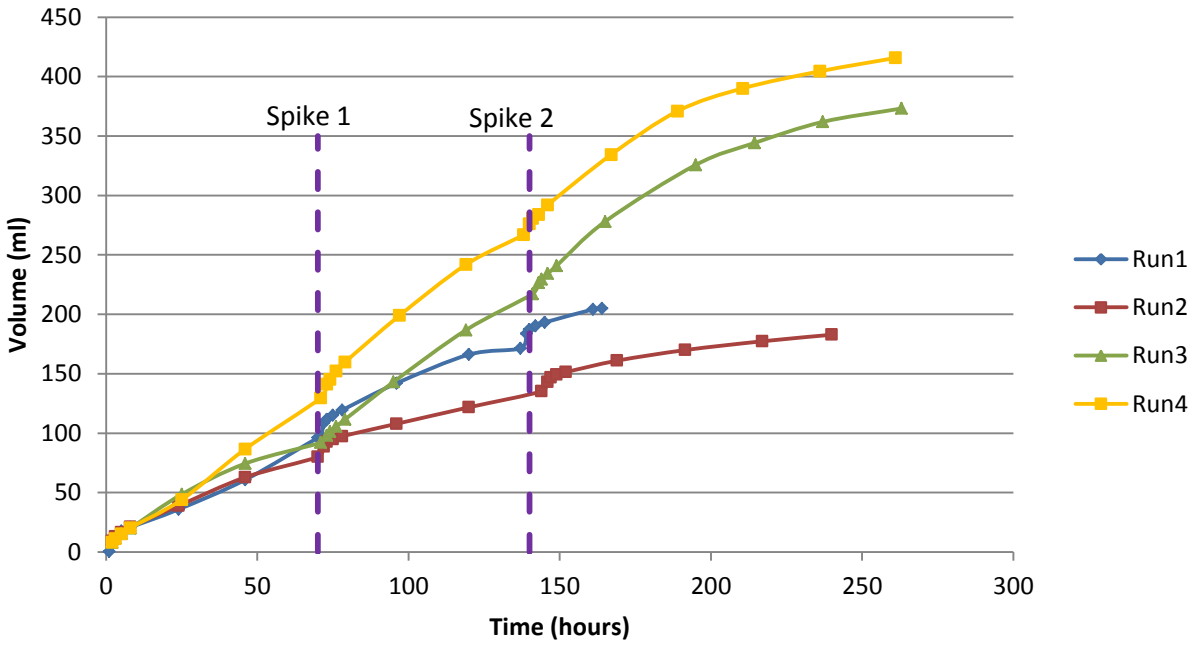

Figure 17 - Graph showing the average gas production from triplicates of the control substrate reactors from each run. Run4 has the desired COD concentration of 4 g/L, while the others are slightly lower due to miscalculations.

4.1.2 Tween 80

From Figure 18 and Figure 19 it is evident that the gas production is considerably lower than that of the control substrate with the same COD concentration. Before spike 1, when all reactors are fed with control, the COD concentrations were lower than desired in runs 1 to 3 resulting in lower gas productions; this is shown in Figure 18 where the gas production of Tween and Control low COD is lower than Control up until Spike 1. In fact, it is evident from these graphs that a COD concentration of 4 g /L in the Tween reactors yielded almost the same amount of gas as the lower COD concentrations of the control substrate. At the time of termination of run 1, 164 hours after the first feeding, the accumulated gas volume produced in the Tween reactors were an average of 539.9 mL/gCOD, while the control substrate reactors at the desired COD concentration had produced an average of 976.9 mL/gCOD at that time. If the pre-spike 1 period is excluded, the accumulated gas volume produced from the tween reactors totaled 291.2 mL/gCOD while the control substrate reactors produced 632.7 mL/gCOD of biogas.

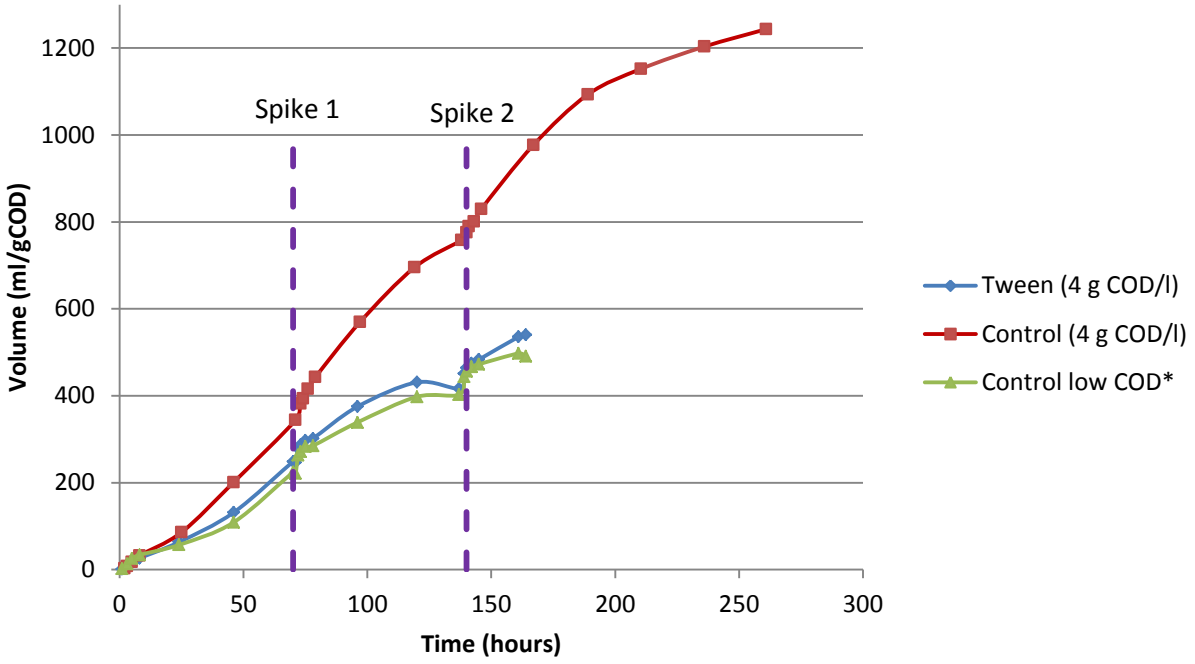

Figure 18 - Graph showing gas production from reactors with tween 80 substrate and control substrate. The period before spike 1 has a COD in the form of control substrate for all reactors. Wastes added at the spikes make the COD concentration in the reactor reach 4 g/L.

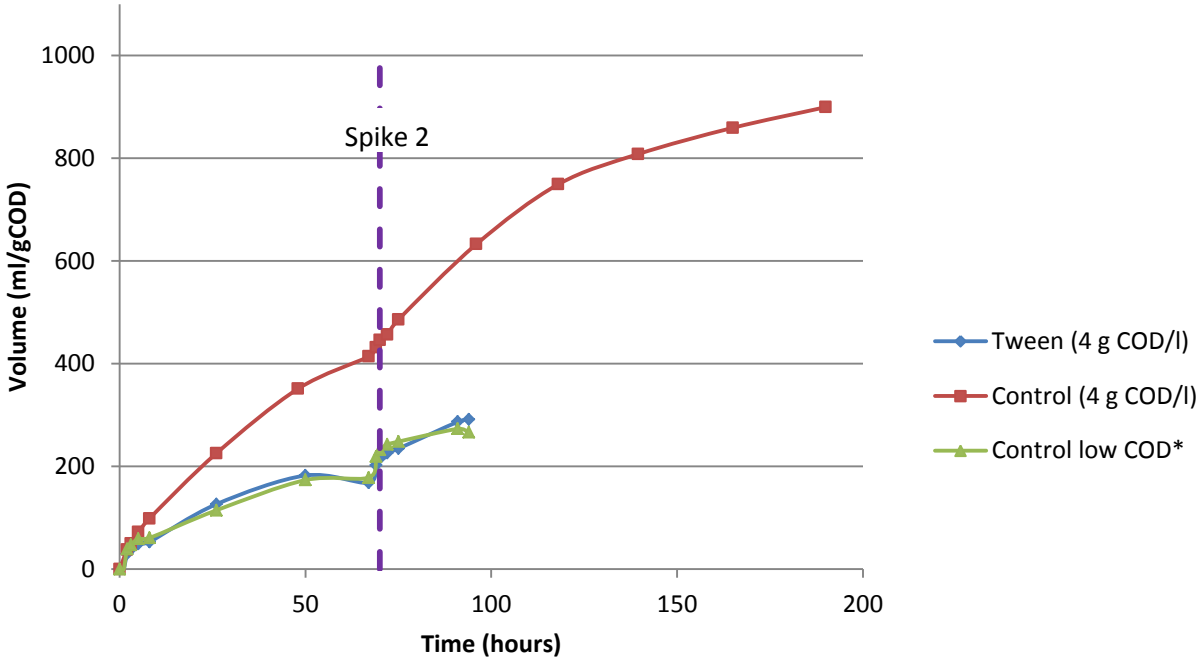

Figure 19 - Graph showing gas production from reactors with tween 80 substrate and control substrate from spike 1 and onwards. In spike 2 the reactors are fed with 10 mL of the wastes. Same volume is removed before.

4.1.3 Starch

Figure 20 shows that the gas production follows the same pattern as well as similar production volumes when comparing the Starch substrate and the control substrate at equal concentrations. Figure 21 shows that gas production from the Starch is slightly slower than the Control of the same COD concentration in the period up until Spike 2, but then picks up and produces more gas as termination is approaching, and ending up surpassing the accumulated volume achieved by the Control at the time of termination. The volumes produced up until termination of run 1 are very similar to volumes measured at the same times in run 4 for the control substrate; 910.7 mL/gCOD at 164 hours for Starch while 976.9 mL/gCOD at 167 hours for the Control, shown in Figure 20. It seems from this, that a slightly larger production can be expected from the Starch, given that from Spike 1 and onwards until termination, the average gas volume produced from the starch reactors is 648.9 mL/gCOD compared to 632.74 mL. Compared with the Control substrate reactors with the miscalculated COD amounts, it is evident that the concentration was too low in those reactors.

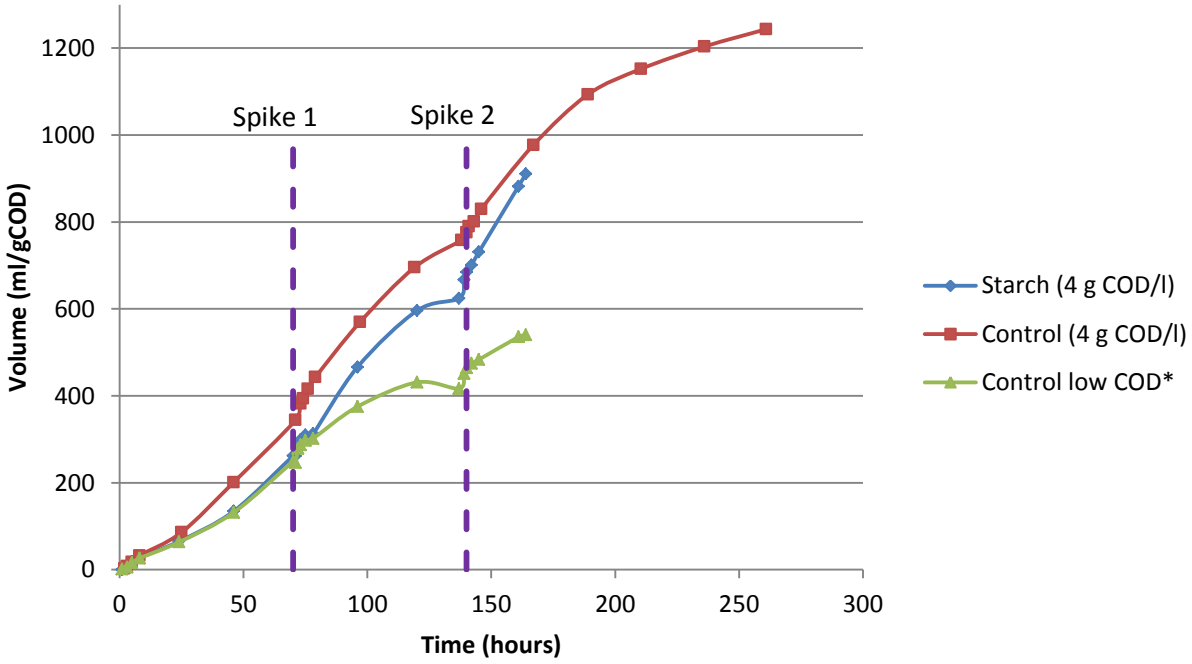

Figure 20 - Graph showing gas production from reactors with starch substrate and control substrate. The period before spike 1 has a COD in the form of control substrate for all reactors. Wastes added at the spikes and the first feed make the COD concentration in the reactor reach 4 g/L.

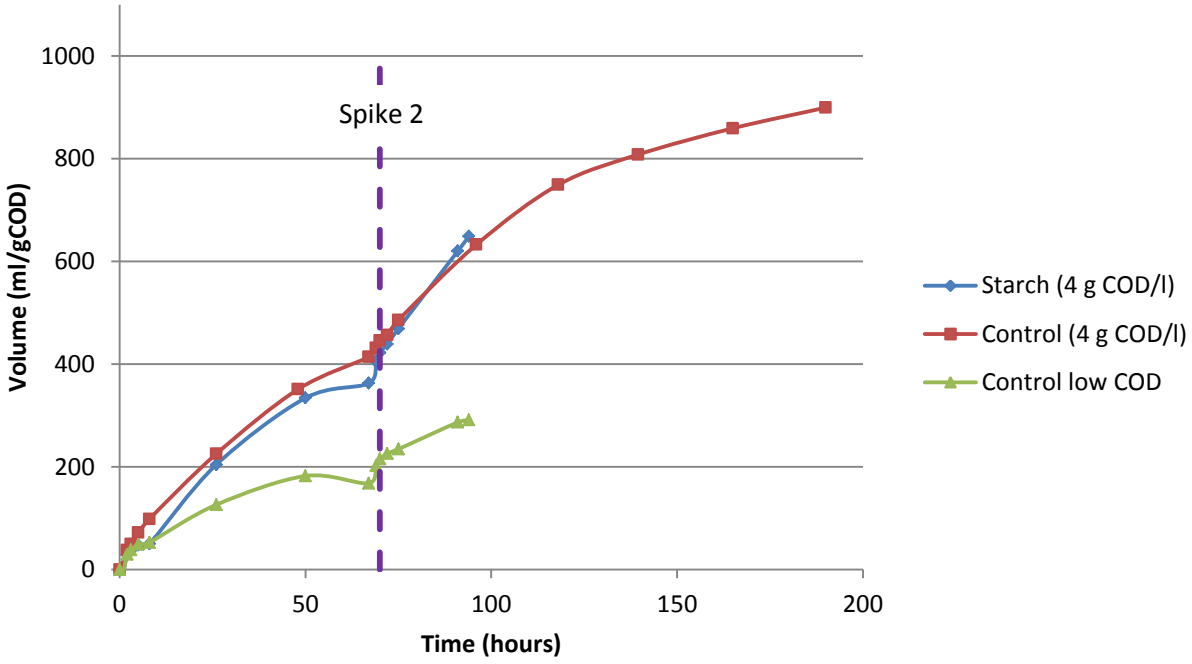

Figure 21 - Graph showing gas production from reactors with starch substrate and control substrate from spike 1 and onwards. In spike 2 the reactors are fed with 10 mL of the wastes. Same volume is removed before.

4.1.4 Gelatin

The gas production from gelatin was measured in two different runs as there was a miscalculation in the first attempt, in run 2. The COD concentration in the gelatin was extremely high in this run resulting in a much higher gas production, as shown in appendix, but a very low yield when the COD is taken into account. With the high concentration like this, the theoretical gas production is far greater than the actual gas produced. From Figure 22 one can, again, see that there are different COD concentrations prior to spike 1 and that this has an effect on the total gas volume produced with the control from run 4 producing more in this period than the reactors from run 2 and 3. From 0 hours until termination of the run with gelatin reactors at the COD concentration of 4 g/L, the accumulated volume produced amounts to 1185.4 mL/gCOD (263 hours) while for the control substrate reactors the volume produced at the termination after 261 hours was a slightly higher 1243.6 mL/gCOD. However, Figure 23 shows, in fact, that the accumulated volume produced from gelatin is higher than that of the control substrate with a total volume produced at termination of 998.7 mL/gCOD compared to 899.4 mL/gCOD. Another interesting observation is that for the first half of the run, the control substrate reactors and gelatin reactors produce very similar amounts, but while the controls keep producing steadily for the rest of the run, the gelatin reactors increase the gas production rate slightly.

As with the gas production, methane production from gelatin was measured in two different runs. Even though the gas production from the high concentration gelatin was higher as seen in appendix, the actual methane volumes measured was lower as the methane content in the gas produced was miniscule. When taking the COD into account, the methane yield was close to 0 as shown in Figure 23. The methane produced from the desired COD concentration gelatin is eight times higher than that of the high COD concentration, both when calculating the accumulated volume from hour 0 and starting from spike 1. When comparing the methane production of gelatin with the production made by the control substrate reactors one can see that the production is similar when comparing same COD concentrations, although it is evident from Figure 23 that methane production is, in fact slightly higher for the gelatin reactors.

