Generation of biogas by the anaerobic digestion of food waste

Selection of the most efficient energy conversion technology for optimal use of food waste converted by anaerobic digestion to biogas

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Abstract

This study aimed at the investigation of food waste (FW) conversion to biogas by anaerobic digestion (AD) and biogas that should be converted to an energy source. The paper is written since it is believed that the tertiary FW from Europa park in Groningen can contribute to the main goal of the "Making City" project, which this research is part of, to transform towards smart and low-carbon cities. Biogas production and conversion to an energy source can contribute to the transition of a future with sustainable energies. The main goal of this project is to obtain the most efficient energy conversion technology for optimal use of FW converted by anaerobic digestion to biogas. This is pursued by doing laboratory experiments on the AD of FW with the addition of trace elements (TEs) and the theoretical upscaling of two pilot plant reactors available at the ENTEG-building in Groningen. A biogas conversion to different energy sources like heat and energy, green gas and proteins with the addition of economic analysis are added to complete the research. The most important results from laboratory experiments are that the addition of FW with an organic loading rate (OLR) of 5 g VS/L/day and a hydraulic retention time (HRT) of 24 days in combination with TEs give promising results. However, microbial adaption seems to impact the AD of FW and acidification occurs at an early stage. A literature review in combination with experiments and the above-mentioned process settings results in a volumetric biogas production (VBP) of 3.75 m^3 biogas/m^3/day with a methane fraction of 58%. The output of the AD of FW in combination with the upgrading of biogas to green gas will give a good working process with an approximate profit of €6500 per year.

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Abbreviations

AD	Anaerobic digestion
BMP	Biochemical methane potential
CA	Chemical absorption
COD	Chemical oxygen demand
ENTEG	Engineering and Technology institute Groningen
FAN	Free ammonia nitrogen
FW	Food waste
HRT	Hydraulic retention time
LHV	Lower heating value
MS	Membrane separation
OLR	Organic loading rate
PED	Positive energy district
PSA	Pressure swing adsorption
TAN	Total ammonia nitrogen
TS	Total solids
UASB	Up-flow anaerobic sludge blanket
VBP	Volumetric biogas production
VMP	Volumetric methane production
VS	Volatile solids
WS	Water scrubbing

1. Introduction

The expectation is that 95% of the Dutch population will live in urban areas by 2035 [1]. So, cities will have an essential role in transitioning from fossil fuel to sustainable energy and preventing climate change. A city of the future with an annual net-zero energy import and net-zero carbon emission could be the solution. Groningen strives to be a city that is energy neutral by 2035. However, research has to be applied in practice to reach a net positive energy district (PED). Groningen North and Groningen South are selected to participate in a project of MAKING-CITY to apply for (new) technologies like geothermal heating, solar panels and retrofitting of residential buildings to come to a PED.

AD of FW is also one of those solutions. FW from the Europa Park in Groningen South is a tertiary FW stream that contributes to a PED. After the digestion of FW, the most efficient use of the biogas generated is determined by considering upgrading and energy conversion. The goal of this research is to come to the most efficient energy conversion technology for the optimal use of FW converted by anaerobic digestion to biogas. This goal leads to different research sub-questions:

- When is there overloading of the AD process in a batch reactor when adding FW?
- Is it feasible in AD of FW to work with high HRTs and low OLRs on a lab scale?
- Does the addition of TEs work at relatively high OLRs and low HRTs?
- What is the most profitable process of upgrading biogas produced by AD of FW?

In this report, lab-scale experiments are performed for the AD of FW with OLRs up to 10 g VS/L/day and HRTs varying from 24 to 583 days. TE addition for higher OLRs in combination with low HRTs is also researched. After the “optimal” process settings are found different conversion technologies are discussed.

2. Theoretical background information

AD of FW to biogas and upgrading this biogas to a sustainable energy source are the main topics of this research. This section will elaborate more on these two main topics.

2.1. Anaerobic digestion

In this research, the AD of FW is researched. In AD the organic-rich feed will be broken down in the absence of oxygen by microorganisms. This results in a combination of methane, CO_2 and digestate. These products can be used as an energy source (methane), greenhouse input (CO_2) or fertilizer (digestate). A series of reactions called hydrolysis, acidogenesis, acetogenesis and methanogenesis can convert the substrate (FW) into these products (see, Figure 1) [2].

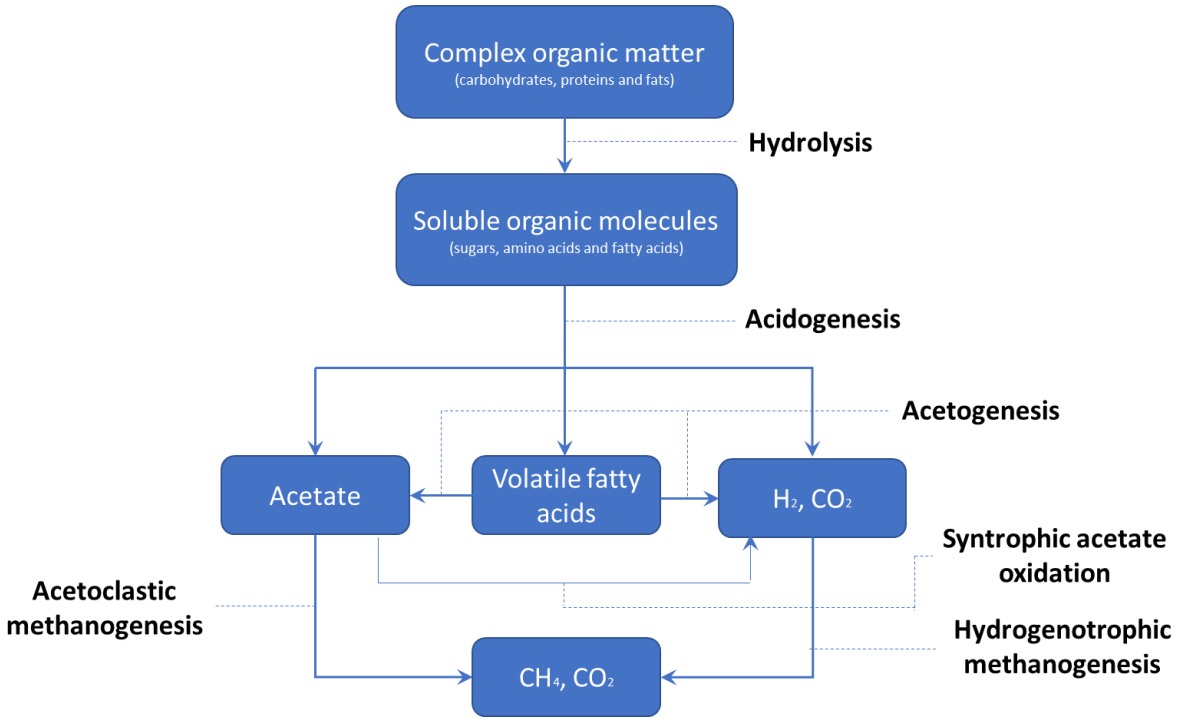

Figure 1 Anaerobic digestion pathway

In the hydrolysis stage, the large organic compounds (proteins, carbohydrates and fats) in the substrate will be broken down into smaller and better soluble molecules (sugars, amino acids and long-chain fatty acids (LCFA)). Looking from a chemical perspective hydrolysis provides the cleavage of chemical bonds by the addition of water. With altering of the pH since O-H bonds were formed *[2]*.

Products generated in the hydrolysis phase as acetate, carbon dioxide and hydrogen can be used in methanogenesis to produce biogas. Large compounds are fermented into volatile fatty acids (VFAs) (by-products like ammonia, carbon dioxide and hydrogen sulphide will also be formed). The stage of the degradation of these compounds is called acidogenesis and is provided by acidogenic (fermentative) bacteria *[3]*.