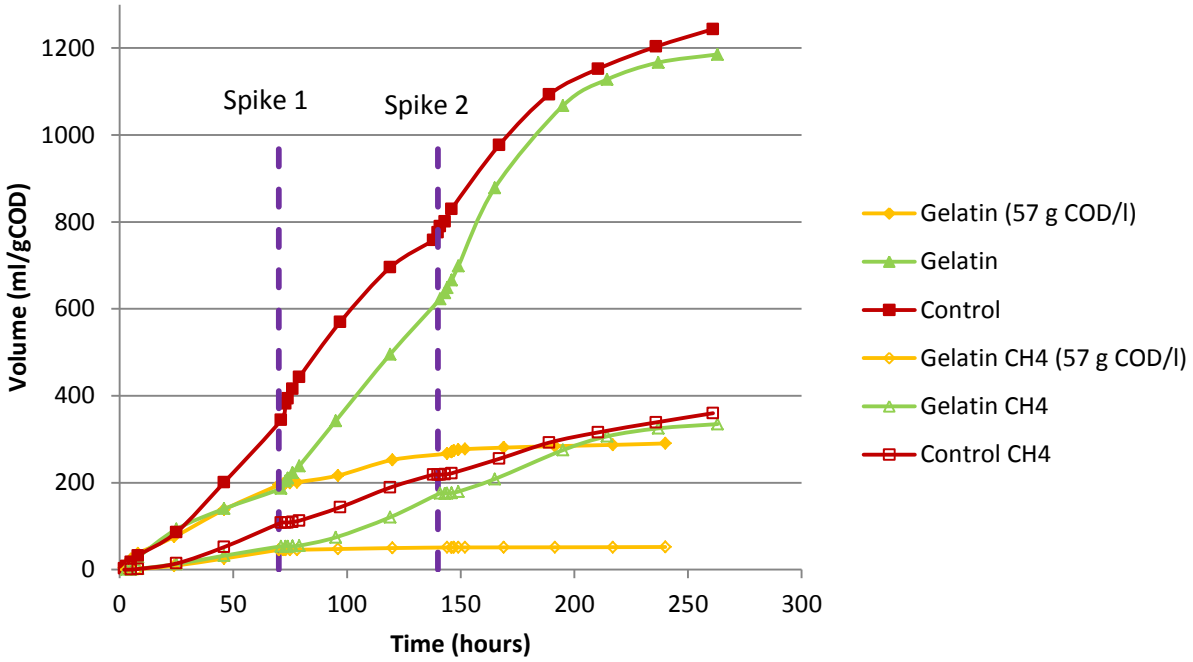

Figure 22 - Graph showing gas and methane production from reactors with gelatin substrate and control substrate. The period before spike 1 has a COD in the form of control substrate for all reactors. Wastes added at the spikes and the first feed make the COD concentration in the reactor reach 4 g/L unless stated otherwise. Methane productions are marked with CH4

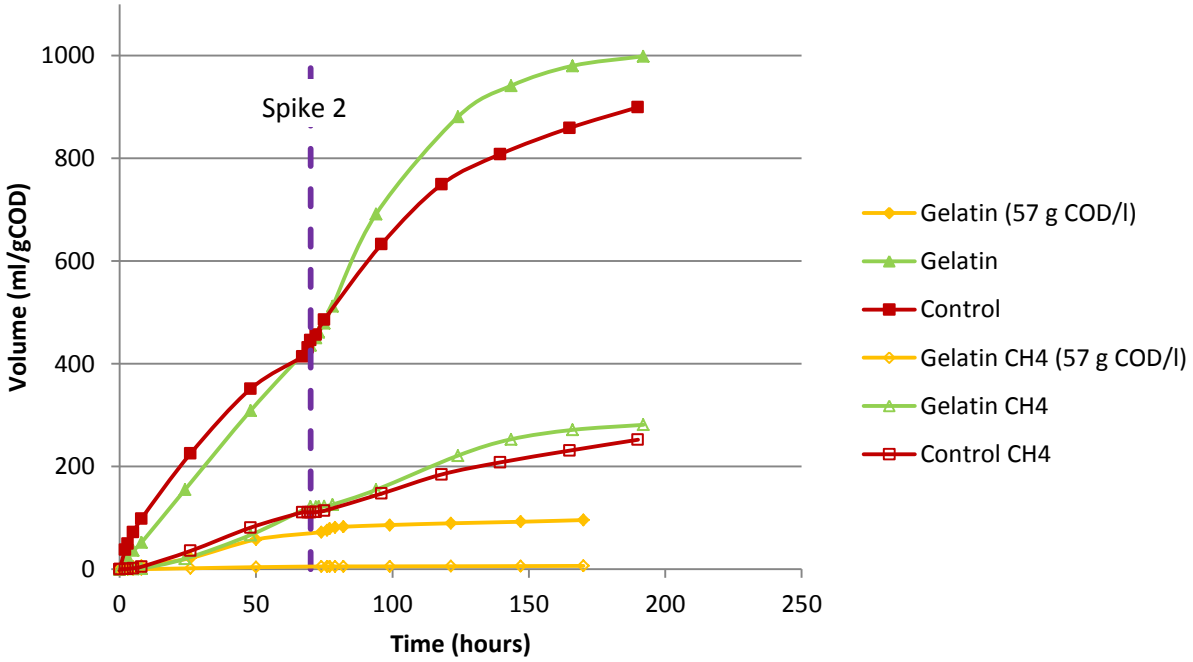

Figure 23 - Graph showing gas and methane production from reactors with gelatin substrate and control substrate from spike 1 and onwards. In spike 2 the reactors are fed with 10 mL of the wastes. Same volume is removed before. If not stated otherwise, the COD concentration is 4 g/L. Methane productions are marked with CH4

4.1.5 Ethylene glycol and propylene glycol

The glycols were run twice each because their high standard deviations. The gas productions varied greatly from reactor to reactor, and in order to verify that everything had been done correctly a second run for each glycol was conducted. The variations were still high, leading to the conclusion that it had to do with the acclimatization of the microbial communities. What is evident from looking at figures 24 to 27 is that the development of the two different glycols are different; while the EG reactors tend to slow their gas production in the same manner as the control substrate reactors, the PG reactors do not seem to slow their production as termination is coming closer. From both figures one can see that in the final run, the ethylene glycol reactors and propylene glycol reactors produce similar amounts up until 130 hours in Figure 25 and Figure 27, with the exception of EG 1. It is also shown in these figures that the PGs from the different runs are similar, a total accumulated volume difference of 85 mL/gCOD which is within the standard deviations of accumulated volumes of PG 1 and PG2. The EGs, on the other hand, have a slightly larger difference in accumulated volumes at the time of termination for run 2 at 240 hours (157 hours after spike 1) of 140 mL/gCOD. This value is within the standard deviation for the EG 1, but well above that of EG 2 at 240 hours in run 4.

Compared with the results of the gelatin, starch and control substrate reactors the glycol reactors do not perform well with respect to biogas production. Even compared with the tween reactors they come out with inferior results with a total average accumulated gas production from spike 1 until the termination time of run 1 at 164 hours (94 hours after spike 1) of 191 mL/gCOD, 301 mL/gCOD, 232 mL/gCOD, and 272 mL/gCOD for EG 1, EG 2, PG 1 and PG 2 respectively.

As with the gas productions, the methane production from the glycol reactors varied greatly from reactor to reactor resulting in large standard deviations and a second run with both chemicals; ethylene glycol and propylene glycol. What is different from the relations in gas production is that the methane production is lower in the EG 1 reactors compared with the other glycol reactors as shown in Figure with an average accumulated volume of 31 mL methane/g COD at its termination. Otherwise the general pattern of accumulated volume over time for the EG reactors follow that of the control substrates, while the PG reactors seem to try and catch up with the control substrates as time passes and termination is approaching, as seen in Figure 27. The total accumulated methane production of the glycol reactors is low compared to the volume accumulated from the control substrate reactors. For EG the volume produced is approximately one third of the volume produced by the control substrates and it seems to keep on being the case, while the trend for PG is a little more uplifting as the accumulated difference is diminishing as termination is approaching

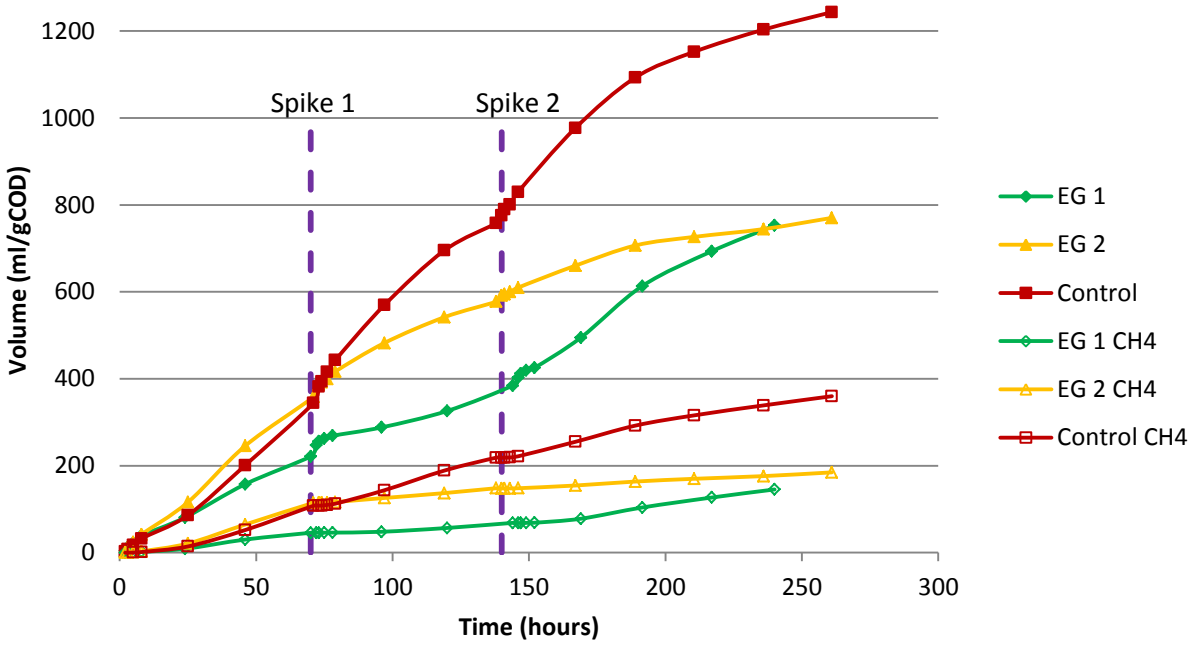

Figure 24 - Graph showing gas and methane production from reactors with ethylene glycol substrate and control substrate. The period before spike 1 has a COD in the form of control substrate for all reactors. Wastes added at the spikes and the first feed make the COD concentration in the reactor reach 4 g/L. Methane productions are marked with CH4. EG 1 is EG from run 2, EG 2 is EG from run 4.

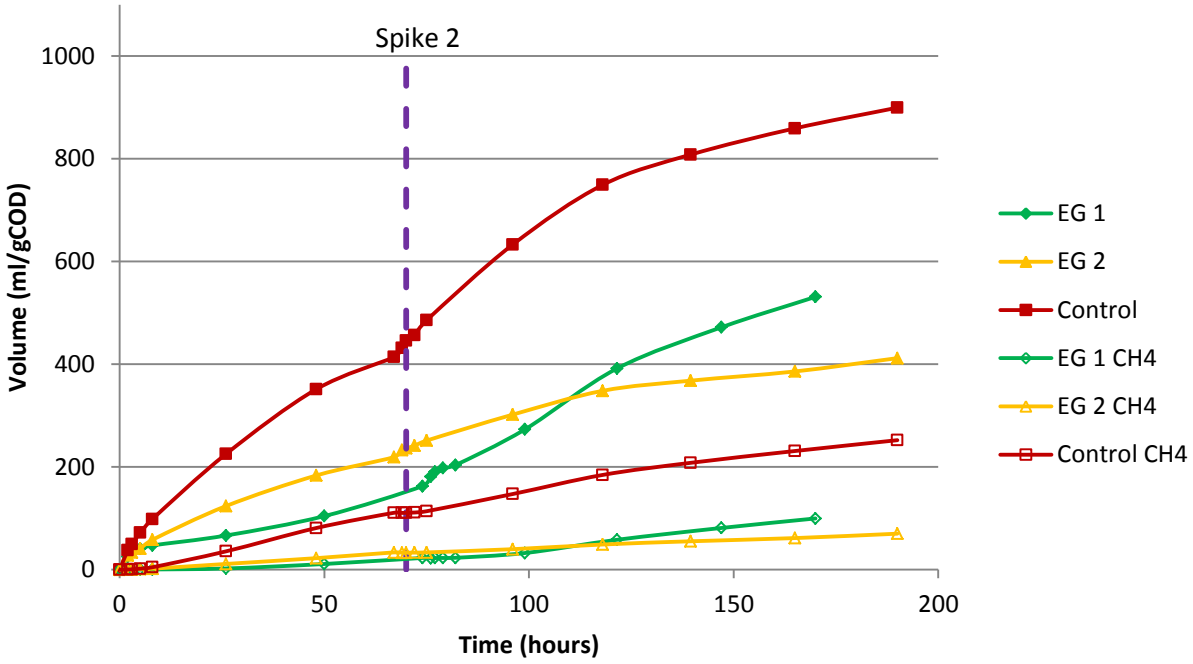

Figure 25 - Graph showing gas and methane production from reactors with ethylene glycol substrate and control substrate from spike 1 and onwards. In spike 2 the reactors are fed with 10 mL of the wastes. Same volume is removed before. If not stated otherwise, the COD concentration is 4 g/L. Methane productions are marked with CH4. EG 1 is EG from run 2, EG 2 is EG from run 4.

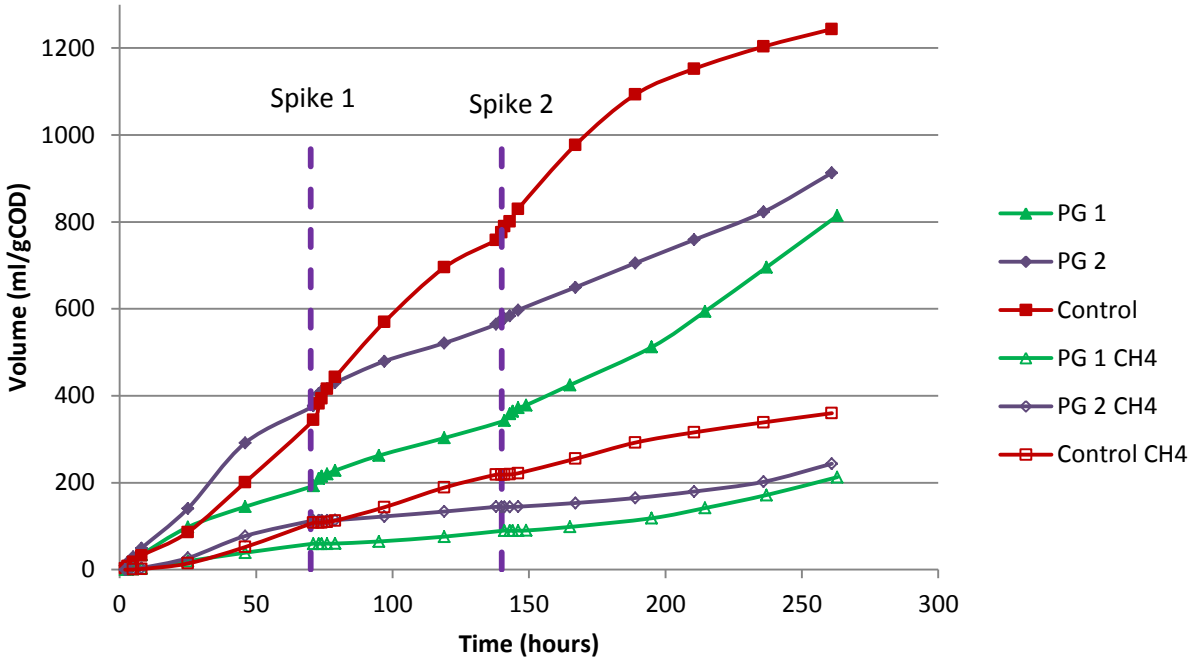

Figure 26 - Graph showing gas and methane production from reactors with propylene glycol substrate and control substrate. The period before spike 1 has a COD in the form of control substrate for all reactors. Wastes added at the spikes and the first feed make the COD concentration in the reactor reach 4 g/L. Methane productions are marked with CH4. PG 1 is PG from run 3, PG 2 is PG from run 4.

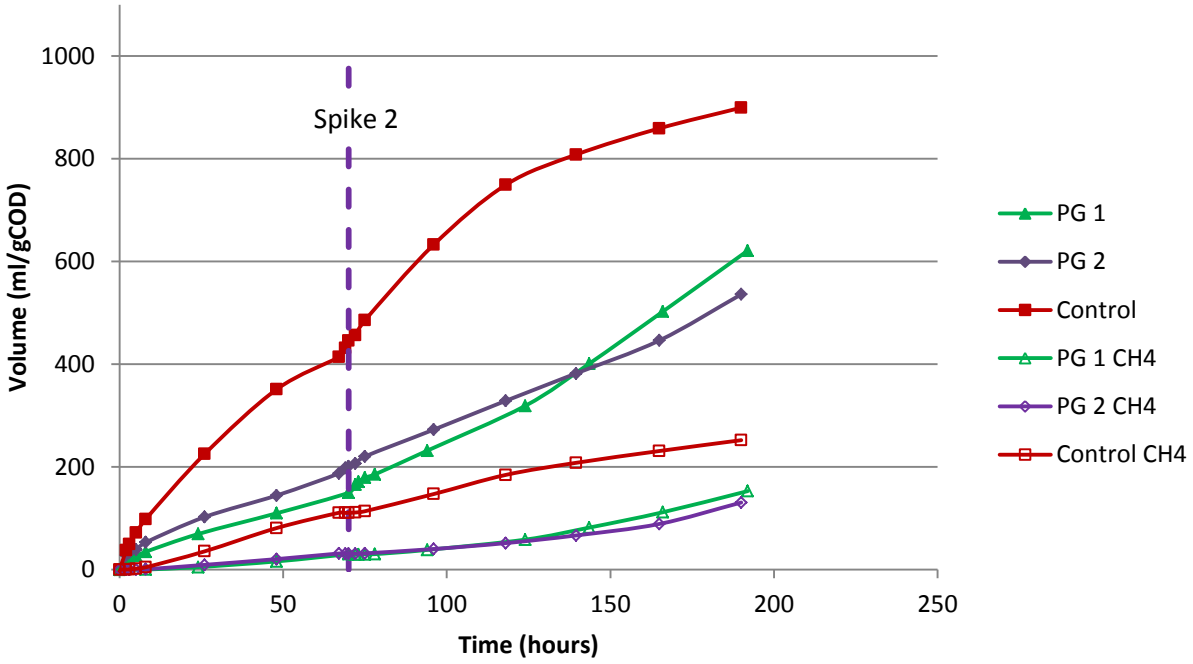

Figure 27 - Graph showing gas and methane production from reactors with propylene glycol substrate and control substrate from spike 1 and onwards. In spike 2 the reactors are fed with 10 mL of the wastes. Same volume is removed before. If not stated otherwise, the COD concentration is 4 g/L. Methane productions are marked with CH4. PG 1 is PG from run 3, PG 2 is PG from run 4.