The acetogenesis provides the digestion of VFAs and other small molecules. Hydrogen is the main product of this digestion process and is thus called the dehydrogenation phase. Since hydrogen will release and exhibits toxic effects on microorganisms in the acetogenesis, it is necessary to do this in symbiosis with the methanogenic archaea (hydrogenotrophic methanogens). Acetic acid and CO_2 are formed next to the production of hydrogen. The dominance of organisms within acidogenic and acetogenic bacteria depends on the substrate *[2]*, *[3]*.

The limiting step in AD of FW is methanogenesis. Acetate, carbon dioxide and hydrogen will be converted to methane and carbon dioxide. Archaea responsible for this conversion are sensitive to oxygen. Two groups of archaea involved are acetoclastic and hydrogenotrophic, which produce methane and carbon dioxide by respective decarboxylation of acetate and the reaction of carbon dioxide with hydrogen. Acetoclastic methanogens are the most dominant since approximately 70% of the methane formed is produced through the reduction of acetate [2], [3].

The final composition of CH_4 from anaerobic digestion is normally in the range of 50-75% and for CO_2 between 25-50% [2].

2.2. Anaerobic food waste digestion

FW is an attractive substrate for anaerobic digestion since it has biochemical methane potential (BMP) of around 460 mL CH_4/g VS. This is a relatively high BMP compared to other substrates like wastewater-activated sludge (157 CH_4/g VS) and dairy cattle manure (243 CH_4/g VS) [4]. Also, the high availability in urban areas makes it an interesting resource for AD. However, there are still some issues with the AD of FW.

2.2.1. High in carbohydrates

A high organic fraction, a structure that is mainly composed of easily degradable carbohydrates and a low pH are characteristics of FW [4]. Due to this easy degradability, an overproduction of VFAs could be obtained at an early stage of the digestion process. This overproduction of VFAs will lead to an overwhelming of methanogens and a decrease in pH if there is no sufficient buffering capacity [5]. Moreover due to this increase of the intermediate VFA products the pH is decreasing and propionic acid will be formed. However, VFAs in low concentrations are not bad for AD and an amount is needed for production since it is an intermediate product [6].

2.2.2. Propionic acid

Propionic acid is known to give extra toxicity since unionized propionic acid can diffuse through the cell membrane (see, Figure 2) [7], [8]. Since only propionic acid can diffuse through the cell membrane and not the conjugated base (propionate), the intracellular pH will decrease in contrast to the extracellular phase. After the propionic acid crossed the cell membrane it will release a proton in response to the pH gradient, resulting in an intracellular pH decrease (recovery of equilibrium inside the cell). Propionic acid can freely transverse through the cell membrane since unionized propionic acid is lipophilic and propionate is lipophobic [9].

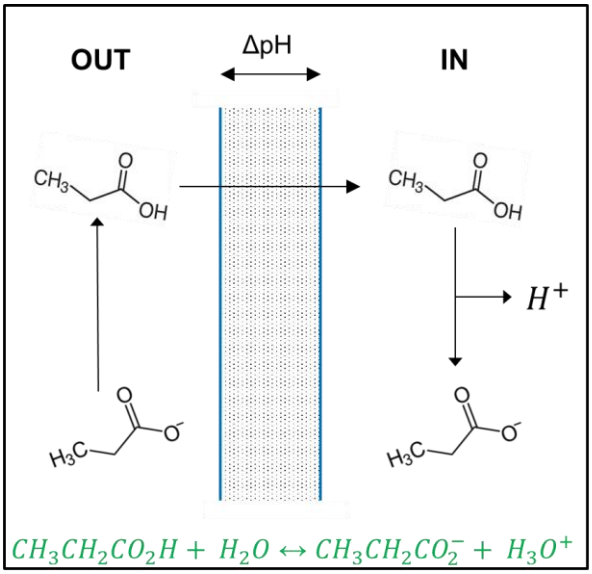

Figure 2 diffusion of unionized propionic acid through cell a membrane

The toxicity is dependent on pH since there is an equilibrium between propionic acid and propionate. With the Henderson-Hasselbach equation, it is possible to determine the ratio between these compounds, propionic acid is a weak acid with a pK_a of 4.87 (see, Figure 3) [10]:

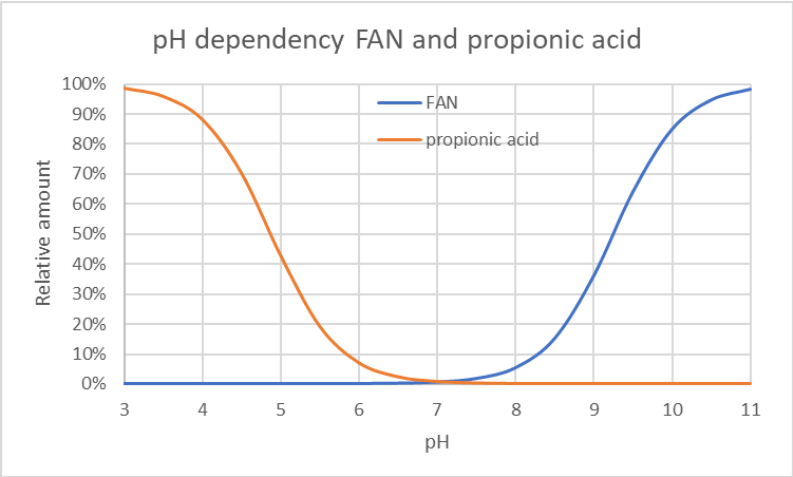

Figure 3 pH dependency FAN and propionic acid

However, it is impossible to say that there is one concentration where the process experiences inhibition of propionic acid or propionate. Literature gives values for no inhibition of propionate on methane yield up to 2600 mg/L [11]. Next to this, propionic acid will be degraded even at high concentrations of up to 4200 mg/L [8]. While in other papers inhibition of propionic acid was even found at low concentrations of 900 mg/L [12]. Since all reactors are in batch configuration, the differences in reported inhibition levels are mainly due to the applied operation times in these research papers. For the highest concentration, there was a longer retention time which results in recovering of the reactor. The degradation of propionic acid has been reported as a slow process that needs a high retention time. In the AD process propionate will be degraded to hydrogen, bicarbonate and acetate by acetogens. This process is highly endergonic and will not occur spontaneously [13], [14]:

$$(1)\ CH_3CH_2COO^- + 3H_2O \leftrightarrow CH_3COO^- + HCO_3^- + 3H_2 + H^+ \ (\Delta G^{\circ\prime} = +76.1 \frac{kJ}{mol})$$

However, this reaction can be maintained by consumption of the products by methanogenesis [15] [11], [13], [14]:

$$(2)\ 4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \ (\Delta G^{\circ\prime} = -135.6 \frac{kJ}{mol})$$

Microbial adaption of the inoculum to propionate seems to improve process characteristics [11]. What is observed in a report by "Han, Green, & Tao, 2020" is that when a concentration of propionic acid is added to the inoculum of a batch reactor that is already fed with FW, the composition will change over time to a digester with a composition that has more hydrogenotrophic methanogens (only when the retention time and propionic acid concentration are high). Also, a shift in syntrophic bacteria is observed. For propionic acid, it is known that degradation occurs through the cooperation of the methanogens with syntrophic acetogens for which the hydrogenotrophic methanogens are crucial. So there is a shift from acetoclastic methanogen to hydrogenotrophic methanogen since acetate degradation becomes less important.