4.1.6 Dimethylformamide

The figures 28 to 31show the gas and methane production from the reactors with the DMF wastes. The two runs with DMF differ because of the COD concentrations in the reactors. The first run with DMF contains the same amounts of COD in the reactors as all the other valid reactors, 4 g/L. The second run with DMF was conducted with a tenth of the concentration to see whether the previous concentration had been too high and resulted in inhibition of the microbial community, and whether the inoculum would be able to adapt to the waste at low concentrations. Both of the runs with DMF show a low gas production, without taking COD into account, compared to all the other wastes shown in appendix. But when taking the COD into account it is clear that the low concentration DMF produced a lot of gas, and also methane, although the methane content in the gas was not incredible. Total gas production at termination of the runs (calculated from spike 1 and onwards) ended at 147 mL/gCOD for the high concentration DMF, while at 2243 mL for the low concentration. The difference here is substantial, with the low concentration DMF producing 45% more gas according to the measurements taken, and that is even without taking the COD into account. When considering the difference in COD, the gas production is very high for the low concentration DMF compared to other wastes.

The two runs with DMF, when looking at the methane production, differ because of the COD concentrations in the reactors. The high concentration DMF show very low methane production compared with all the other wastes, while the low concentration is similar to the control in volume, but then considerably lower in content. They seem to follow the same pattern of production relative to their own respective gas productions. But compared with the control substrate reactors the methane production of the DMFs is considerably lower than their gas production. While in the gas production shown in Figure 29, the DMF produced about one sixth of the gas production for the control substrates, the methane production of the DMFs amounted to only 5% of the methane produced by the control substrate reactors.

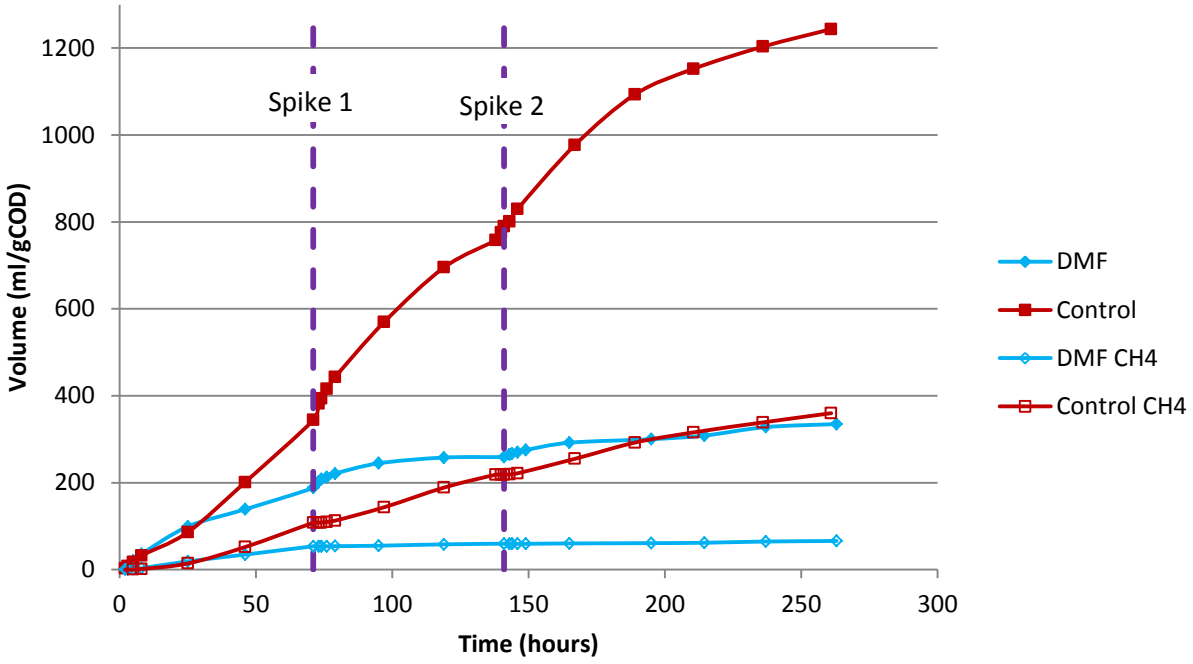

Figure 28 - Graph showing gas and methane production from reactors with DMF substrate and control substrate. The period before spike 1 has a COD in the form of control substrate for all reactors. Wastes added at the spikes and the first feed make the COD concentration in the reactor reach 4 g/L. Methane productions are marked with CH4.

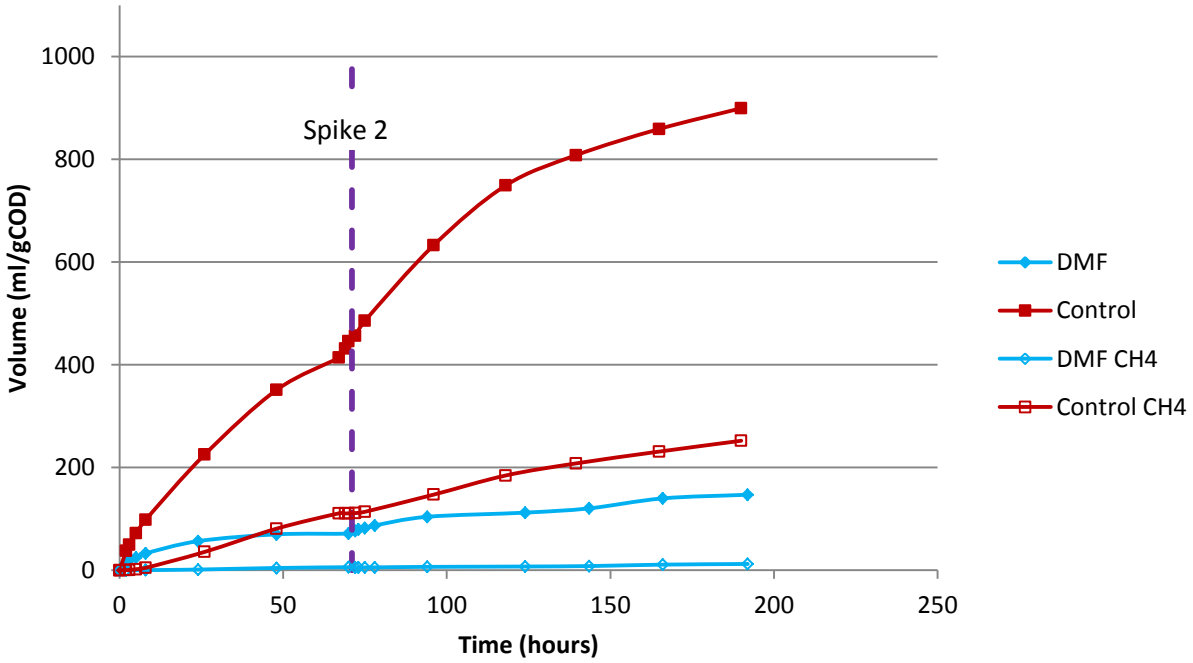

Figure 29 - Graph showing gas and methane production from reactors with DMF substrate and control substrate from spike 1 and onwards. In spike 2 the reactors are fed with 10 mL of the wastes. Same volume is removed before. If not stated otherwise, the COD concentration is 4 g/L. Methane productions are marked with CH4.

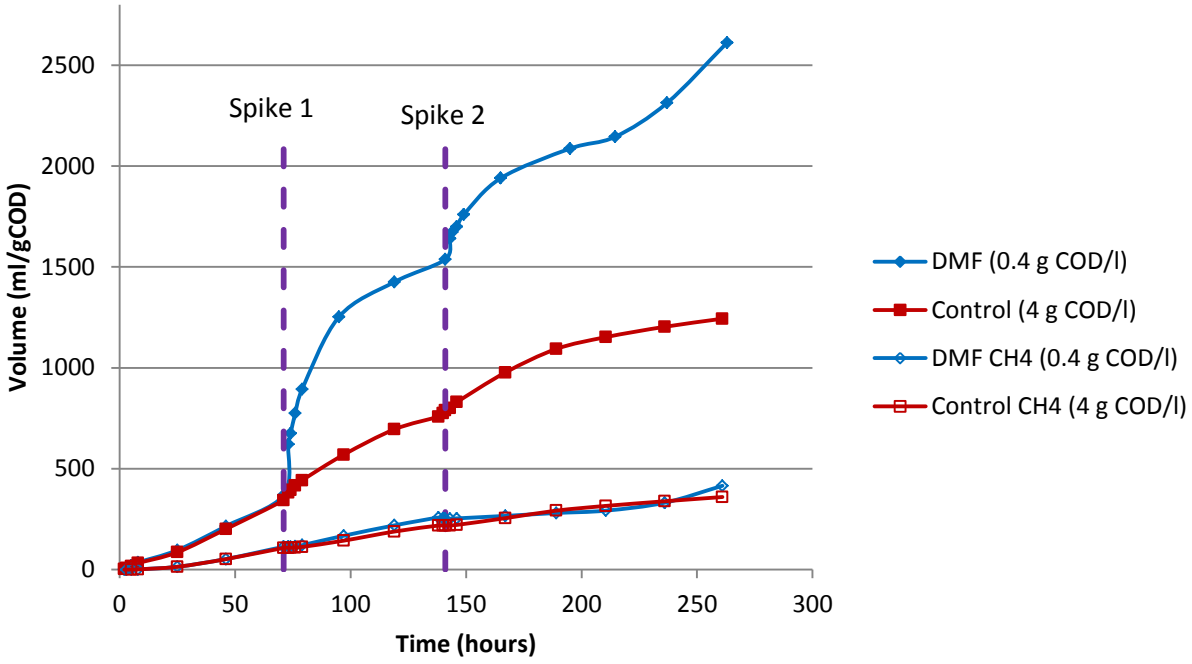

Figure 30 - Graph showing gas and methane production from reactors with DMF substrate and control substrate. The period before spike 1 has a COD in the form of control substrate for all reactors. Wastes added at the spikes and the first feed make the COD concentration in the reactor reach 4 g/L unless stated otherwise. Methane productions are marked with CH4.

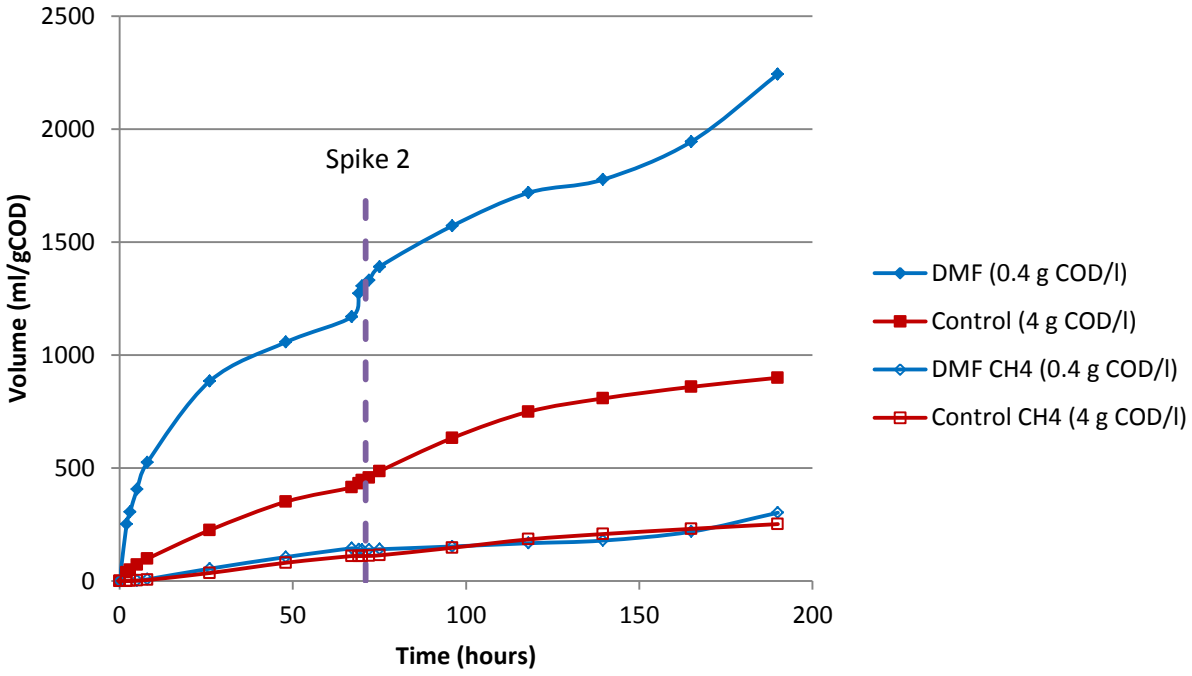

Figure 31 - Graph showing gas and methane production from reactors with DMF substrate and control substrate from spike 1 and onwards. In spike 2 the reactors are fed with 10 mL of the wastes. Same volume is removed before. If not stated otherwise, the COD concentration is 4 g/L. Methane productions are marked with CH4.

4.2 Production rates

The gas and methane production rates of each waste have been looked at and are shown in graphs sorted with the production rates from each waste. When looking at production rates of methane compared to those of biogas it is clear that the rates are much higher when talking about the biogas production rates.

4.2.1 Tween 80

The gas production rate of tween reactors and the control substrate reactors generally follow the same pattern with peaks soon after feeding and spiking. This can also be seen in the previous graphs by the slope of the graphs being steeper immediately after spiking. Some odd results are seen here in Figure 32 as with the peak around spike 1 where the rate of production is much higher for the tween reactors even though they in the long run produce less gas, or the point prior to spike 2 where the production rate drops to 0. These aspects will be discussed in a later chapter.

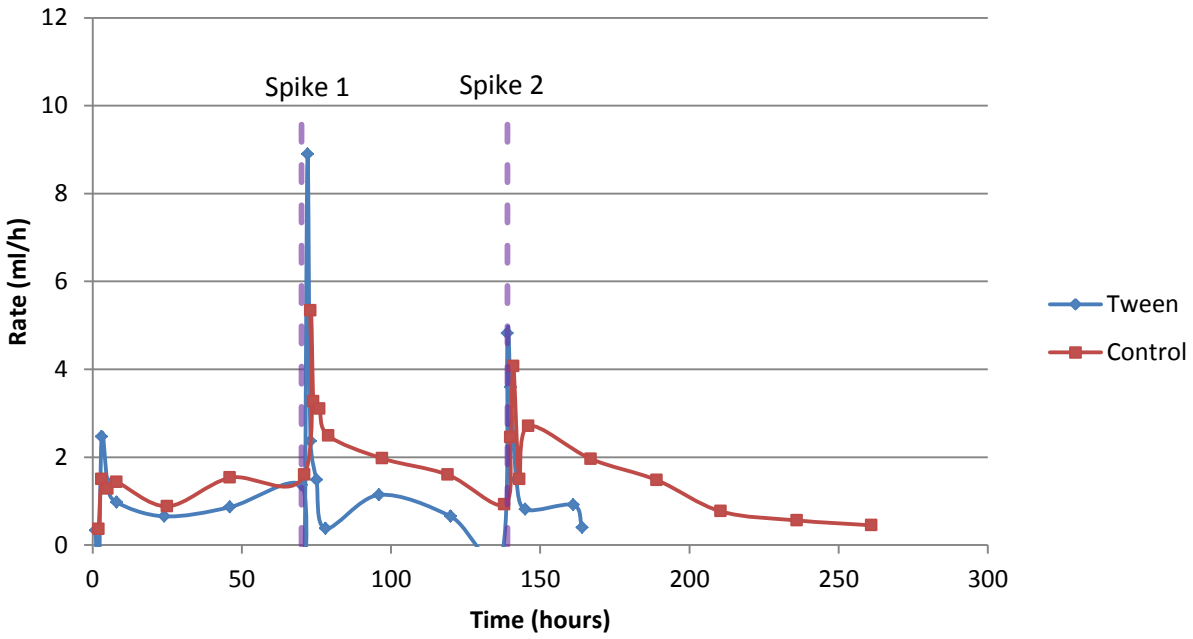

Figure 32 - Graph showing the change in gas production rate (mL gas/hour) over time for Tween 80 in a 70 mL reactor. The spikes represent removal of 10 mL effluent and feeding of 10 mL more waste. Both spikes and the first feeding brings has a COD concentration that brings the COD concentration in the reactor to 4 g/L

4.2.2 Starch

The gas production rate from the starch reactors also follows the same path as the tween reactors shown previously. The reason why the peaks are generally high in the measurements taken from the first run will be discussed. Compared with the production rate of the control substrate reactors from run 4, Figure 33 shows that the rate varies more with the starch reactors, with a higher secondary peak between spike 1 and 2 as well as with an increasing rate immediately before termination.

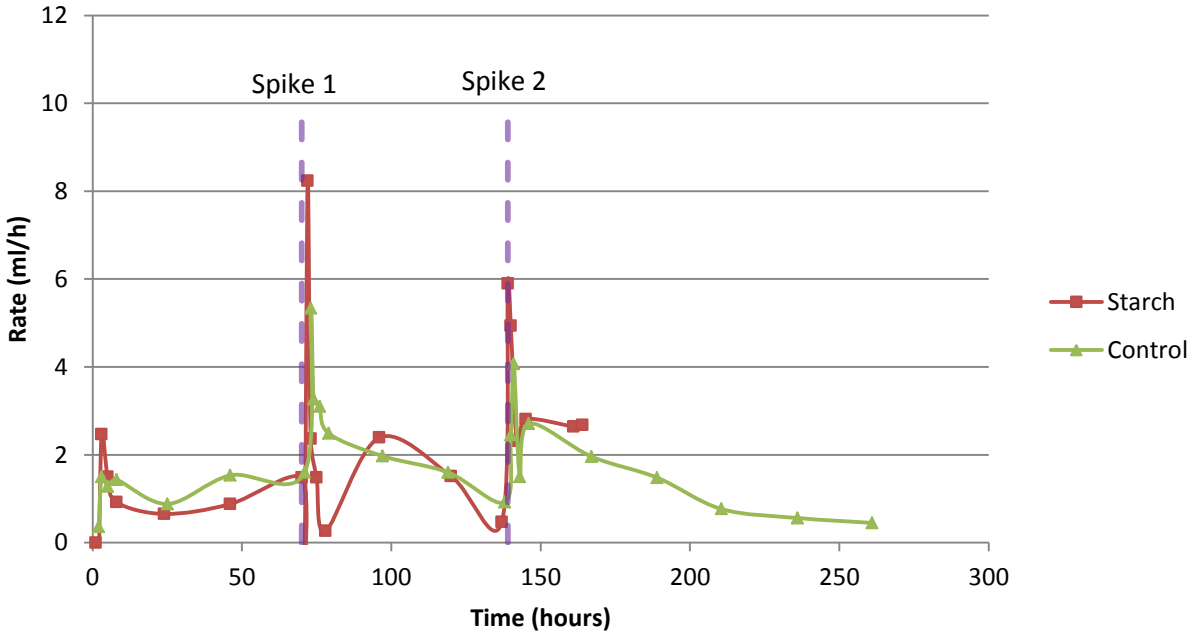

Figure 33 - Graph showing the change in gas production rate (mL gas/hour) over time for starch in a 70 mL reactor. The spikes represent removal of 10 mL effluent and feeding of 10 mL more waste. Both spikes and the first feeding brings has a COD concentration that brings the COD concentration in the reactor to 4 g/L

4.2.3 Gelatin

From Figure 34 it is evident that the control substrate reactors and the gelatin reactors with the same COD behave similarly. For the high concentration gelatin the peaks are clearer and higher, reaching a maximum production rate of near 12 mL/h which is double the peak production rate registered for the control substrate reactors in run 4.

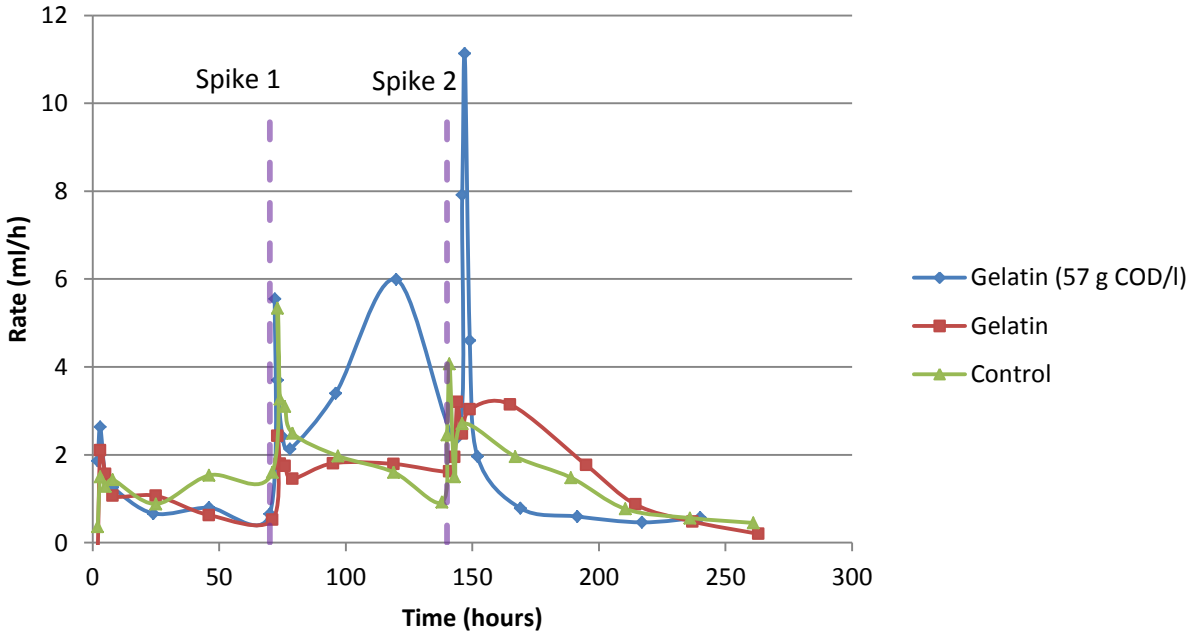

Figure 34 - Graph showing the change in gas production rate (mL gas/hour) over time for gelatin in a 70 mL reactor. The spike represents removal of 10 mL effluent and feeding of 10 mL more waste. Both spikes and the first feeding brings has a COD concentration that brings the COD concentration in the reactor to 4 g/, unless stated otherwise.

From Figure 35one can see that the methane production rate is very dependent on the COD levels for the control substrate phase before spike 1. The peaks there are lower for the lower concentrations used in runs 2 and 3. When using the same COD concentrations of gelatin and control substrates, the reactors behave similarly with respect to methane production rate, although the peaks are higher for the gelatin than for the control. Also, the peaks tend to go lower for each spike.

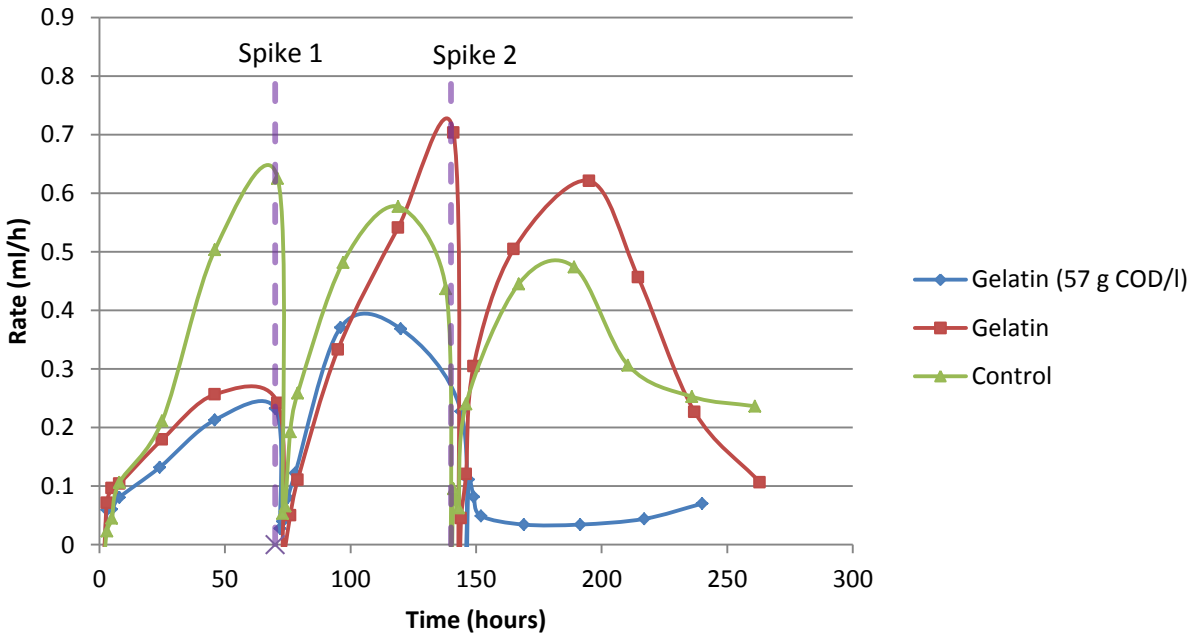

Figure 35 - Graph showing the change in methane production rate (mL gas/hour) over time for gelatin in a 70 mL reactor. The spikes represent removal of 10 mL effluent and feeding of 10 mL more waste. Both spikes and the first feeding brings has a COD concentration that brings the COD concentration in the reactor to 4 g/L, unless stated otherwise.

One notable aspect of the production rates are shown in the Figure 36 and Figure 37. The peaks of production rates do not coincide. In fact they are opposite, even though the gas production is slower towards the end, the methane production is higher (in volume and content) and peaking right before the spikes, while the peaks of biogas production as a whole tend to peak right after feeding the reactors.

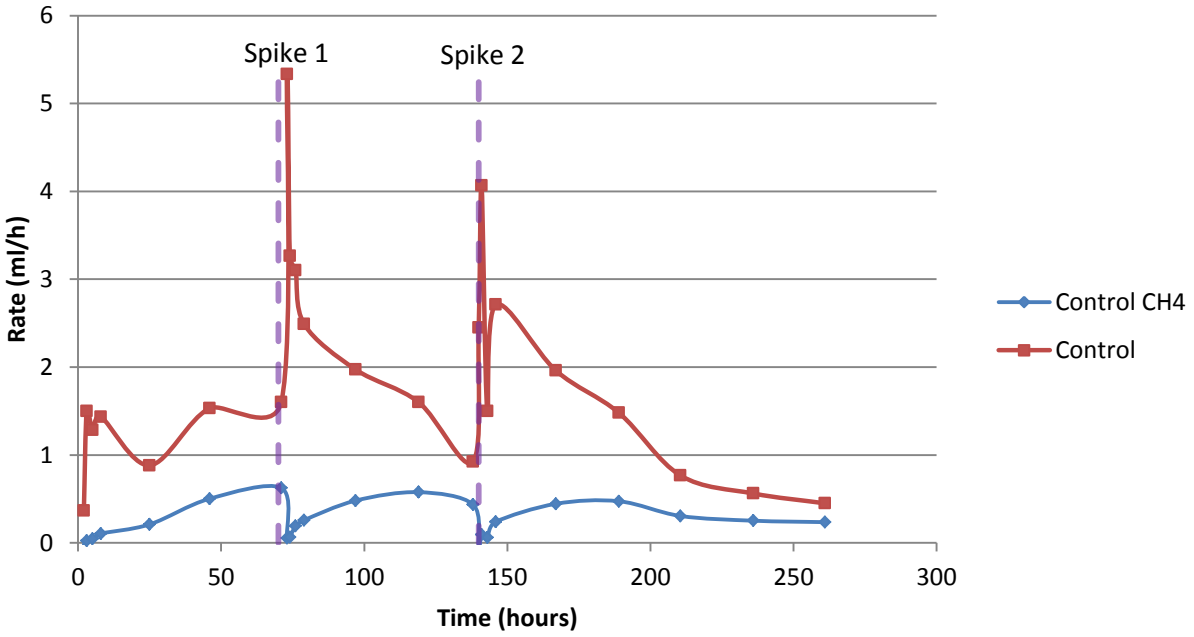

Figure 36 - Comparison of production rates of methane and biogas. The COD concentration in the reactors is 4 g/L in the feeding at hour 0 as well as the spikes

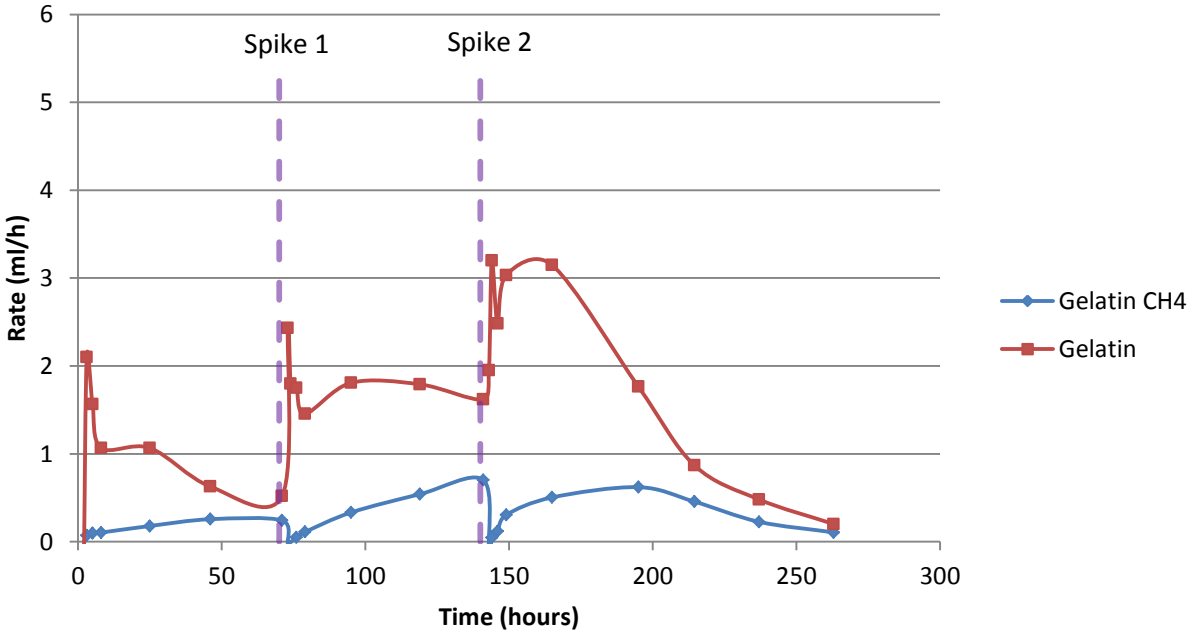

Figure 37 - Comparison of production rates of methane and biogas. The COD concentration in the reactors is 4 g/L in the feeding at hour 0 as well as the spikes. In the period before spike 1, the COD is in the form of control substrate. Spike 1 and 2 are both with gelatin

4.2.4 Ethylene glycol and propylene glycol

The gas production rates from the EG reactors shown in Figure 38 show that they generally lie beneath the rate of the control substrate reactors and consequently lower than those of the starch and gelatin reactors as well. They peak immediately after spikes and have a general tendency of lowering the rates as time goes by, with lower peaks after Spike 2 compared after Spike 1. The PG reactors behave exactly the same up until 200 hours where the production rate flattens out rather than decrease as most of the other wastes tend to. The PG reactors even increase their production rates towards termination as shown in Figure 39, surpassing the production rates of the control substrate reactors.

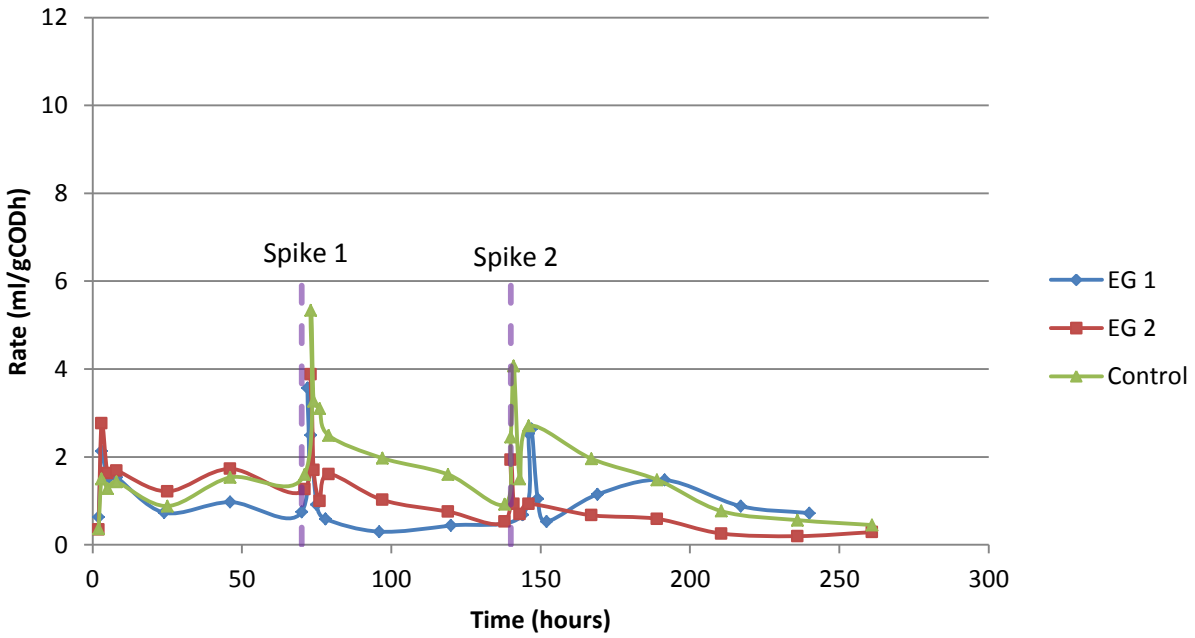

Figure 38 - Graph showing the change in gas production rate over time for ethylene glycol in a 70 mL reactor. The spikes represent removal of 10 mL effluent and feeding of 10 mL more waste. Both spikes and the first feeding brings has a COD concentration that brings the COD concentration in the reactor to 4 g/L

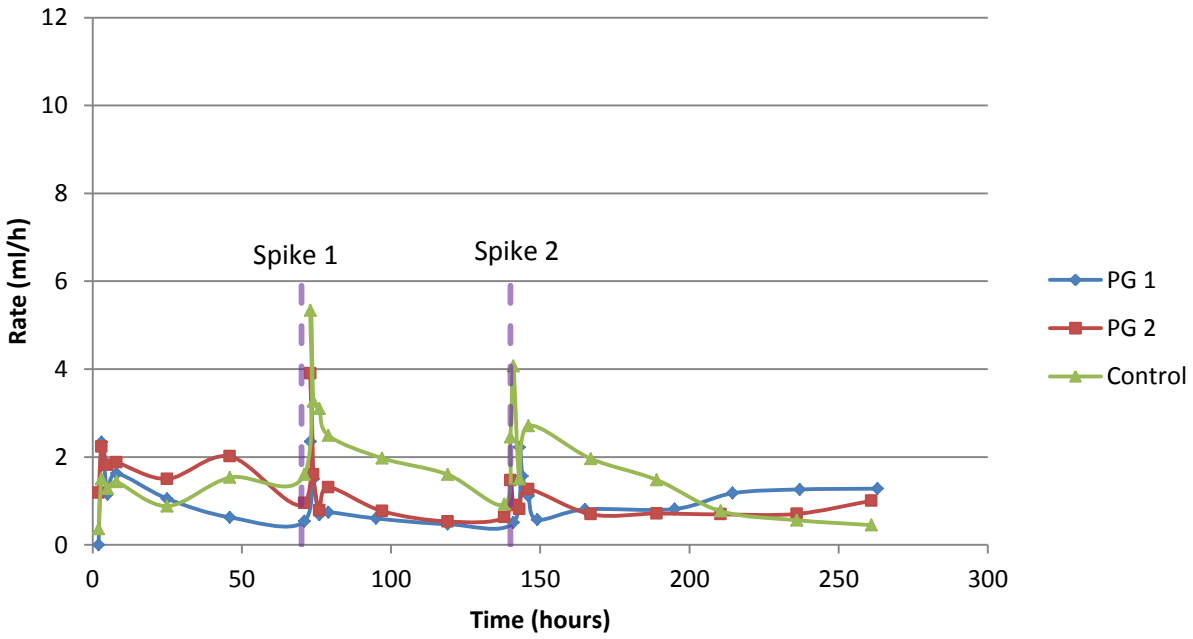

Figure 39 - Graph showing the change in gas production rate over time for propylene glycol in a 70 mL reactor. The spikes represent removal of 10 mL effluent and feeding of 10 mL more waste. Both spikes and the first feeding brings has a COD concentration that brings the COD concentration in the reactor to 4 g/L

The methane production rates of the glycols differ from the control substrate in the fact that they do not decrease prior to spikes. Both increase steadily between spike 1 and 2 peaking at very similar rates shown in Figure 40 and Figure 41 . However, after the second spike, the methane production rates of the EG reactors, peak and flatten out, while the rates of the PG reactors keep soaring, up past the rates of the control substrate reactors up until termination.