2.2.3. Free ammonia nitrogen

A problem that arises with the relatively high protein content of FW is a reduction in the C/N ratio. In a continuous process with high retention times, this results in the accumulation of total ammonia nitrogen (TAN). In combination with a high pH, this results in the accumulation of free ammonia nitrogen (FAN) (see, Figure 3). The pK_a of ammonia is 9.25 and the equilibrium between ammonia (FAN) and ammonium is given below [16]:

$$(3)\ \ NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$$

High concentrations of FAN are known as an inhibitor for methanogenesis, especially acetoclastic, the dominant pathway in producing biogas in AD. In unprotonated form, FAN is possible to diffuse through the cell membrane like for the propionic acid (see, Figure 2) [5], [17], [4]. Due to this inhibition, the syntrophic acetate-oxidizing bacteria (SAOB) will become predominant and acetate will be oxidized to H_2 and CO_2 (see Figure 1) [17], [18]:

$$(4)\ \ CH_3COO^- + H^+ + 2H_2O \rightarrow 4H_2 + 2CO_2\ (\Delta G^{\circ,} = +95\frac{kJ}{mol})$$

This process is thermodynamically stimulated by the removal and consumption of products by the hydrogenotrophic methanogens generating methane to make it sufficiently exergonic (the sum of these reactions will not result in an exergonic process) [17], [19]:

$$(5)\ \ 4H_2 + CO_2 \rightarrow 2H_2O + CH_4\ (\Delta G^{\circ,} = -32.7\frac{kJ}{mol})$$

Eventually, the process will break down due to the accumulation of VFAs (propionic acid) since the hydrogenotrophic methanogens cannot handle the high loadings regenerated through the absence of the acetoclastic methanogens [7]. The process will not suffer from the inhibition of hydrogen since the acetoclastic methanogens are already inhibited by the high FAN concentrations.

Different ammonia concentrations are given in the literature to be toxic reaching from 1000 – 3000 mg TAN/L [7], [6], [20], [3], [21]. Reactors where *Methanosarcina* are dominant (the case for FW) have moderate inhibition limits to FAN compared to reactors where the *Methanosaeta* or hydrogenotrophic methanogens are dominant [21]. This is due to the different microbial communities used, the variances in reactor parameters like pH or temperature and the different methods used for the calculation of the FAN concentration [21]. The main reason why it is not possible to use too high HRTs is that the accumulation of FAN will result in intoxication.

2.2.4. Low in trace elements

FW also has a relatively low amount of TEs compared to other substrates for AD, like dairy cattle manure and wastewater activated sludge [4], [5]. In several studies, this lack of TEs is seen as the fundamental reason for the accumulation of VFAs in the AD of FW over time, since TEs are essential in methanogenesis [15], [5].

Background information on the methanogens that consist of multiple microorganisms is required to understand the necessity of TEs. *Methanosarcina* are the dominant methanogens in AD with an abundance of 70-80% followed by other methanogens like *Methanosaeta* (± 10%) and other archaea mainly for the hydrogenotrophic methanogens [15]. *Methanosarcina* produces methane from all three metabolic pathways: acetate, CO_2+H_2 and methyl compounds. Moreover, *Methanosarcina* are assumed to have a relatively high tolerance to high concentrations of ammonia, salt and acetate [15]. Acetate is the sole substrate of *Methanosaeta,* and are the only methanogens that use acetate as the sole substrate. Other archaea contribute to hydrogenotrophic methanogenesis. In good-running digesters, the acetate-consuming methanogens are well available [15]. However, with the addition of

FW this composition of methanogens will shift to a composition that results in digester failure. This is caused by the low TE concentration in FW [15]. From research is observed that the composition will shift to a composition where the *Methanosaeta* are dominant (± 80%) and *Methanosarcina* will slowly disappear [15], [8]. Once these *Methanosaeta* are dominant and the hydrogenotrophic methanogens are oppressed the methanogenesis will slow down. The reaction for the syntrophic acetogens to convert propionate is not favourable anymore since the product will be slower removed [15].

In Table 1 the micronutrient composition of the methanogens is given. For nutrient concentration in the feed of the AD, a value equal to or twice the minimal nutrient concentration is required [22]. However, the total amount of methanogens in the reactor first has to be known to do something with this composition (dependent on the substrate). A nutrient concentration that is too high can also lead to digester failure, however, such high values (e.g. Co = 35-950 mg/L and Ni = 35-1600 mg/L) are not obtained for AD of FW [5]. Observed from different experiments is that Fe is an element that is required in high concentrations, without the addition of Fe the digestion will fail [23].

Table 1 element composition of methanogens

	Units	*Concentration*
Fe	mg/kg	1800
Ni	mg/kg	100
Co	mg/kg	75
Mo	mg/kg	60
Zn	mg/kg	60
Mn	mg/kg	20
Cu	mg/kg	10

Since the concentration of TEs in FW and the TE requirements for methanogens differ a lot there is a need for the addition of these elements. This can be done by the addition of TEs to the substrate (FW) or by co-digestion with a TE-rich substrate.

First, the addition of TEs to the FW is discussed. The main trace elements are Ni, Zn, Co, Mo, Fe, Se and W all with their strengths in the AD process (see Appendix A) [5]. OLR will play an important role in the determination of the addition of TEs in the AD of FW. A common issue with the addition of TEs to a reactor is that they exit the reactor with effluent [5]. The TE concentrations are based on reports from “Zhang, et al., 2019”, ”Zhang & Jahng, 2012” and “Banks, Zhang, Jiang & Heaven” since the same characteristics of the substrate, inoculum and digester parameters are observed (see Appendix A)

Co-digestion is less discussed in this report but is not less attractive for the digestion of food waste. In co-digestion, two or more organic substrates are added to a digester that fills in each other shortcomings, e.g. in micro- and macronutrients, C/N ratio or buffer capacity. Possible co-substrates for co-digestion with FW are sewage sludge, dairy cattle manure, paper waste, rice etc.. [4].

2.2.5. Digester foaming

Digester foaming is a problem that can cause the failure of the digestion process. Foaming is a result of the accumulation of gas in the liquid phase when it is not possible to rise. As a consequence, the volume of the digestate keeps increasing and eventually digester failure due to blockage of gas tubes occurs. There are multiple causes for digester foaming. Surface active materials come in with the substrate or are formed in the digester by microorganisms and can be proteins, fatty acids, detergents and other compounds. They are formed when there is an overfeeding and input of inhibitors [5]. Another reason is the sudden change in physiochemical conditions like a rise in temperature and/or a decrease in pH. Due to this sudden change the CO_2 that is dissolved as carbonic acid and HCO_3^- will be released. It is possible to control the foaming with anti-foaming additives that contains inert chemicals and has surface active properties.

2.3. biogas as an energy source

This report intends to come to the most efficient conversion of FW to an energy source. At the start of the project, the conversion of biogas into an energy source was also the focus of the report. However, due to problems with financing and regulations, the upgrading is based only on theory. It is important to not only take the energy conversion efficiency but also the possibility of storage [24]. The discussed solutions are heat and electricity, green gas and proteins.

2.3.1. biogas to electricity and heat

The upgrading of biogas to electricity and heat is a method widely used for the conversion of biogas produced at wastewater treatment plants (WWTPs). The generation of heat and electricity is done by a combined heat and power (CHP) system. For these CHP units, the biogas does not need any upgrading requirements and thermal energy will be used for heating (otherwise wasted thermal energy). Produced heat can be used for the plant itself or heating buildings (in this case campus Zernike). CHP units have an approximate conversion efficiency of 30% for electricity and 55% for heat [25]. However, heat is a source that needs to be immediately used. Electricity can also be generated from other sources like solar panels and wind turbines (there is already an overloading of the net at peak moments).