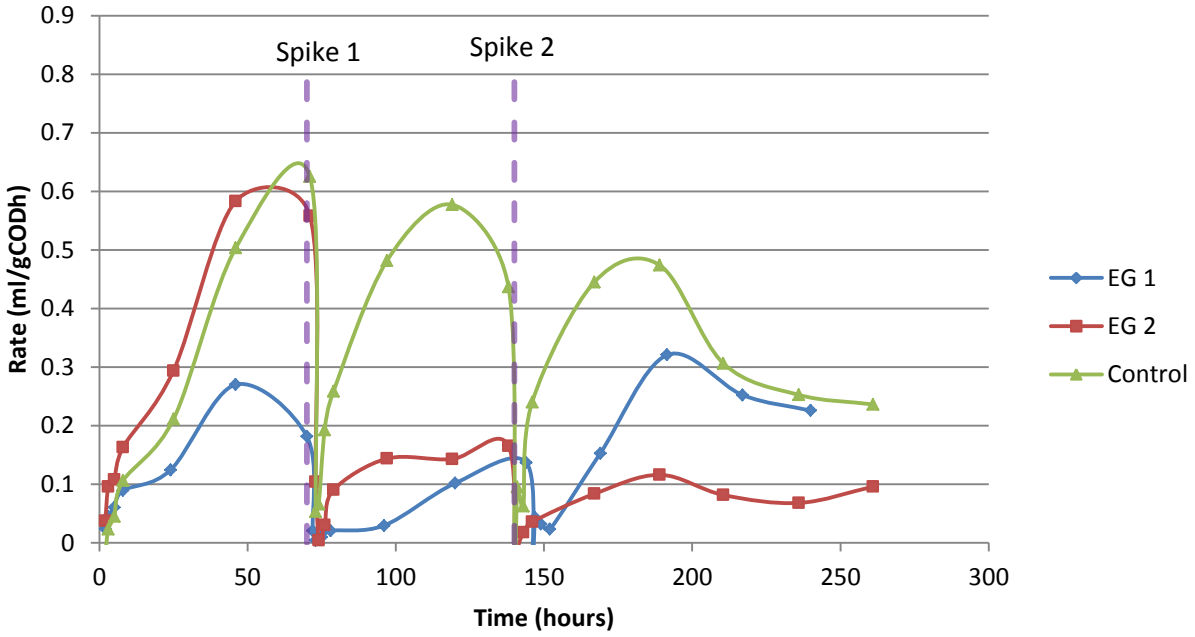

Figure 40 - Graph showing the change in methane production rate over time for ethylene glycol in a 70 mL reactor. The spikes represent removal of 10 mL effluent and feeding of 10 mL more waste. Both spikes and the first feeding brings has a COD concentration that brings the COD concentration in the reactor to 4 g/L.

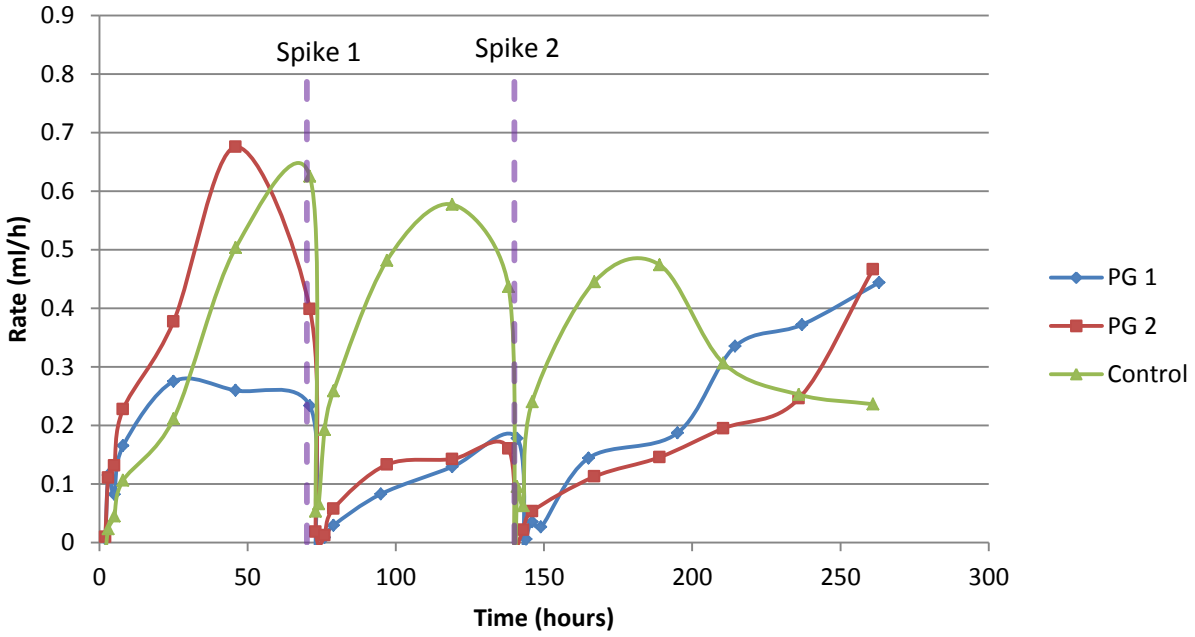

Figure 41 - Graph showing the change in methane production rate over time for propylene glycol in a 70 mL reactor. The spikes represent removal of 10 mL effluent and feeding of 10 mL more waste. Both spikes and the first feeding brings has a COD concentration that brings the COD concentration in the reactor to 4 g/L

4.2.5 Dimethylformamide

Figure 42 below shows that the gas production rates of the DMFs follow the same general pattern that most wastes follow, including the control substrate shown in the figure. The production rates peak at the same time dropping fast and evening out at very low levels for both concentrations of DMF, although one should consider that the difference in COD concentrations of the DMFs would play a big role. The difference in rates between the two concentrations is bigger between the two spikes than after the second.

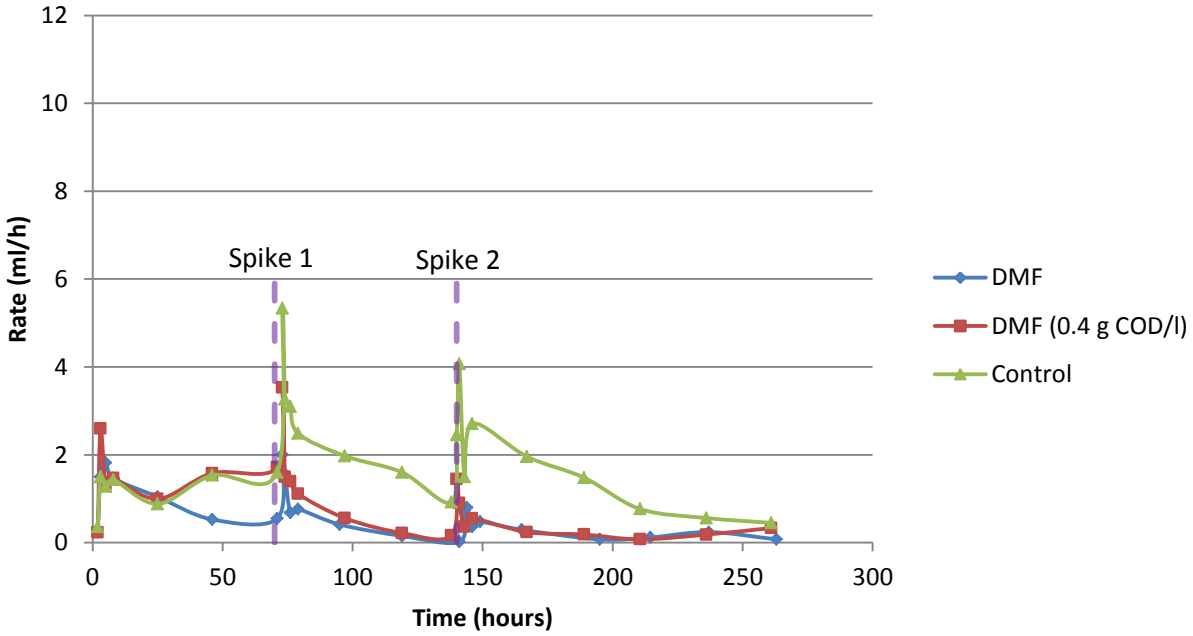

Figure 42 - Graph showing the change in gas production rate over time for DMF in a 70 mL reactor. The spikes represent removal of 10 mL effluent and feeding of 10 mL more waste. Both spikes and the first feeding brings has a COD concentration that brings the COD concentration in the reactor to 4 g/L, unless stated otherwise.

The same can be said about the methane production rates shown in Figure 43, the rates are by far the lowest observed from any of the model wastes, together with the highly concentrated gelatin, and the rates from the different concentrations of DMF seem to differ slightly more early on. Also, a rise in the production rate is observed after a long steady period on the reactors with low concentration DMF right before termination.

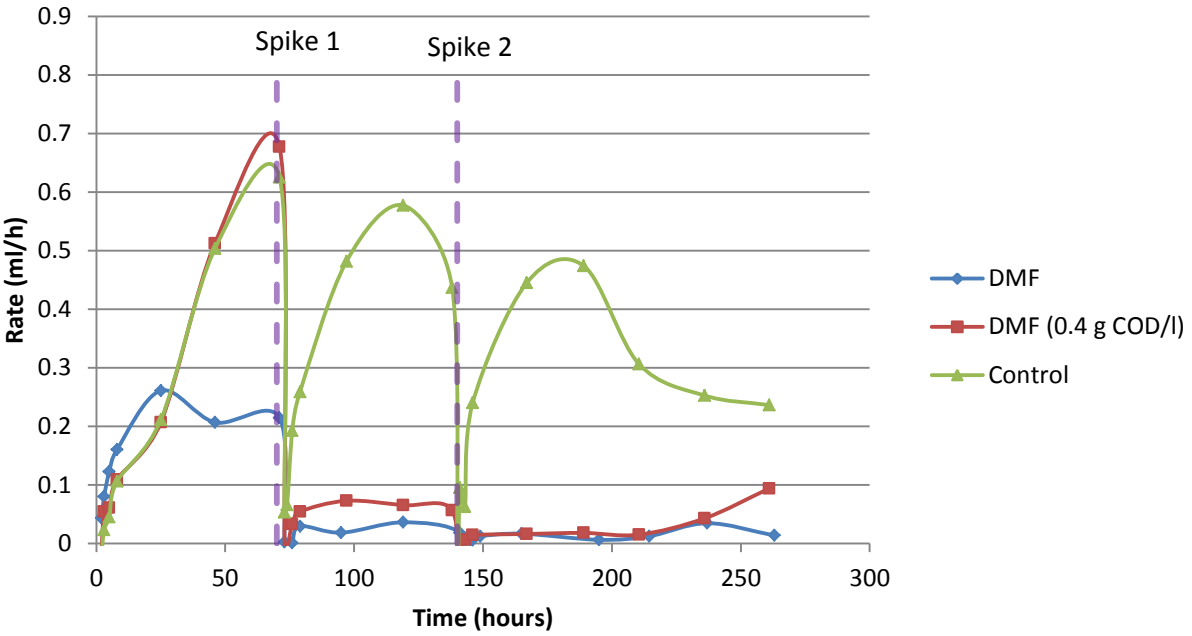

Figure 43 - Graph showing the change in methane production rate over time for DMF in a 70 mL reactor. The spikes represent removal of 10 mL effluent and feeding of 10 mL more waste. Both spikes and the first feeding brings has a COD concentration that brings the COD concentration in the reactor to 4 g/L, unless stated otherwise

4.3 Summary and methane content

Table 7 - Accumulated gas and methane productions and total methane content from the gas produced. COD concentration in the reactors is 4 g/L. The asterisks represent different COD concentrations; 57 g/L and 0.4 g/L for gelatin and DMF respectively.

	Total accumulated gas (mL/gCOD)	Total accumulated methane (mL/gCOD)	Time of termination (hour)	Methane content	Methane content last measurement
Control	1243.57	359.91	261	28.94 %	52 %
Gelatin*	290.37	52.13	240	17.95 %	12 %
Gelatin	1185.36	334.91	263	28.25 %	52 %
EG 1	752.74	145.39	240	19.32 %	21 %
EG 2	770.24	184.66	261	23.97 %	33 %
PG 1	814.17	212.78	263	26.14 %	34 %
PG 2	912.74	243.73	261	26.70 %	45 %
DMF	335	65.97	263	19.69 %	18 %
DMF*	2611.43	415.65	261	15.92 %	28 %

Table 8 - Accumulated gas and methane productions, and total methane content from the gas produced from spike 1 and onwards. COD concentration in the reactors is 4 g/L. The asterisks represent different COD concentrations; 57 g/L and 0.4 g/L for gelatin and DMF respectively.

	Accumulated gas after spike 1 (mL/gCOD)	Accumulated CH_4 after spike 1 (mL/gCOD)	Methane content	Methane content last measurement
Control	899.40	252.12	28.03 %	52 %
Gelatin*	95.72	6.53	6.82 %	12 %
Gelatin	998.69	281.15	28.15 %	52 %
EG 1	531.07	99.73	18.78 %	21 %
EG 2	411.79	70.09	17.02 %	33 %
PG 1	621.19	152.98	24.63 %	34 %
PG 2	535.95	130.72	24.39 %	45 %
DMF	147.02	12.23	8.32 %	18 %
DMF*	2242.86	302.47	13.49 %	28 %

5. Discussion

The theoretical methane yield in the serum bottles at the working temperature is 350 mL/g COD for each spike, but the highest yield achieved from one spike is a much lower with 159 mL/g COD for the gelatin with the COD concentration of 4 g/L. An argument for that are the relatively short retention times, but with a maximum yield amounting to only 45% of the theoretical yield, one should expect that a higher methane yield and thus a higher removal of COD would be possible. In addition to this another cause could be the short start-up time for the bigger reactors (4 weeks) from where the inoculum was collected. This might not have been sufficient in order to achieve complete stabilization. These reactors had been going through a gradual start-up time of 4 weeks prior to the first run of the serum bottle reactors. A long start-up time for anaerobic digesters is important because of the desire of having a population of methanogenic organisms as high as possible. The start-up period normally needs a long time in this sense as the growth rate of methanogens is low (Tan et al., 2006). Also, the type of substrate is important with respect to how well the methanogens perform, according to Tan et al, more so than with the quantity of substrate, which according to their study generally show the same trends in COD removal for the same substrates (Tan et al., 2006).

5.1 Tween 80

As shown in the result chapter, the gas production from the tween 80 reactors was lower than the control substrate reactors. When looking at previous studies done on fat, oil and grease wastes, one can see that LCFAs tend to have a negative effect on methanogenesis, as they inhibit the methanogens and acetogens important to produce methane. The study done by Long et al. on LCFA methanogenic activity inhibition shows that loading reactors with a COD concentration of 3500 mg/L resulted in 50% methanogenic activity loss, when the COD loaded was in the form of oleate (Long et al., 2012a). Oleate and tween 80 are both derivatives of oleic acid (Partanen et al., 2001), an acid which according to Chen et al is an inhibitory acid (Chen et al., 2008). The tween waste in this experiment does, however, produce gas on its own, so arguing that it is completely inhibitory is difficult. At the time of termination, the gas produced is about 50% of the gas produced by the control substrate reactors, meaning there is a 50% activity loss in gas producing organisms. But here a possible residual COD content added in the first feed might have an effect on the gas production. According to their study, Long et al have found from various other studies that results on inhibition involving various LCFAs are inconsistent with some studies showing greater inhibition with a mix of LCFAs while others observed a greater COD removal. The results with tween

show that there is a gas production to some degree, but a 50% reduction in gas production compared to the control is a poor performance, and one could conclude that tween is unsuitable as a waste to produce biogas on its own. In co-digestion with other wastes, fatty wastes have proven to increase the methane yield, and ways to implement this kind of waste in co-digestion with other wastes should be considered.

5.2 Starch

Although the results on gas production from starch are a somewhat inadequate with an early termination of the run, one can see the trend this kind of waste follows. Figure 21 shows that it follows the same trends as control substrate and in fact has produced more gas than the control at the time of termination. The gas production is more rapid after the last spike. This confirms the studies presented earlier that shows how starch wastes and food wastes are easily degraded, with rapid gas production. In fact, this model waste is the one that produces most biogas together with gelatin in these experiments, as shown later. This fits well with the studies presented earlier. Starch in particular, has a high methane yield as well. Unfortunately, due to problems with GC, the methane content was not determined for the starch and tween reactors, but with approximate methane content in biogas around 50% normally, shown for example by the methane contents in biogas produced in anaerobic digestion of sugar and potato pulp that ranged between 50.8% and 54. 1% (Kryvoruchko et al., 2009), one would expect starch to produce this. However, as we can see from Table 7 and Table 8, the total methane content in the biogas samples that were analyzed in the GC, is low, with the maximum total accumulated methane contents of 26 % from the gelatin reactors and the control substrate reactors, one could predict around the same for starch. What is worth pointing out with the methane contents in general is that they increased with time with the last measurements showing methane contents of 50% which confirms the theory. Actually, in a study on biomethanation of cassava starch, the authors Malial et al found an average methane content of 59% in the biogas produced from batch digesters (Manilal et al., 1990). This was methane content from a run that lasted 60 days. With these high methane contents yielded from other starches, one could argue that the methane content from the starch reactors would have the potential to surpass the gelatin and control substrates. Also, with methane contents in general increasing with each measurement, it seems the methanogens are acclimating to the wastes and one could possibly, with long runs, achieve similar contents for these reactors. When looking at gas production from starch compared to the controls one can see that they are virtually the same from spike 1 until spike 2, but then starch accelerates relative after spike 2. The reason here could be, as previously discussed, that the residual COD originating from the first feed has an impact, and the COD concentration was lower in the first feed of starch, than the control substrate as shown in part 4.1 of this report. Another reason is that the inoculum has been using the

control substrate as the continuous feed in the start-up period of the project so the microbial community should be acclimated for that kind of waste, meaning that even though the COD have easier accessibility in the starch, the bacteria will easier utilize the COD in the waste they are used to, until they figure out how to best use the COD in the starch, resulting in a delayed production from the *unknown* wastes.

A way to increase gas yield from starch wastes would be to add other types of wastes that provide essential nutrients absent in the starch. One of these nutrients is nitrogen, and in the case of this model waste, the inoculum must provide the process with nitrogen. Once this has been used, one would expect the methane production to diminish.

The potential of carbohydrate wastes are immense, as these wastes are readily available, making up 20% of landfill wastes. Degrading these anaerobically means an increase in biogas production which is important in order to move away from the use of fossil fuels as well as diminishing the problems related to these landfills such as contamination of surface and ground waters as well as spreading of diseases (Smith and Almquist, 2014).

5.3 Gelatin

The gas production from gelatin is strikingly similar to that of the starch, with equal production as the control substrate between spike 1 and 2 before accelerating the production and ending up with slightly higher production. When looking at the studies conducted by Elbeshiby et al, the gelatin here performs better relative to the carbohydrates, as the cumulative gas production at the time of termination of the starch reactors is virtually the same whereas, in the their study the cumulative methane volumes were 20% higher for the pure starch waste than for the pure proteinaceous waste(Elbeshbishy and Nakhla, 2012). A way to explain this could possibly be that the composition of the gelatin used has a C:N ratio that is advantageous for gas production, more advantageous than the proteinaceous waste used by Elbeshiby.

The hydrolysis of proteins is slower than the hydrolysis of carbohydrates, which means that the gas production should be lagging more for the gelatin than that of the starch. A reason why this is not the case here, could be due to relatively low retention times in the reactors, giving the microbial community little time to adjust. If the protein content in the control substrate is high, the inoculum might need more time to maximize the gas production from the starch, than it does before maximizing production from gelatin wastes.

In comparison to the control, the gelatin behaves similar to starch and the arguments for the accelerated production could be due to residual COD, acclimation or a combination of the two.

When looking at the high concentration, it is evident that the microbial population was not able to handle the extremely high concentration, although the accumulated gas production was high, it was very low considering the amount of COD that was available in the reactor as shown in Figure 23. The methane content was also the lowest recorded. Complete inhibition is evident; the ammonia concentration in the reactors is obviously well above the threshold for viable reactors, resulting in ammonia inhibition. In the paper *Inhibition of anaerobic digestion process: A review* Chen et al makes it clear that inhibition of methanogens is also related to acclimation. In order to avoid inhibition, feeding with low concentrations of substrates will help methanogens utilize the new type of substrate, and once acclimated far higher concentrations of the substrate will be viable for methane production. In the case of the high concentration gelatin, it is evident that the concentration used exceeded the limit concentration where an acclimation is possible (Chen et al., 2008), and the large amounts of nitrogen lead to a high concentration of ammonia.

5.4 Ethylene glycol and propylene glycol

The gas and methane production from the glycols varied a lot from each bottle, leading to two runs for each waste; ethylene glycol and propylene glycol. The standard deviations were still high, meaning the inoculum developed differently in each bottle, and it became particularly evident with glycols. The glycol substrates are not nearly as efficient as the more well-known wastes in anaerobic digestion. With a gas production amounting to 50% of the production from the control substrate reactors between spike 1 and 2, these wastes are not initially well suited for biogas production with this inoculum. However, as discussed by Chen et al, the microbial community is able to adapt to a number of potentially inhibitory substrates as long as the concentration in the start-up is low. The up-lifting element with the glycol wastes is that they seem to adapt more as termination is approaching, especially to the PG wastes. The gas and methane production rate is never higher relative to the control substrate than at the time of the last measurements. This shows that the inoculum is adapting and is increasingly able to utilize the COD in the form of glycols. The adaption is clearly easier with PG as shown in Figure 27, but there is also, as shown in Figure 25, a slight increase in gas production towards the end for EG 2. The observation of the propylene glycol is confirmed Zitomer et al, as they found that methane production could be slow with high organic loading rates of propylene glycol aircraft deicing fluids. They discovered that loading rates higher than 1.6 g COD/L·d resulted in decreased digester pH. This, in turn, lead to a hydraulic residence time of more

than 15 days needed in order to prevent inhibition (Zitomer et al., 2001). This could very well be the case here too, with the gas production picking up because the rate-limiting step, propionate fermentation to acetate, is getting close to being done. These glycols would also be very suitable to mix with other wastes that need more carbon in order to increase the gas yield, as these wastes are carbon-rich.

5.5 Dimethylformamide

The gas and methane production from the high concentration DMF shown in Figure 28 and Figure 29 show that there is hardly any gas and, particularly, methane production once the inoculum has been spiked with the DMF. After the spike there is clear evidence of inhibition. The inhibition is most likely due to the high concentration of the waste leading to toxic quantities of ammonia. As reported earlier in the literature review, an ammonia concentration exceeding 200 mg/L in the digester would lead to negative effects. The theoretical ammonia concentration in the digester after spike 1 is more than twice the threshold, with a concentration of 404.84 mg/L (see appendix C); this concentration is clearly too high to be handled by the inoculum. DMF is also a problematic waste due to its composition with a high nitrogen content; the C:N ratio in the waste itself is 3:1, while the ideal ratios for anaerobic digestion range between 20:1 and 30:1.

For the DMF with low concentration, a massive production of gas is shown in both Figure 30 and Figure 31. The reason for the great production shown especially just after spike 1 is probably due to the residual COD from the part of the run before spike 1. All serum bottles were fed with the control waste with a COD concentration of 4 g/L at hour 0 shown in Figure 30. COD remaining from this period will have a great effect on the development of the graph as it is shown with gas volume produced per gram of COD. The residual COD will have an effect that looks ten times higher once the spike only contains a COD concentration of 0.4 g/L. However, gas is produced, and from Figure 31 it is clear that production is increasing as termination is approaching. By this time the COD used to produce the gas probably originates from the DMF in spike 2, meaning the inoculum has been able to adapt to the waste. Although the DMF has a C:N ratio far from the ideal, the low concentration of the waste has resulted in successful acclimation. If one looks at the theoretical ammonia concentration after adding the DMF with low concentration, one can see that it is well below the threshold on 200 mg/L; with a concentration of 40.48 mg/L (appendix C). This fits well with the literature saying ammonia acclimation is possible using low concentrations.

From Figure 31 one can also read that low concentration of wastes in general, result in higher gas yield per gram of COD added. However, this is inaccurate due to the high COD concentration added in the

beginning, in order to investigate that further, one would have to start with the same low concentration. If it proves to be the case that low COD concentration yield more methane, it would not necessarily mean that it would be profitable in a large scale due to the fact that larger digesters would be needed. Looking into that is a different study discussing the pros and cons with large digesters and large volumes of waste versus higher yields of methane gas.

5.6 Production rates

The trends in gas and methane production rates are peaking right after feeding, as shown in the graphs with the well-known wastes like gelatin, starch and tween. This was anticipated and was the reason for the frequent measurements in the first 24 hours after feeding the reactors. An interesting observation is that the production rate of the methane increases towards every spike, and is slow again right after the spike. It is clear that the added substrate has a negative effect on the methane production rate as the methanogens to adapt to the new substrates and concentration added in spike 1 and a change in concentration in spike 2.

The model waste that most clearly differs from the general trend is propylene glycol, where the gas production rate is close to the maximum rate observed from this waste. When looking at the methane production rate the difference from the others is even more evident as the peak production rate, measured at 260 hours, is twice as high as the peak between spike 1 and 2 and at that time the methane production rate is significantly higher than for that of the control substrate, this shows a clear acclimation. This can lead to the conclusion that propylene glycol at the COD concentration of 4 g/L does produce biogas after a long acclimation period. A change in retention times might be needed to maximize this gas production.

6. Summary and Conclusion

In these experiments gas and methane production, as well as the production rates, have been found from anaerobic digestion of different model wastes simulating the core part of wastes such as ADF waste, effluent from film processing, diary and meat wastes, and FOG wastes. The results show that methane production is lagging compared to the gas production for all wastes, meaning that methanogens need time to adapt to new concentrations of waste as well as new kinds of wastes. Some wastes have proven to have inhibitory effects, while others yield more gas than the control substrate.

From the results obtained it is evident that, compared to the gas and methane production of the control substrate that is already in use in the plant, gelatin and starch are well suited for degradation and gas production, as they perform better with respect to gas production. Knowing this, one could argue that there is a potential for improvement in the biogas production at the treatment plant as these wastes are singular and homogeneous, meaning that there are limiting factors due to absence of essential nutrients. The inoculum has proven to be able to adapt to the PG wastes, and there are indications of the same for EG wastes. The gas production for the tween 80 waste produce the same amount of gas as the EG and PG before the inoculum has adapted, and all these wastes would be considered unsuitable for gas production in anaerobic digesters as long as the inoculum is not adapted.

For the DMFs, it is evident that the high concentration wastes are completely inhibitory to the methanogens and therefore unsuitable. But with low concentrations the digester was able to handle the waste and produce gas. Therefore, one could say that the starch and gelatin wastes are suitable, the tween and glycols have potential if an acclimation time is allowed, and the DMF is completely inhibitory at the COD concentration of 4 g/L, but at low concentrations DMF is degradable. As this part of the project has solely focused on singular model wastes, and some of them have proven to yield more gas than the control, there are many ways to advance this research in terms of investigating the troublesome wastes like tween, EG, PG and DMF further, as well as optimizing the gas production of all wastes, including the control waste, by mixing different wastes.

6.1 Recommendations for further work

There are many ways to advance this research. From this, the well-known wastes have confirmed the theory and hypothesis to some extent with gas production. However, to find a way to make the reactors more efficient in terms of degrading the wastes and utilizing the COD for methane production, different ways of treating the wastes should be explored; longer runs could result in higher methane contents, like the studies on starch wastes and proteinaceous wastes that achieve a biogas production with a methane

content approaching up to 60%(Kryvoruchko et al., 2009, Fang and Chung, 1999, Elbeshbishy and Nakhla, 2012). Longer times in the reactors will also shed more light upon the question of acclimation of the inoculum as the glycol based wastes seem to require a long acclimation time. It would be interesting to see the further development in gas and methane production as from these wastes, together with the low concentration of DMF, to see if they eventually would reach the same gas production as the conventional wastes.

Different COD concentrations of the various wastes should be investigated in order to find the concentration of each waste that yield the most in terms of COD added. When doing this the same COD concentration should be used from hour 0 with the feed of control substrates. However, one should also take into consideration the full-scale operation when choosing the desired concentration of the waste added, as too low concentrations might lead to less waste being processed, or a need for larger digesters. It is also important to check different concentration in order to find the concentration most suited to lead to acclimation to possibly inhibitory substrates as DMF, FOG and the glycols.

Another recommendation is, as most of the studies have shown that, co-digestion of different wastes is important to find the maximum methane potential of the different wastes, even studies with pure carbohydrate wastes found a better methane yield if that was mixed with protein wastes (Elbeshbishy and Nakhla, 2012). Zitomer found that co-digestion with municipal wastes and aircraft deicing fluids would increase the potential organic loading rate from 0.65 to 1.6 COD/L·d(Zitomer et al., 2001), and co-digestion of FOG-wastes have been proven to be economically and environmentally sustainable method of waste disposal as well as increasing digester gas production with for example in co-digestion with municipal sewage sludge; a feed of 10-30% of FOG wastes with the rest being municipal sewage sludge gave an increase of 30-80% in gas production.(Long et al., 2012b)

A natural path to further the research is testing in lab-scale reactors using proper wastes; dairy, fat, oil and grease, meat, film processing chemicals, and airplane de-icing. These would probably behave slightly differently than the model wastes as the COD there are not completely homogeneous like the model wastes. It is also important to test lab-scale reactors with wastes that are intended for the full-scale digester, but the more knowledge acquired in the field prior to full-scale digestion, the better. That way, the best combinations and concentrations of the wastes are known and one can maximize the production of biogas and degrade as much waste as possible.

So, in short, the further research should include:

- Different retention times, especially a pro-longed period after the last spike in order to exhaust the inoculum and utilize as much of the COD as possible
- Different concentration of wastes
- Mix the wastes to find the best possible combination and contents of different wastes in co-digestion.
- Investigate the proper wastes as they come to the treatment plant.

When this has been investigated, one has to look at the practical aspects of implementing this in the treatment plant; the capacity of existing anaerobic digesters, seasonal differences (aircraft deicing fluids will mainly be a concern in the winter), and what concentrations one should aim to digest in order to be able to keep the tanks at a reasonable size, but still have a methane yield as high as possible.

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Appendix A - Calculations

Calculations

In order to obtain a COD concentration of 4000 mg/L, the different chemicals had to be diluted with PBS.

Control feed

The solution of the control feed consisted of 50% of thickened waste activated sludge and 50% of thickened primary sludge.

Initial COD value of control feed: 50000 mg/L, value given by the treatment plant

Total volume of inoculum and feed in serum bottle: 70 mL

$$\frac{Needed\ amount\ of\ COD}{Total\ volume\ in\ serum\ bottle} \cdot Initial\ COD\ concentration = Wanted\ COD\ concentration$$

$$\frac{X\ mL}{70\ mL} \cdot 50000 \frac{mg}{L} = 4000 \frac{mg}{L}$$

$$X = 5.6\ mL$$

The amount of needed feed for the control bottles is 5.6 mL

The inoculum is fed by 10 mL of feed, giving a total volume of 70 mL

Therefore the feed added consists of:

Constituents of feed	Volume Necessary
TWAS	2.8 mL
TPS	2.8 mL
PBS	4.4 mL

Tween 80

Polyoxyethylene (20) sorbitan monooleate

Chemical formula: $C_{64}H_{124}O_{26}$

Reaction with oxygen: $C_{64}H_{124}O_{26} + 82O_2 = 64CO_2 + 62H_2O$

Total volume of inoculum and feed in serum bottle: 70 mL

Molecular Weight O_2	32 g/mol
Desired COD concentration	4000 mg/L
COD additive	$\frac{4\ g/L}{32\ g/mol} = 0.125\ mol/L$
COD added	$0.125\ mol/L \cdot 0.070\ L = 0.00875\ mol$

From the reaction: 1 mol $C_{64}H_{124}O_{26}$ to 82 mol O_2

Needed $C_{64}H_{124}O_{26}$	$0.00875\ mol \cdot \frac{1mol}{82mol} = 0.1067\ mmol$
Molecular weight $C_{64}H_{124}O_{26}$	1310 g/mol
Needed $C_{64}H_{124}O_{26}$ (mass)	$0.1067\ mmol\ \cdot 1310\ g/mol = 0.1398\ g$
Density $C_{64}H_{124}O_{26}$	1.06 g/mL
Volume $C_{64}H_{124}O_{26}$	0.148 mL

Constituents of solution	Volume Necessary
Tween 80	0.148 mL
PBS	9.852 mL

Starch

Chemical formula: $C_6H_{10}O_5$

Reaction with oxygen: $C_6H_{10}O_5 + 6O_2 = 6CO_2 + H_2O$

Total volume of inoculum and feed in serum bottle: 70 mL

Molecular Weight O_2	32 g/mol
Desired COD concentration	4000 mg/L
COD additive	$\frac{4\ g/L}{32\ g/mol} = 0.125\ mol/L$
COD added	$0.125\ mol/L \cdot 0.070\ L = 0.00875\ mol$

From the reaction: 1 mol $C_6H_{10}O_5$ to 6 mol O_2

Needed $C_6H_{10}O_5$	$0.00875\ mol \cdot \frac{1mol}{6mol} = 1.4583\ mmol$
Molecular weight $C_6H_{10}O_5$	162 g/mol
Needed $C_6H_{10}O_5$ (mass)	$1.4583\ mmol\ \cdot 162\ g/mol = 0.2362\ g$

Constituents of solution	Volume Necessary
Starch	0.2362 g
PBS	10 mL

Gelatin

Chemical formula: Mixture of various peptides and proteins

Total volume of inoculum and feed in serum bottle: 70 mL

1 g gelatin	1.08 g COD
Desired COD concentration	4000 mg/L
COD additive	4 g/L
COD added	$4\ g/L \cdot 0.070\ L = 0.28\ g$

1 g gelatin =1.08 g COD

Needed Gelatin	$\frac{0.28\ g\ COD \cdot 1\ g\ gelatin}{1.08\ g\ COD} = 0.2593\ g\ gelatin$

Constituents of solution	Volume Necessary
Gelatin	0.2593 g
PBS	10 mL

Propylene Glycol

Chemical formula: $C_3H_8O_2$

Reaction with oxygen: $C_3H_8O_2 + 4O_2 = 3CO_2 + 4H_2O$

Total volume of inoculum and feed in serum bottle: 70 mL

Molecular Weight O_2	32 g/mol
Desired COD concentration	4000 mg/L
COD additive	$\frac{4\ g/L}{32\ g/mol} = 0.125\ mol/L$
COD added	$0.125\ mol/l \cdot 0.070\ l = 0.00875\ mol$

From the reaction: 1 mol $C_3H_8O_2$ to 4 mol O_2

Needed $C_3H_8O_2$	$0.00875\ mol \cdot \frac{1mol}{4mol} = 2.1875\ mmol$
Molecular weight $C_3H_8O_2$	76 g/mol

Needed $C_3H_8O_2$ (mass)	$2.1875\ mmol\ \cdot 76\ \mathrm{g}/mol = 0.16625\ g$
Density $C_3H_8O_2$	1.036 g/mL
Volume $C_3H_8O_2$	0.1605 mL

Constituents of solution	Volume Necessary
Propylene Glycol	0.1605 mL
PBS	9.8395 mL

Ethylene Glycol

Chemical formula: $C_2H_6O_2$

Reaction with oxygen: $2C_2H_6O_2 + 5O_2 = 3CO_2 + 6H_2O$

Total volume of inoculum and feed in serum bottle: 70 mL

Molecular Weight O_2	32 g/mol
Desired COD concentration	4000 mg/L
COD additive	$\frac{4\ g/L}{32\ g/mol} = 0.125\ mol/L$
COD added	$0.125\ mol/L \cdot 0.070\ L = 0.00875\ mol$

From the reaction: 1 mol $C_2H_6O_2$ to 2.5 mol O_2

Needed $C_2H_6O_2$	$0.00875\ mol \cdot \frac{2mol}{5mol} = 3.5\ mmol$
Molecular weight $C_2H_6O_2$	62 g/mol
Needed $C_2H_6O_2$ (mass)	$3.5\ mmol\ \cdot 62\ \mathrm{g}/mol = 0.217\ g$
Density $C_2H_6O_2$	1.1132 g/mL
Volume $C_2H_6O_2$	0.1949 mL