2.3.2. biogas to green gas

The upgrading of biogas is an alternative that is attractive since the storage of green gas will not be a limiting factor. The total storage capacity of gas in the Netherlands include 12.5 billion m^3 [26].

There are several applied technologies for the upgrading of biogas to green gas. In this report, there is a focus on the more well established technologies in the industry. The discussed technologies are pressure swing adsorption (PSA), membrane separation (MS), water scrubbing (WS) and chemical absorption (CA).

PSA is a technique based on the adsorption of gasses on an adsorbent bed through pressure fluctuations. For biogas, the CO_2 will adsorb in the pores of the adsorbent material at relatively high pressure resulting in a gas stream enriched with the less strongly adsorbed components (methane). This is because there is a difference in molecular dimensions of methane (0.38 nm) and CO_2 (0.34 nm). For adsorbent material, there can be made use of zeolites, activated carbon, silica gels and activated alumina. PSA technology shows a methane slip of 1.8% to 2% [27], [28], [29].

MS is a technique that removes gasses such as CO_2, H_2O and NH_3 from biogas and is already widely used in the natural gas industry. In this technique, a membrane made of polymeric materials is exposed to biogas. These membranes are permeable to CO_2, H_2O and NH_3, which results in a gas rich in methane

that is possible to flow through this methane without being removed. Membrane technology shows a methane slip of 1% to 2% and a CO_2 removal efficiency of 98% [28], [30].

WS and CA are techniques based on the differences in solubility between CO_2 and methane in water or amine solvents. In these techniques, the biogas is compressed and injected at the bottom of a washing column filled with water or amine solvents. The scrubber packing facilitates contact between gas and liquid resulting in the adsorption of CO_2 and methane coming out of the top of the reactor. A difference between water and amine solvents is the difference in selectivity to CO_2, which makes it possible to operate chemical absorption in smaller units. Next to this amine solvents are effective at almost atmospheric pressure while water scrubbing is at an elevated pressure between 4 and 6.5 bar [28], [31].

2.3.3. biogas to proteins

In the Netherlands, there is a lot of livestock but there is a lack of land, so importing protein from Brazil is an applied option. Another option is to add a protein production plant in addition to anaerobic digesters. This sounds like a strange way of producing protein since the protein first will be demolished and then will be produced again. However, since this can be done in a way through the gas phase quite pure products can be obtained without contaminants. In this field, there are multiple options for the production of a protein with substrates that are (mostly) available at an AD plant. Currently, multiple companies are focussing on the production of proteins in this way (*Feedkind (Calysta) and uni bio*) and research currently going on in the Netherlands called “power-to-Protein”.

The research of “Power-to Protein” with close relations to different WWTPs in the Netherlands is focussed on the recovery and valorisation of ammonia from the wastewater cycle. The ammonia that is recovered from the rejected water of a WWTP is used in combination with hydrogen, oxygen and CO_2 to produce proteins. These single-cell proteins are produced by lithotrophic hydrogen oxidizing bacteria. The equation is as follows:

$$21.36\ H_2 + 6.21\ O_2 + 4.09\ CO_2 + 0.76\ NH_3 \rightarrow C_{4.09}H_{7.13}O_{1.89}N_{0.76} + \ O_2$$

The ammonia can be recovered by air stripping and is based on the increase of pH and temperature. An increase in the pH can be obtained with the stripping of CO_2. An advantage of FW is that it has a high protein content resulting in high ammonia concentrations. Moreover, higher retention time in combination with an OLR of 5 g VS/L/day will result in higher ammonia levels, which favours the reclaim of ammonia and reduces costs [32], [33]. For the increase of pH, it might be interesting to have a look at the process of the tanks in series since the obtained pH of the second tank is then 8.4 instead of 7.32 in only 1 tank [34]. A benefit of ammonia stripping is a reduction of TAN in the digestate which reduces costs in the processing of digestate. For this protein production, the CO_2 can be recovered by potential stripping from the biogas [35]. There is also a need for a large amount of hydrogen. Hydrogen can be produced from biogas by steam methane reforming (SMR) [35].

The final product has a protein content of 70% and can be used as an alternative to fish meal and soja proteins [35]. If the product will be used for human consumption there first have to be focussed on food regulations within “Novel Food Verordering (EG) nr. 258/97)”, this is not the case for this protein yet [35].

There is also the possibility to make microbial protein from natural gas by *Methylococcus capsualtus*. This is already done by FeedKind® where they obtain 3-4 kg microbial protein per m^3 reactor volume per hour. In this project, there has been made use of a U-Loop fermentation to make sure the gasses have a lot of surface area to react with. The produced proteins are already used as pig feed [36].

3. Materials and methods

The setup of the different experiments and the analysing methods are discussed in the materials and methods section. Starting with the analytical methods for the experiments supplemented with the characteristics of the reactor sludge and substrate.

3.1. Analysing methods

In this subsection, the different analysis methods are discussed with compact background information on why these factors are of importance. For a more extensive working procedure appendices, B and C can be consulted. All measurements are done in triplicate if not mentioned otherwise. The samples are taken from three different tubes with different lengths to make sure a good reflection of the true reactor sludge is given. The samples are analysed on the same day as the samples were taken, except for the continuous experiments with a HRT of 24 days. These samples are stored in the freezer until analysis.

3.1.1. COD, TS, VS and ash

For determination of the amount of available substrate that can be used for the production of biogas the COD, TS, VS and ash contents are measured. COD and VS are reflections of the organic fraction in the substrate, which is the feed of the micro-organisms in the bioreactor. TS, VS and ash contents are determined by the APHA standard methods, 2540 E [37]. For the COD, the substrate sample is diluted and homogenized before measurements. There is made use of COD measuring kits LCK 514 and LCK 304 following protocol (Hach, US).

3.1.2. FAN and TAN

For the determination of the FAN and TAN, it is possible to measure the ammonium concentration (NH_4^+). Before measurement, the sample is first centrifugated (15763 RCF, 30 min, 4 °C), filtrated (45 µm) (to avoid scattering of the particles by light [38]) and diluted. Hereafter the ammonium measuring kits LCK 303 and LCK 503 are used following protocol (Hach, US). To determine the FAN concentration from the ammonium measurements there is made used the ideal equilibrium between NH_3 and NH_4^+:

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$$

To come from NH_4^+ to FAN this equation can be used (determined for 25 °C, since this is the room temperature):

$$FAN = \frac{K_A * (TAN)}{K_A + 10^{-pH}} \; (with: TAN = FAN + NH_4^+) \rightarrow FAN = \frac{K_a * NH_4^+}{10^{-pH}}$$

Where K_a is the acid dissociation constant at temperature T. The TAN value is used to determine the FAN concentration at working temperature (37 °C). From this equation, it can be observed that the impact of the pH on FAN is large. The pH is measured with a VOS-70002 for every sample and is also measured in-line in the reactor itself.

This method, where there is made use of the ideal equilibrium, overestimates the FAN concentration up to 37% when compared to the "MINTEQA2 Equilibrium Speciation Model". However, it is possible to use the modified Davies equation which gave better results (only a 1% difference from the model). For this equation, the measurement of different ions like unprotonated VFAs and metals is necessary [21].

3.1.3. HPLC

HPLC measurements make it possible to detect and quantify the amount of VFA. The measured VFAs in the samples are formic acid, acetic acid, propionic acid and butyric acid. The column used for these experiments is the: "Rezex™ ROA-Organic Acid H + (8%)" with the dimensions: 300*7.8 mm. The settings of the HPLC are:

- Flow: 0.5 mL/min
- Mobile phase: 2.5 mM H_2SO_4
- Temperature: 50 °C
- Run time: 60 min
- Detection: UV 210 nm

The VFA concentration is measured by making stock solutions of the four VFAs before every measurement of 0.1 M. Comparison of the measured samples with the surface area of the peaks in the stock solution results in a final concentration of these VFAs. It can be assumed that the VFAs are detected in their protonated form since the mobile phase is 2.5 mM H_2SO_4. To see if the VFA is in its conjugated base form there can be made use of the Henderson-Hasselback equation:

$$pH = pK_a + log\frac{[A^-]}{[HA]}$$

Where the pKa is the acid dissociation constant that describes the acidity of a particular molecule, HA is the acid and A^- is its conjugated base. Since the sum of the acid and its conjugated base is known it is possible to determine the concentrations of each separately.