Constituents of solution	Volume Necessary
Ethylene Glycol	0.1949 mL
PBS	9.8051 mL

Dimethylformamide

COD = 4000 mg/l

Chemical formula: C_3H_7NO

Reaction with oxygen: $4C_3H_7NO + 21O_2 = 12CO_2 + 14H_2O + 4NO_2$

Total volume of inoculum and feed in serum bottle: 70 mL

Molecular Weight O_2	32 g/mol
Desired COD concentration	4000 mg/L
COD additive	$\frac{4\ g/L}{32\ g/mol} = 0.125\ mol/L$
COD added	$0.125\ mol/L \cdot 0.070\ L = 0.00875\ mol$

From the reaction: 1 mol C_3H_7NO to 21/4 mol O_2

Needed C_3H_7NO	$0.00875\ mol \cdot \frac{4mol}{21mol} = 1.667\ mmol$
Molecular weight C_3H_7NO	73 g/mol
Needed C_3H_7NO (mass)	$1.667\ mmol\ \cdot 73\ g/mol = 0.122\ g$
Density C_3H_7NO	0.950 g/mL
Volume C_3H_7NO	0.1281 mL

Constituents of solution	Volume Necessary
Dimethylformamide (DMF)	0.1281 mL
PBS	9.8719 mL

COD = 400 mg/l

Chemical formula: C_3H_7NO

Reaction with oxygen: $4C_3H_7NO + 21O_2 = 12CO_2 + 14H_2O + 4NO_2$

Total volume of inoculum and feed in serum bottle: 70 mL

Molecular Weight O_2	32 g/mol
Desired COD concentration	400 mg/L
COD additive	$\frac{0.4\ g/L}{32\ g/mol} = 0.0125\ mol/L$
COD added	$0.0125\ mol/L \cdot 0.070\ L = 0.000875\ mol$

From the reaction: 1 mol C_3H_7NO to 21/4 mol O_2

Needed C_3H_7NO	$0.000875\ mol \cdot \frac{4mol}{21mol} = 0.1667\ mmol$
Molecular weight C_3H_7NO	73 g/mol
Needed C_3H_7NO (mass)	$0.1667\ mmol\ \cdot 73\ g/mol = 0.0122\ g$
Density C_3H_7NO	0.950 g/mL
Volume C_3H_7NO	0.01281 mL

Constituents of solution	Volume Necessary
Dimethylformamide (DMF)	0.01281 mL
PBS	9.98719 mL

Ammonia concentration in DMF reactors:

$$C_7H_3NO + \frac{6}{4}H_2O \rightarrow \frac{14}{8}CH_4 + \frac{10}{8}CO_2 + NH_3$$

DMF (4 g COD/L):

1.667 mmol DMF $\rightarrow$ 1.667 mmol NH_3

Molecular weight NH_3: 17 g/mol

Amount of NH_3 in the reactor: 17 mg/mmol · 1.667 mmol = 28.33 mg

Volume reactor: 0.07 L

Concentration NH_3: 28.33 mg / 0.07 L = 404.77 mg/L

DMF (0.4 g COD/L):

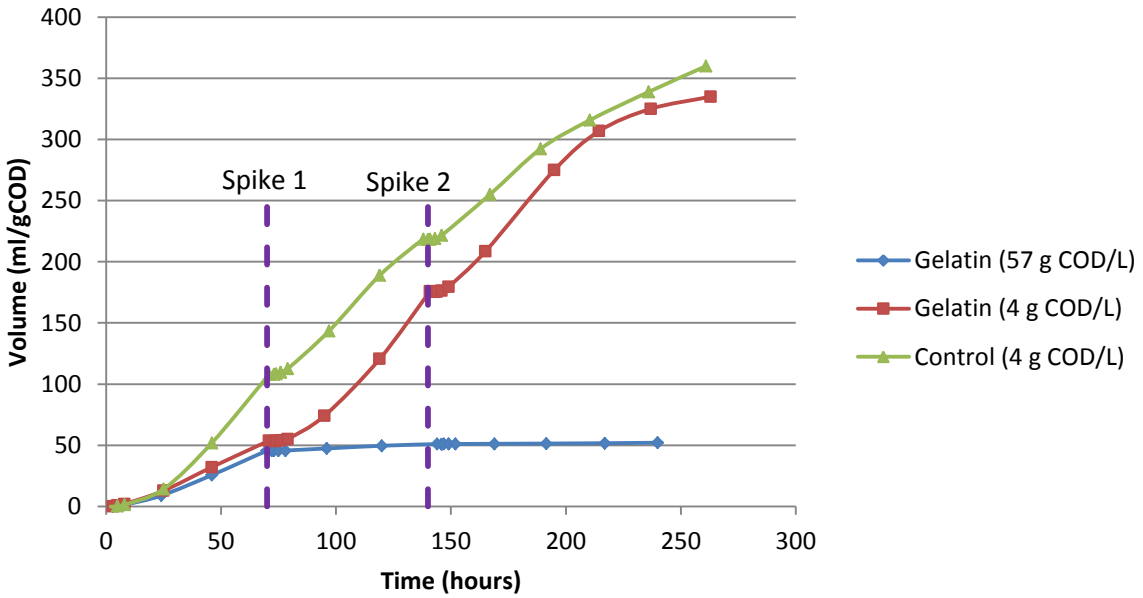
0.1667 mmol DMF $\rightarrow$ 0.1667 mmol NH_3

Molecular weight NH_3: 17 g/mol

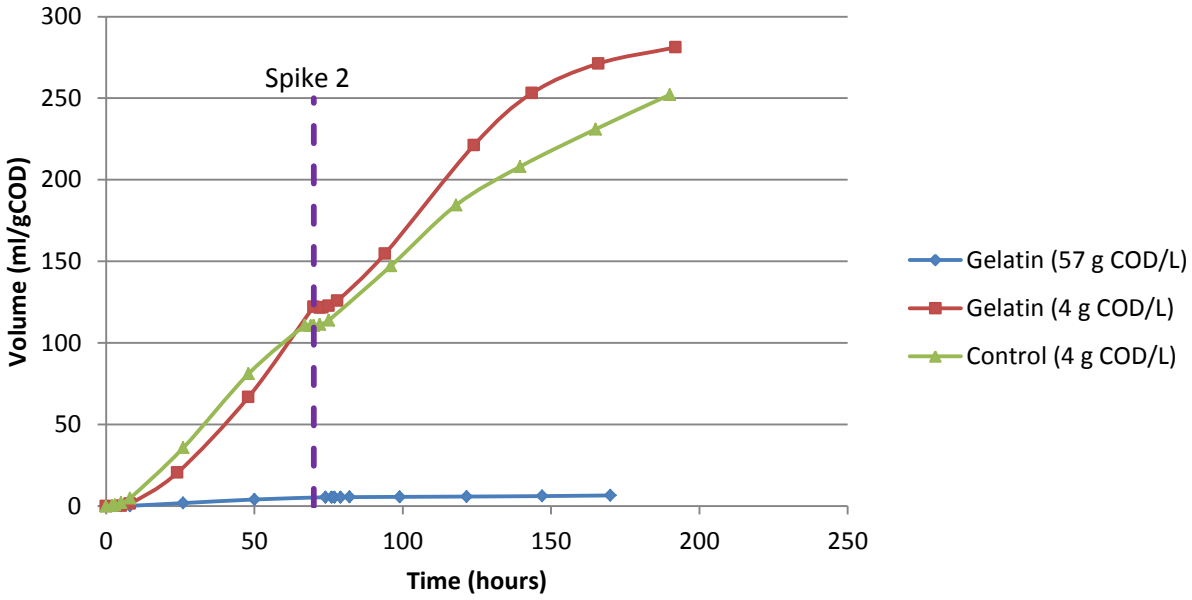
Amount of NH_3 in the reactor: 17 mg/mmol · 0.1667 mmol = 28.33 mg

Concentration NH_3: 2.833 mg / 0.07 L = 40.477 mg/L

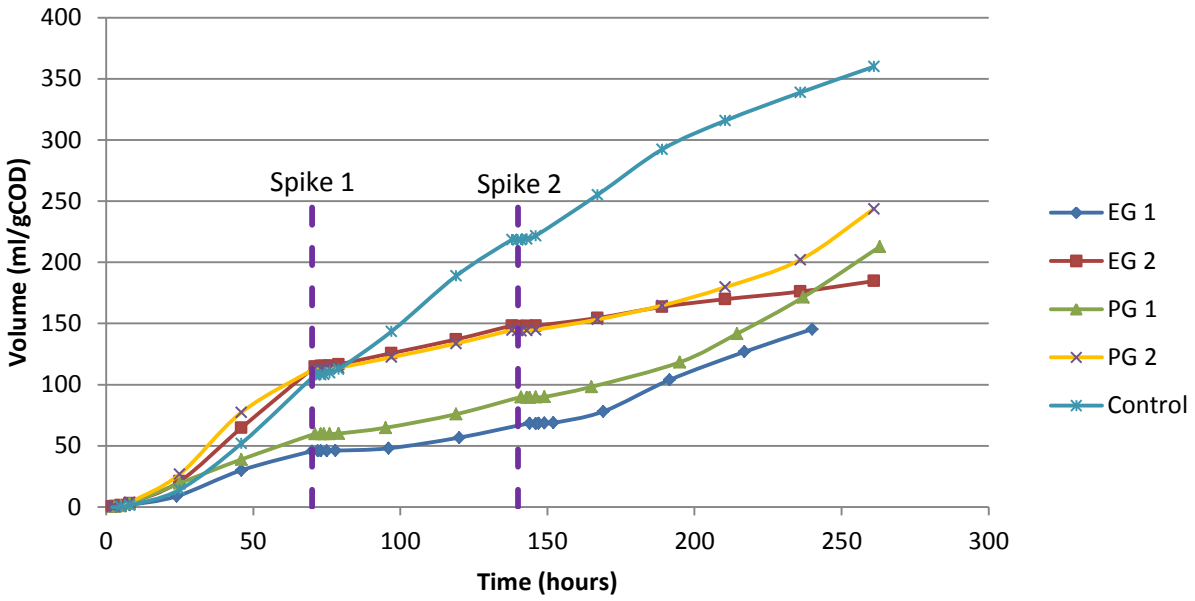
Appendix B – Methane Production



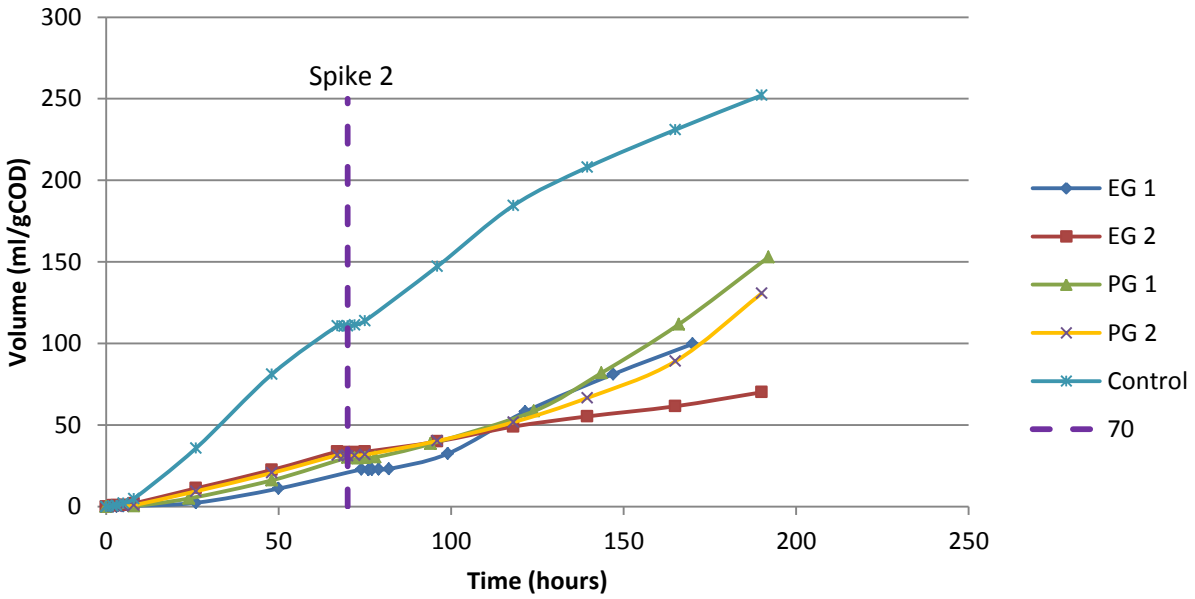
Graph showing methane production from reactors with gelatin substrate and control substrate. The period before spike 1 has a COD in the form of control substrate for all reactors. Wastes added at the spikes and the first feed make the COD concentration in the reactor reach 4 g/L unless stated otherwise.



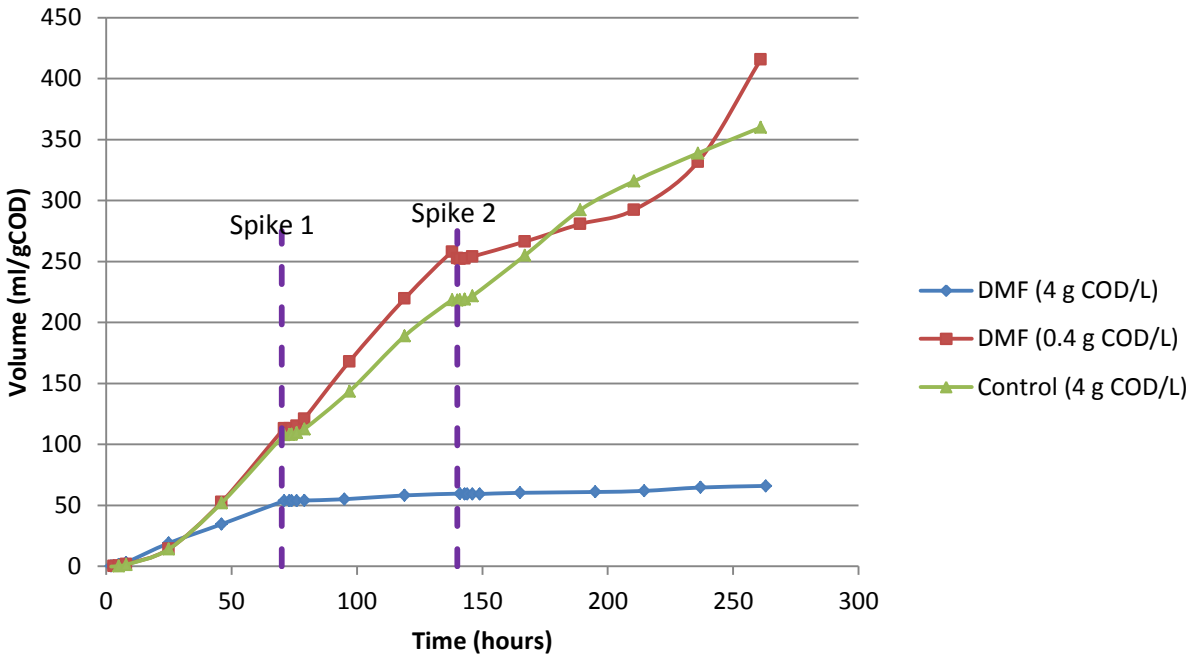
Graph showing methane production from reactors with gelatin substrate and control substrate from spike 1 and onwards. In spike 2 the reactors are fed with 10 mL of the wastes. Same volume is removed before. If not stated otherwise, the COD concentration is 4 g/L.



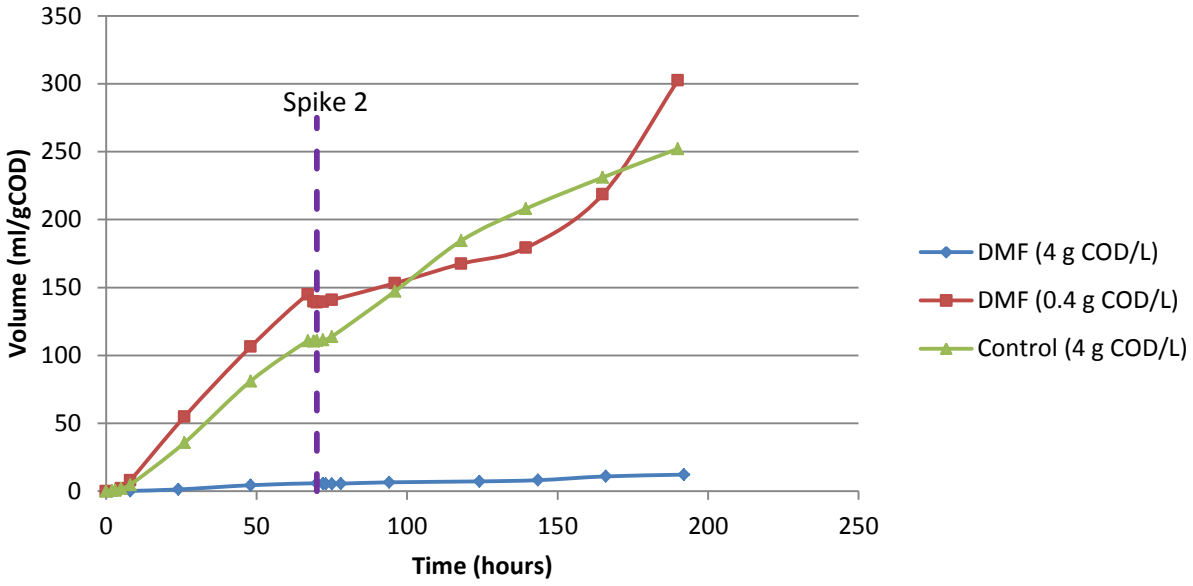
Graph showing methane production from reactors with glycol substrates and control substrate. The period before spike 1 has a COD in the form of control substrate for all reactors. Wastes added at the spikes and the first feed make the COD concentration in the reactor reach 4 g/L unless stated otherwise.



Graph showing methane production from reactors with glycol substrates and control substrate from spike 1 and onwards. In spike 2 the reactors are fed with 10 mL of the wastes. Same volume is removed before. If not stated otherwise, the COD concentration is 4 g/L.