3.1.4 GC

The focus of this research is on the total production of methane, it is important to determine the biogas composition of the anaerobic digesters. The biogas samples are taken with "E-Switch®" PVDF gas sampling bags after the gas meter instrument.

The "Thermo Scientific C2V-200" micro GC is used to measure these biogas compositions. The settings of the GC are:

- Flow: 3 mL/min
- Mobile phase: Helium
- Column temperature: 60 °C
- Injection/detection temperature: 120 °C
- Run time: 20 seconds
- Detection: thermal conductivity
- Column: GCC200-U-BND

The working principle of the GC is the same as for an HPLC. There are chosen only select samples 11-15 to make sure the gas from the last sample is flushed out the GC.

3.1.5. FOS/TAC

A FOS/TAC measurement is based on the ratio between volatile fatty acids (FOS) and total inorganic carbon (TAC) and can also be used as individual parameters. The TAC parameter is based on the basic buffer capacity and is determined through titration of a fermentation substrate sample from its original pH to a pH value of 5 (mg/L $CaCO_3$). The FOS value is determined by a second titration step from a pH of 5 to a pH of 4.4 (mg/L CH_3COOH). The FOS/TAC measurements are based on the Nordmann method and are easy to use for fast evaluation of the reactor sludge [6]. An advantage of this method is that

there can be a response to the acidification of the reactor before the pH decreases. The FOS/TAC measurement is done by AT1102 - Titralab AT1000-titrator (Hach, US).

This method is used to check if it works and is only possible with “big” samples (15 mL of filtrated sludge) and therefore not applicable to most of the experiments in this report

3.1.6 Total gas batch reactor

The total gas in the batch reactor experiments is determined and is calculated by looking at when the gas production was the same as in the 24 h before the measurement (see figure.

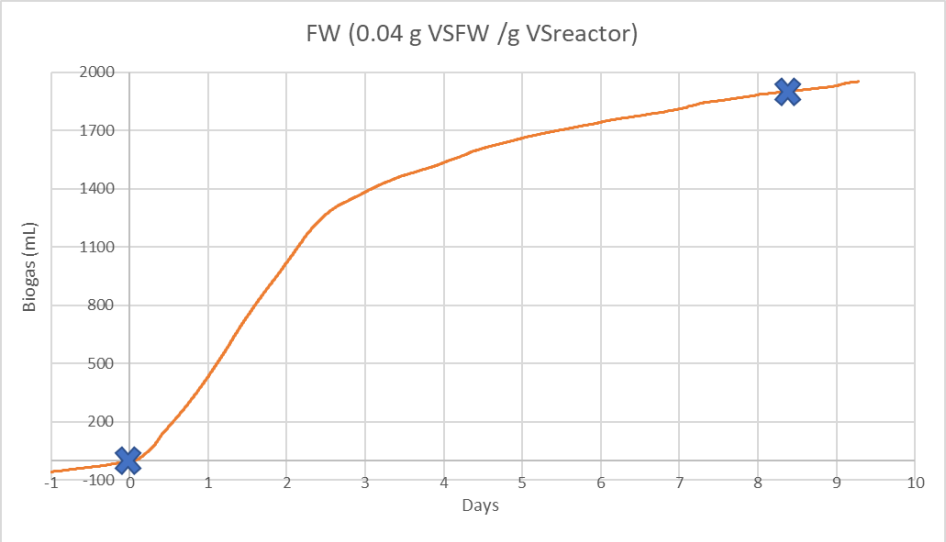

Figure 4 gas production with FW addition of 0.04 g VS/ g VS

3.1.7. Metal analyses

For the detection and quantification of TEs (Mn, Fe, Se, Co, Ni, Zn, Cu, W and Mo) there has been made use of an ICP measurement, performed by “Wetsus”. However, the destruction of the material by a microwave is included in this study.

For this destruction, there has been made use of a “MARS 5” (Microwave Accelerated Reaction System) by CEM Corporation with “XP-1500 Plus” tubes.

. The settings are:

- Max. Power: 1200 W
- Ramp time: 15 min
- Hold time: 15 min
- Temperature: 200 °C

As a reagent, there is made use of the minimal addition of 10 mL 65% HNO_3. Before the samples are added to the tube there has been made use of a 105 °C oven to make sure all water is evaporated and it is possible to add more organic material since the maximum load is 0.5 g.

After the degradation, the samples need to be diluted to a 2% HNO_3 matrix. Which results in dilution factors of 124 and 188 for FW and sludge.

3.2. Substrate and inoculum

The inoculum that is used is anaerobic sludge from the WWTP of Garmerwolde. Characteristics of samples are given in the tables below:

Table 2 Anaerobic sludge Garmerwolde

Parameters	*Units*	*Anaerobic sludge Garmerwolde*
Density	g/cm^3	1.00 ± 0.02
Total solids (TS)	%	1.8 ± 0.3
Volatile Solids (VS)	%	1.3 ± 0.2
VS/TS	%	71.2 ± 4.4
COD	mg/L	21213 ± 7947
TAN	mg/L	1837 ± 161
FAN	mg/L	158 ± 14
pH	-	7.85 ± 0.02
Formic acid	mg/L	0 ± 0
Acetic acid	mg/L	33 ± 46
Propionic acid	mg/L	21 ± 30
Butyric acid	mg/L	0 ± 0

This research there is made use of a FW sample that is prepared by blending a collection of household- and restaurant FW. This FW is first kept in a freezer and thawed in a cooling room (8 °C) 6 days before measurements were done.

Table 3 FW

Parameters	*Units*	*Diluted FW*[a]	*FW*[b]
Density	g/cm^3	-	1.10 ± 0.03[b]
Total solids (TS)	%	21.6 ± 0.75	39.1 ± 1.4
Volatile Solids (VS)	%	20.3 ± 0.1	36.9 ± 0.2
VS/TS	%	94.4 ± 2.9	94.4 ± 2.9
COD	mg/L	227525 ± 62049[c]	431524 ± 117682
TAN	mg/L	180 ± 2[c]	341 ± 4
FAN	mg/L	0 ± 0[c]	0 ± 0
pH	-	4.18[d]	3.90
Formic acid	mg/L	5108 ± 379[c]	9688 ± 719
Acetic acid	mg/L	0 ± 0[c]	0 ± 0
Propionic acid	mg/L	113 ± 1[c]	214 ± 2
Butyric acid	mg/L	66 ± 114[c]	125 ± 216

[a] There was only taken 1 big sample where all measurements are done in triplicate.
[b] The normal FW was based on the diluted FW
[c] Duplicate
[d] One measurement

3.3.1. Batch reactor set-up

The first experiment is executed in an airtight 2L “Applikon” reactor configurated as a batch reactor. The working volume of the reactor is 1700 mL and is filled with anaerobic sludge of the WWTP of Garmerwolde (see Table 2). The temperature of the reactor is regulated by a “Tamson TC.3” water bath and a temperature sensor inside the reactor of “applikon®” displayed at an “applikon® ADI 1010” bio controller. The temperature is set to 37.1 ± 2.4 °C (from 30 random observations, one per day). An “IKA® Eurostar 40 digital” laboratory stirrer provides the stirring and is changed to a “P100 applikon®” stirring motor controlled by an “ADI 1012 applikon®” motor controller for the last batch experiment. The speed of the stirring motor is set to 120 rpm. The gas flow is measured by a “Ritter MGC-1 V3.4 PMMA_R” (1 tick per 0.94 mL) flowmeter. A “Consort SP22x ” glass electrode is used for measuring pH and an “SKxxT” electrode for conductivity, displayed both at the “Consort C3060”. The run of the reactor before the first addition of an energy source is 34 days. A syringe was used to add the undiluted FW to the reactor. Before each experiment, the amount of volume that is added is also rejected from the reactor to maintain a constant working volume.

Figure 5 Applikon reactor

3.3.2. Continuous reactor (syringe pump)

The second run is performed in an airtight “applikon®” reactor, continuously fed by a “Syringepump.com NE-1000X” syringe pump. The working volume used is in the same ratio as for the potential upgrading to a pilot plant reactor (1400 mL) and is filled with anaerobic sludge of the WWTP of Garmerwolde (see Table 2). The temperature of the reactor is regulated by a “Tamson TC.3” water bath and temperature is controlled by a temperature sensor inside the reactor of “applikon®” displayed at an “applikon® ADI 1010” bio controller. The reactor temperature is 36.4 ± 1.4 °C (from 28 random observations, one per day). The mixing is provided by a “P100 applikon®” stirring motor controlled by an “ADI 1012 applikon®” motor controller. The mixing speed of the anaerobic digester is set to 120 rpm. The gas measurement is done by a “Ritter MGC-1 V3.4 PMMA_R” (1 per 0.91 mL). The pH is measured with a “Consort SP94Y” glass electrode and is displayed at the “Consort C3060”. The experiment started with diluted food waste (50% v/v) added by a “MONOJECTTM” 140 mL piston syringe and set to a flow rate of 0.2 ml/h (after filling the tubes with FW). After 46 days the syringe is changed to a smaller “Terumo®” 30 mL syringe, new tubing and FW that is not diluted. This is done due to the blockage of the FW and the settlement of the FW in the suspension after a while. The flow rate is changed from 0.2 to 0.1 mL/h to keep the OLR constant (tubes were empty when the flow rate was started).

3.3.3. Continuous reactor (TE addition)

In the final experiment, an airtight “applikon®” is fed by a “Syringepump.com NE-9000” programmable peristaltic pump with 1/8 inch “Norprene® A-60-F” tubing. The flow rate for both in- and outflow is set to 0.04 mL/min (HRT= 24.3 days). The feed is from a reactor with food waste diluted with water and includes a mixture of TEs. The inflow of both peristaltic pumps is connected by a two-inlet system that minimizes the possibility of failure. The working volume of the reactor is 1400 mL filled with anaerobic sludge of the WWTP of Garmerwolde (see Table 2) and is also sieved (3 mm). The anaerobic digester is temperature regulated with a “LKB BROMMA 2219 MULTI TEMP II” thermostatic circulator and

temperature controlled by a temperature sensor inside the reactor of "applikon®" displayed at an "applikon® ADI 1010"bio controller. The temperature was registered every week and regulated to an average of 37.0 °C ± 0.2 °C. The mixing for both the FW buffer and the anaerobic digester is provided by a "P100 applikon®" stirring motor controlled by an "ADI 1012 applikon®" motor controller. The mixing of the anaerobic digester is set to 120 rpm and is changed to 160 on 05-08-2022 since some foaming seems to occur. For the FW this changes from 200 rpm to 300 rpm if worse mixing was observed. The biogas flow is measured by a "Ritter MGC-1 V3.4 PMMA_R" with 1 tick for every 0.91 mL. The pH is measured by a "Consort SP22x" glass electrode and is displayed at the "Consort C3060".

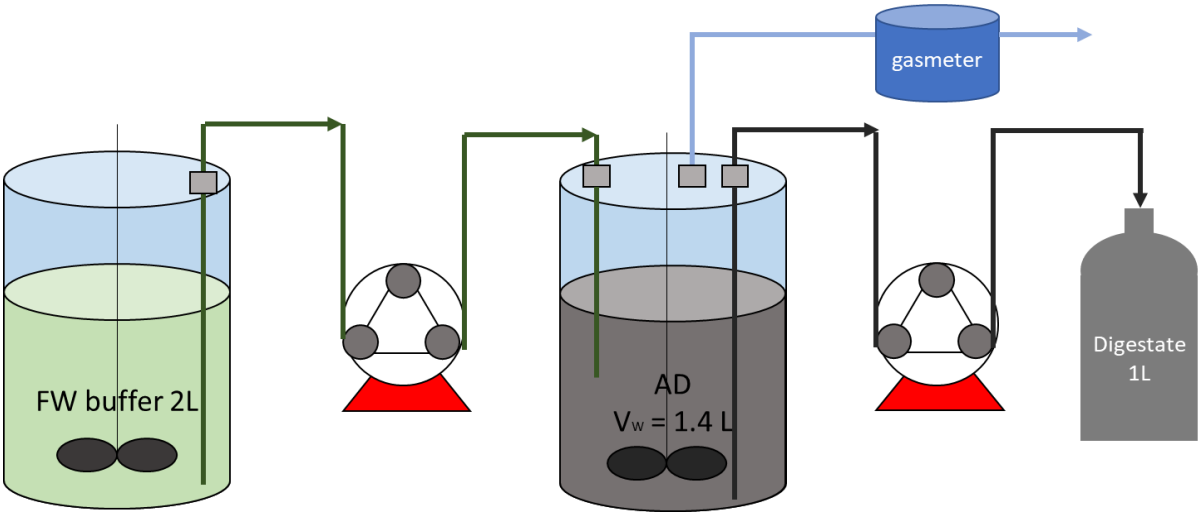

Figure 6 reactor set-up for continuous reactor

The reference reactor without the addition of TEs has the same configuration as the reactor with TE addition. Differences are in the water bath (Tamson TC.3) and the FW stirrer (IKA® Eurostar 40 digital). Assumed is that these changes do not have impacts on the results.

The food waste is fed from an "applikon®" reactor that is closed but not airtight. The food waste buffer is prepared by adding FW from the cooling room that is there for a maximum of 15 days with distilled water and eventually some TEs. The food waste is also sieved (3 mm mesh).

The continuous experiments with a HRT of 24.3 days and an OLR of 10 g VS/L/day. The first sample is taken after 5 days whereafter the samples are taken every week. The pH and T are registered every week before sample taking. The values are determined for an OLR of 10 g VS/L/day, for Mo the value was held at 5 mg/L since no seed sludge was used this was higher than 3 mg/L initially.

Table 4 TE additions

Element	*Units*	*TE addition OLR =10 g VS/L/day*	*TE addition OLR = 5 g VS/L/day*
Fe	mg/L	250	125
Co	mg/L	2.5	1.3
Se	mg/L	1	0.5
W	mg/L	1	0.5
Ni	mg/L	12.5	6.3
Mo	mg/L	5	2.5

3.3. Experimental design

The laboratory work done in this research is divided into three experiments with different setups.

3.4. Pilot plant reactor

For this research, an upscaling for the laboratory reactor will be taken into account. Two single-stage up-flow anaerobic sludge blanket (UASB) reactors are used as pilot plants. Both reactors are operated parallel and have a total volume of 5 m^3 with a working volume of 3.5 m^3. The reactors are fed with an extruder that is fed by “flood feeding” where the hopper is directly above the feed throat. This allows gravity and the screw to feed the FW to the extruder [39].