Graph showing methane production from reactors with DMF substrates and control substrate. The period before spike 1 has a COD in the form of control substrate for all reactors. Wastes added at the spikes and the first feed make the COD concentration in the reactor reach 4 g/L unless stated otherwise.

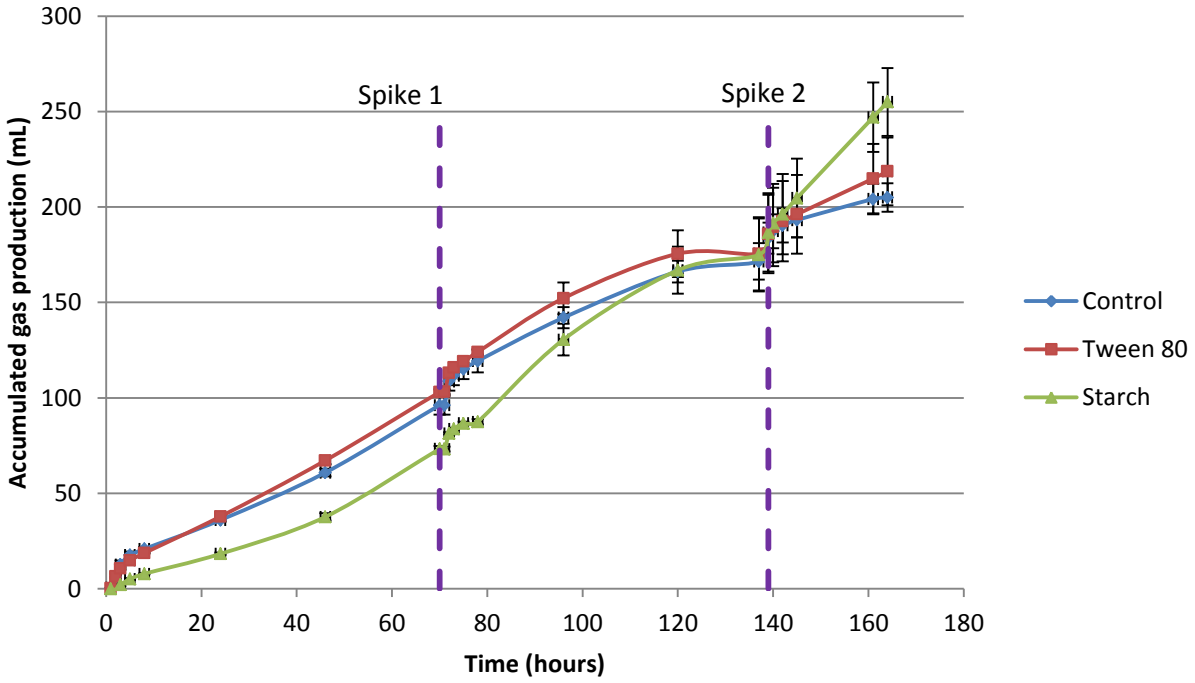


Graph showing methane production from reactors with DMF substrates and control substrate from spike 1 and onwards. In spike 2 the reactors are fed with 10 mL of the wastes. Same volume is removed before. If not stated otherwise, the COD concentration is 4 g/l.

Appendix C – Measured Accumulated Volumes

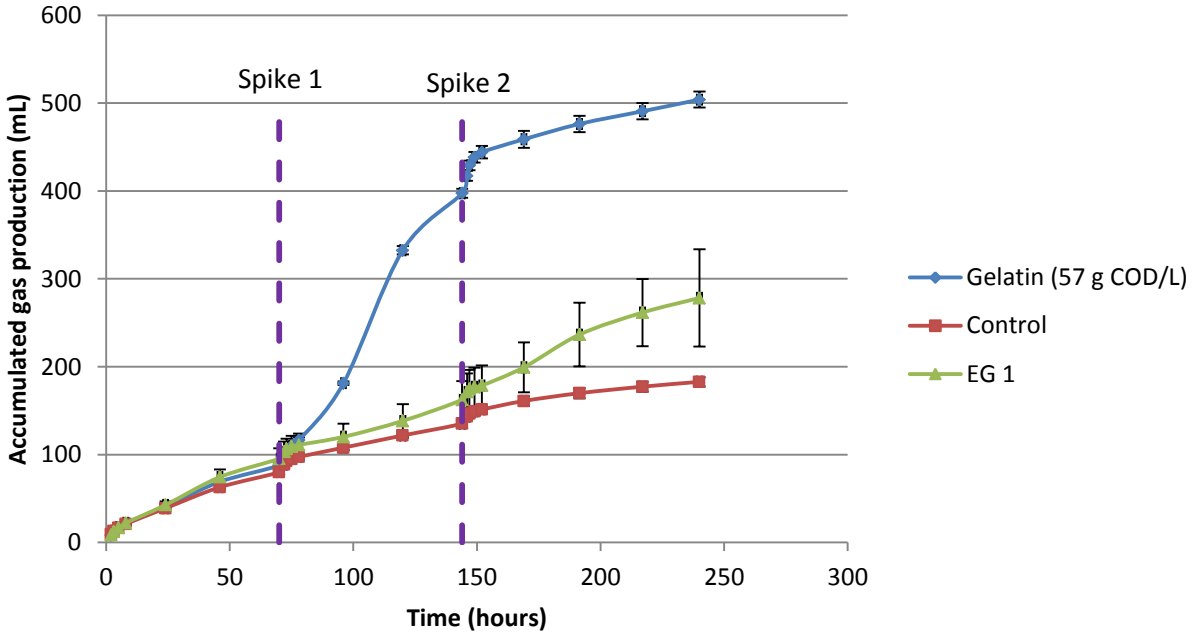
Graphs showing the accumulated volumes (gas and methane) recorded for each waste from each run.

Run1

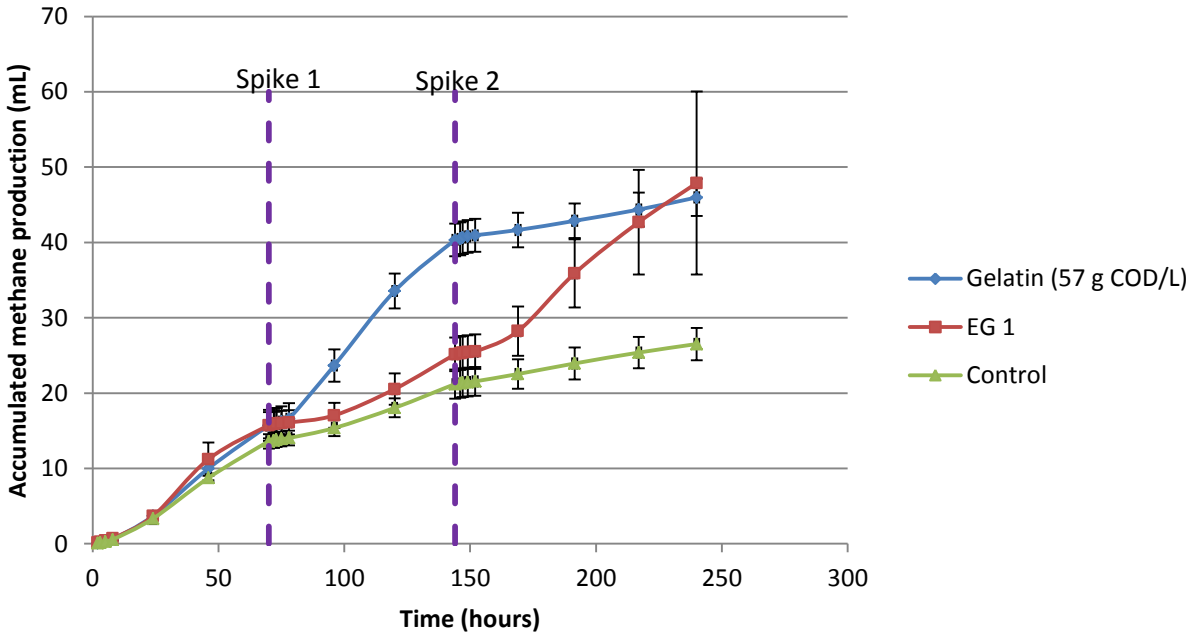


Graph showing the average accumulated gas production recorded from the reactors in Run 1. Volumes are shown with standard deviations

Run 2

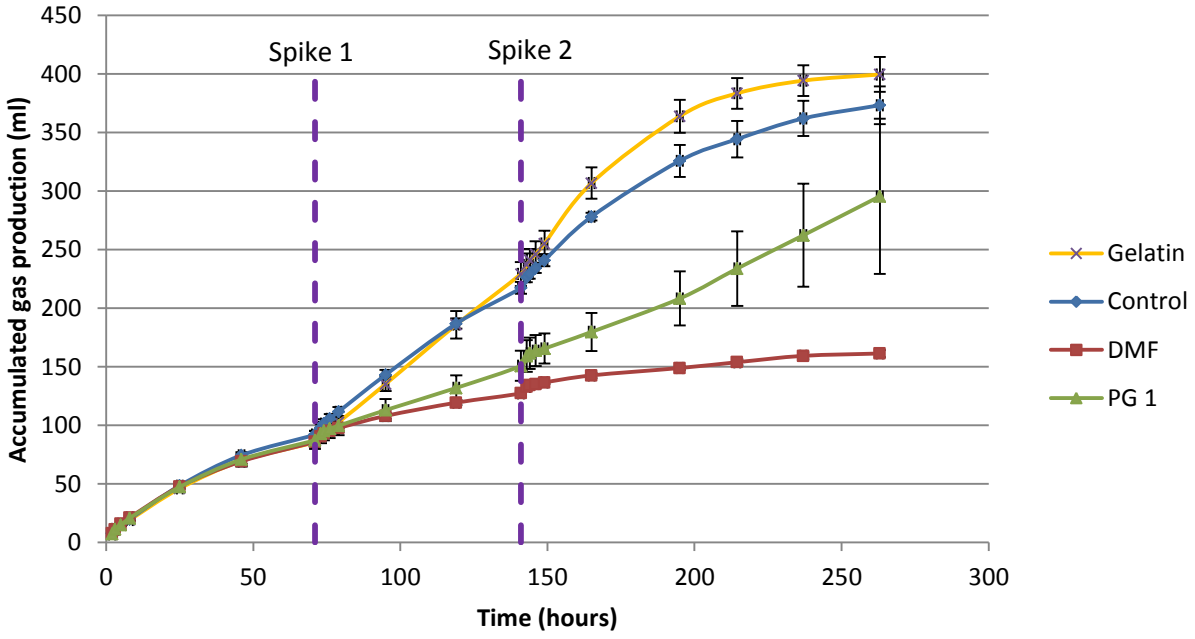


Graph showing the average accumulated gas production recorded from the reactors in Run 2. Volumes are shown with standard deviations

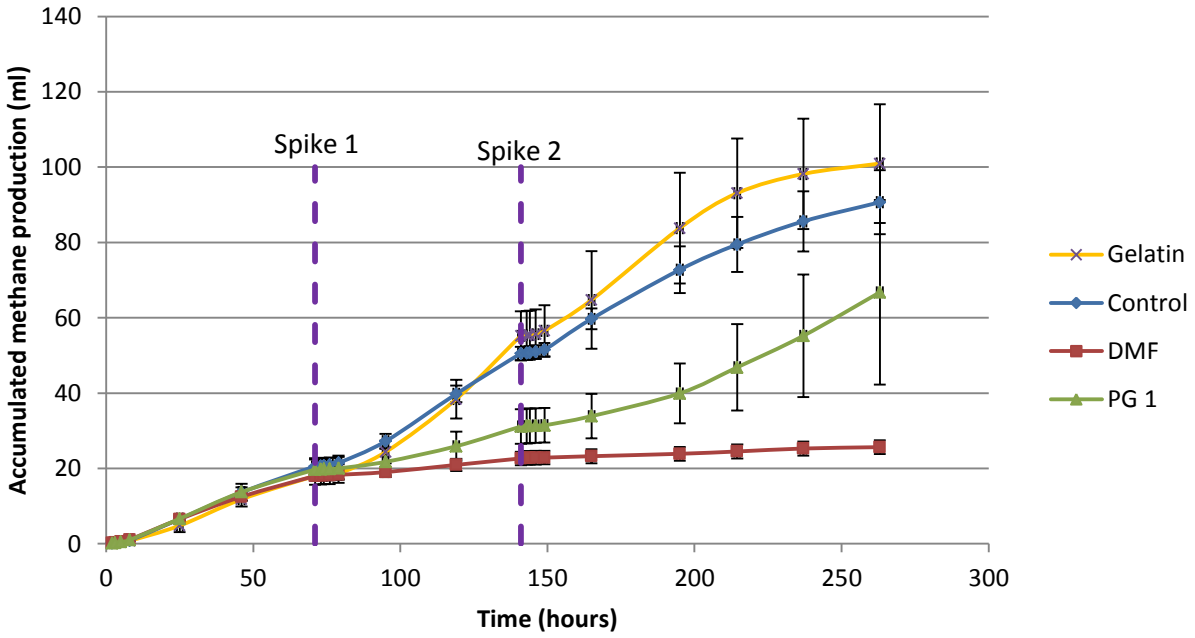


Graph showing the average accumulated methane production recorded from the reactors in Run 2. Volumes are shown with standard deviations

Run 3

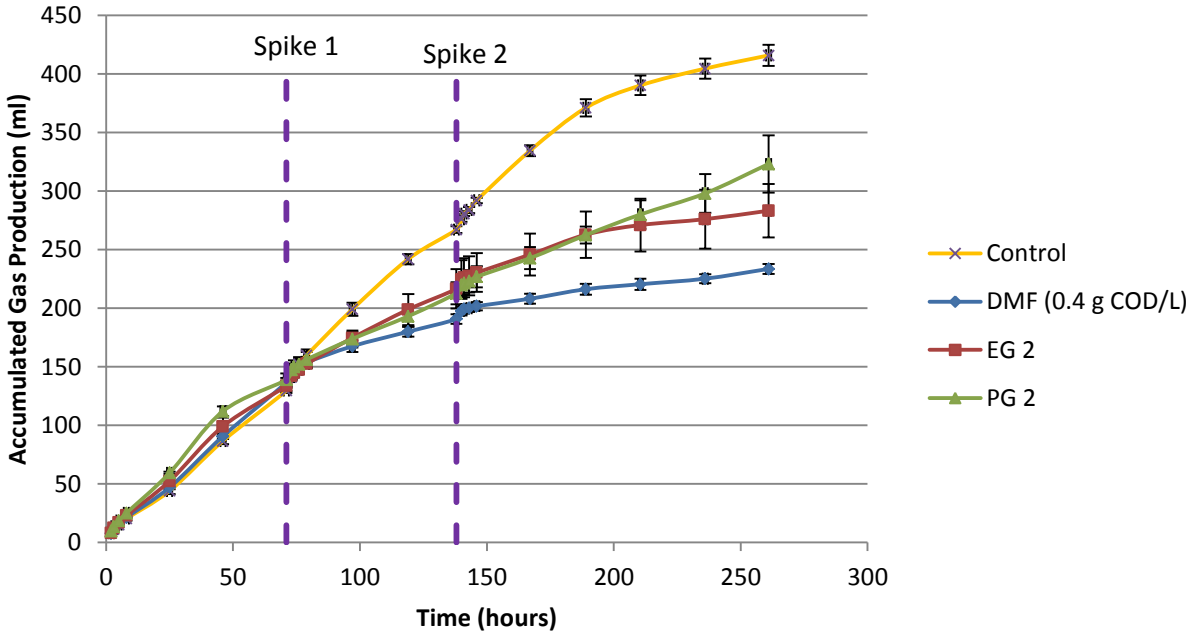


Graph showing the average accumulated gas production recorded from the reactors in Run 3. Volumes are shown with standard deviations

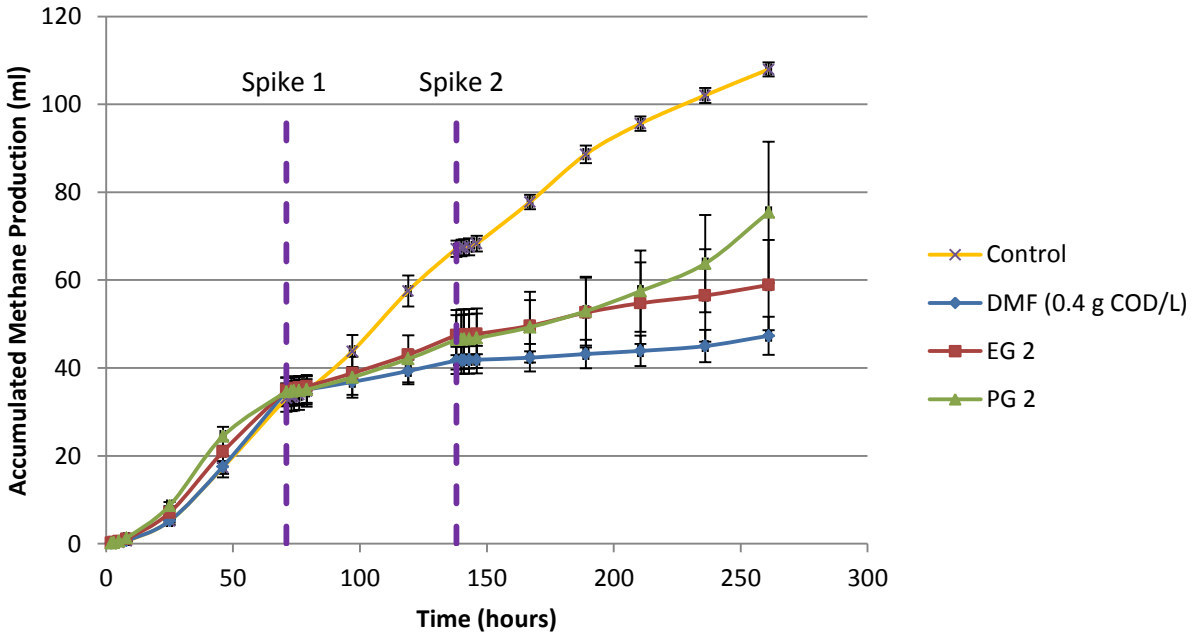


Appendix figure 1 - Graph showing the average accumulated methane production recorded from the reactors in Run 3. Volumes are shown with standard deviations

Run 4



Graph showing the average accumulated gas production recorded from the reactors in Run 4. Volumes are shown with standard deviations



Graph showing the average accumulated methane production recorded from the reactors in Run 4. Volumes are shown with standard deviations