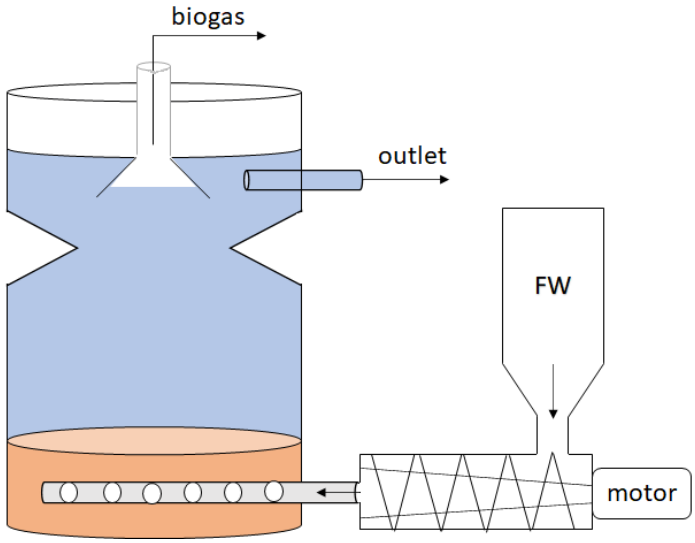

Figure 7 pilot plant reactor ENTEG building

4. Results and discussion

Different experiments are performed to obtain insights into the processing of FW by AD. The experiments are divided into batch and continuous lab-reactor experiments. For the batch lab-reactor experiments the main goal is to get the maximum S/X ratio for which no acidification of the reactor occurs. For the continuous lab-reactor experiments the main goal is to observe if it is possible to do AD of FW on a lab scale in continuous reactors and to obtain prove TE effectivity.

4.1. Batch lab-reactor experiments

There is a relation to the previous adaption of a substrate to inoculum in batch reactors. The initial bacterial community tolerance is of importance since VFAs and TAN can accumulate in the reactor. Also, microbial adaption plays an important role when several loadings of a substrate are added to the same digester. For this reason, it is hard to say what the overloading of such batch reactors is, from previous research S/X ratios of 0.3-1.35 VS_{FW}/ $VS_{reactor}$ are obtained at mesophilic conditions [4], [40].

Before the addition of FW to the reactor, an amount of 1 g of D-(+)-glucose dissolved in 70 mL water is used to perform the first experiment (S/X = 0.02 VS_{FW}/ $VS_{reactor}$). Glucose is used since the exact composition of this molecule is known and it is easily degradable. The total produced biogas by glucose is 775 mL. The maximum amount of gas that can be produced following the ideal gas law is 858 mL. This is 90%, as expected that 10% is for the bacteria to grow as energy and the rest is produced as gas.

Three experiments are performed with FW in one batch reactor. Loadings are based on the VS loading in the reactor. Based on the loadings in "Capson-Tojo, et al., 2017" there is chosen to make use of X/S ratios of 0.04, 0.19 and 0.89 g VS_{FW}/ g $VS_{reactor}$. The S-curve-like reaction of the gas production on the FW addition can be found in Figure 4. What can be observed in Figure 8 is that the total methane produced increases with the increase of VS. However in the last experiment with an addition of 0.89 g VS_{FW}/ g $VS_{reactor}$ this increase is minimal due to digester foaming after 8 days. The methane production is faster when higher loadings are added to the reactor with a maximum of ± 7000 mL/day and is at the start of the experiment with the S/X loading of 0.89 g VS_{FW}/ g $VS_{reactor}$. This is probably not only due to the higher loading but the microorganisms can adapt to FW as a substrate which is also seen in several papers, also called microbial acclimatization [8]. There is also a relationship between the first and second experiment since the production and loading both will increase by a factor of five.

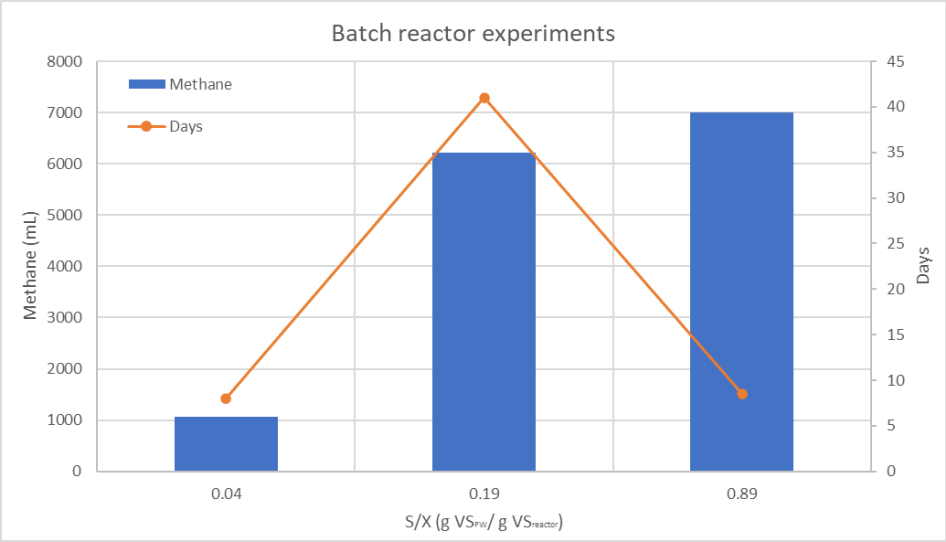

Figure 8 total methane production and days of production batch reactor experiments

In comparison with the papers of “Capson-Tojo, et al., 2016” and “Khadka, et al., 2022” with a BMP of ± 450 mL CH_4/g VS the batch reactor with different loadings in Figure 9 gives higher yields. Since the measurements of the reactor sludge are done at the end of every run the total VFA is expected to be zero. However, since the last experiment is interrupted by foaming there are still some VFAs left. From the diagram, it can be observed that the VFAs mainly consist of acetate followed by a smaller amount of propionate and butyrate. The relatively higher abundance of acetate could be because the reactor is not stopped after acidification (pH was at 7.33 ± 0.07) but in the middle of the process. Acetate is an intermediate product for the acetoclastic methanogens and propionic acid will be higher after digester failure The ratio of the VFAs is normal for a working anaerobic digester: 30-75% acetic acid, 5-30% propionic acid, 10-30% butyric acid and 2-30% valeric acid [11]. pH is still high the amount of unionized VFAs is very low (0.3%) and the toxicity of the VFAs is negligible. After the addition of the FW, the pH will decrease (the last loading by 1.2 and this was the maximum) whereafter it will go back to the initial pH of ± 7.5.

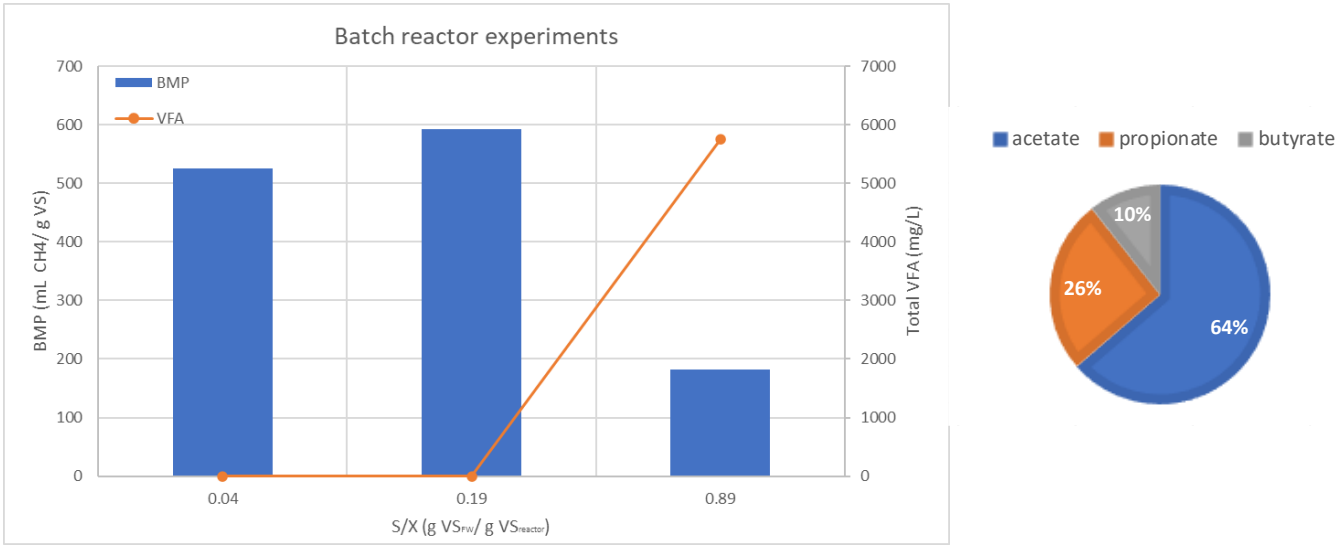

Figure 9 BMP and total VFA (including composition) batch reactor experiments

The difference in FAN between the samples is mainly because of the pH. However, the FAN is still in a safe range [7], [6]. A complete table including TAN, FOS/TAC and CO_2 composition can be found in Appendix D.

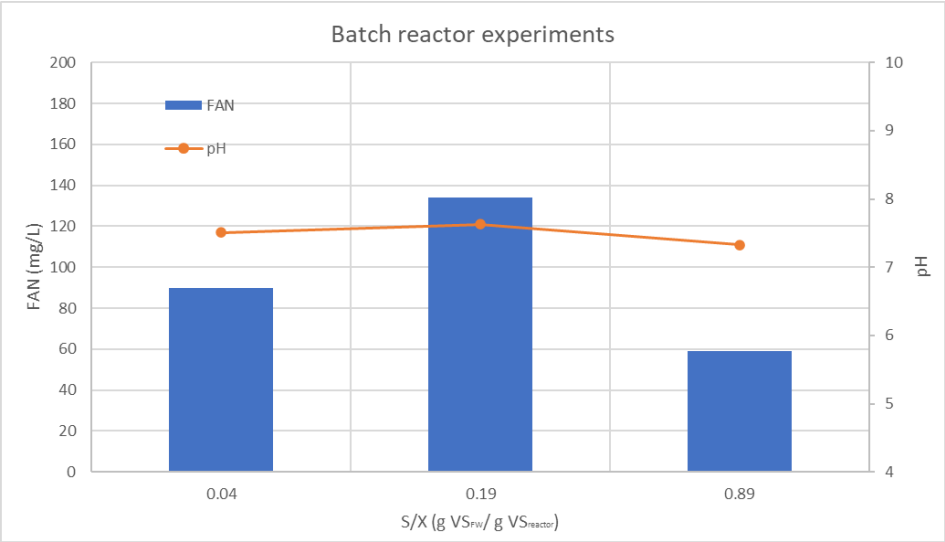

Figure 10 FAN and pH batch reactor experiments

4.2 Continuous lab-reactor experiments

In the continuous lab-reactor experiments, the BMP of a FW stream with an OLR of 1.45 g VS/L/day and a difference in HRT are compared. For continuous AD the reactor loading is based on an OLR and HRT instead of the S/X ratios seen by the batch experiments [4]. For continuous reactors, it is possible to obtain BMPs of 400 mL CH_4/ g VS for high HRTs of 180 days and low OLRs of 1.45 g VS/L/day, which is high compared to a BMP of 300 mL CH_4/ g VS for lower HRTs of 25 days [41]. A process with high HRTs results in the accumulation of ammonia and VFAs since methanogen will slowly disappear and nitrogen is raising [41]. An ”inhibited-steady state” in which the high TAN concentration will buffer the high VFA concentrations which avoids a pH drop is the only reason why such a process still works. For these high concentrations of VFAs and TAN low methane yields and unstable operations have been reported [4]. It is known that for an HRT of up to 100 days the reactor will fail after a while (with TEs above 50 days will work). For an HRT of 180 days, it seems more promising since the high VFA concentrations are buffered by the high TAN concentrations [41].

The experiments done in this section were mainly to find a good method to test the AD of concentrated (or at least minimal diluted) FW and to observe as such an “inhibited-steady state” can be observed.

The first loading is set to an OLR of 1.36 g VS/L/day and a HRT of 292. The reactor is started immediately with the addition of FW. This experiment ran for 46 days and only the first 14 days give a good reflection of the production since the in and outflow of the reactor gave some issues with blockages of the tubes. The whole gas production can be observed in Appendix E.

In the first 14 days, there was a mean biogas production of 740 ± 253 mL/day. On day eight a GC analysis for the gas composition is performed, 76% methane (26% CO_2). What gives an average BMP over the first 14 days of 577 ± 198 mL CH_4/g VS. Following batch experiments a BMP between 500-600 mL CH_4/g VS can be expected. What can be observed is that the biogas production is already decreasing which indicates that the FW on itself doesn’t produce that much but it is also plus the initial reactor sludge gas potential. A solution for this is a reference reactor where no FW is added and the produced biogas from this reactor can be subtracted. However, no other reactors were available.

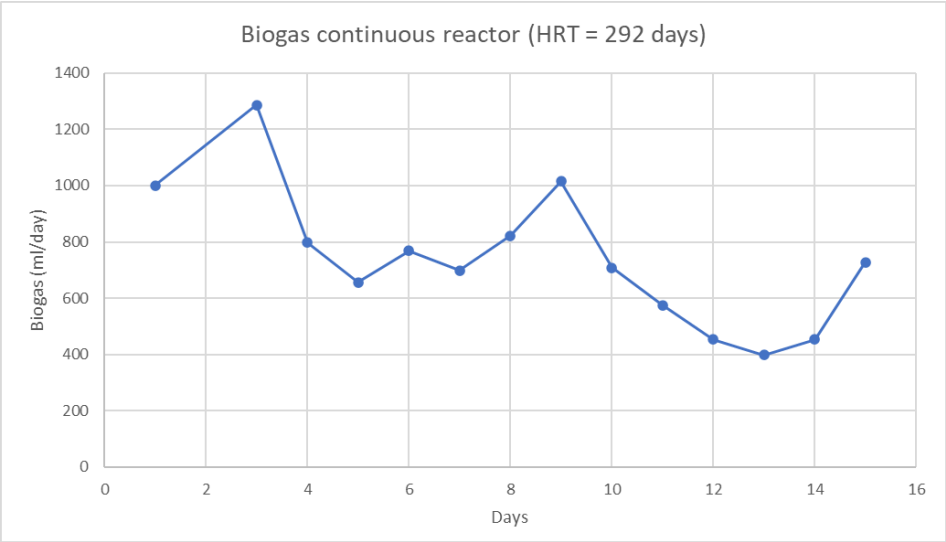

Figure 11 biogas production continuous experiment (HRT = 292 days)

The second run started after 46 days of running with diluted FW (with disturbances). In this run, the syringe, tubes and dilution of FW were changed for improvement of the process. The run lasted for 45 days whereof only the first 20 days give a good view of the system since blockages occur. The whole gas production can be observed in Appendix E.

The first 20 days give an average biogas production of 794 ± 124 mL/day (without days 3 and 4). On day 5 a GC analysis was done that give a methane fraction of 77% (27% CO_2). The BMP of the undiluted FW is almost the same as for the diluted and is 612 ± 96 mL CH_4/ g VS (without days 3 and 4).

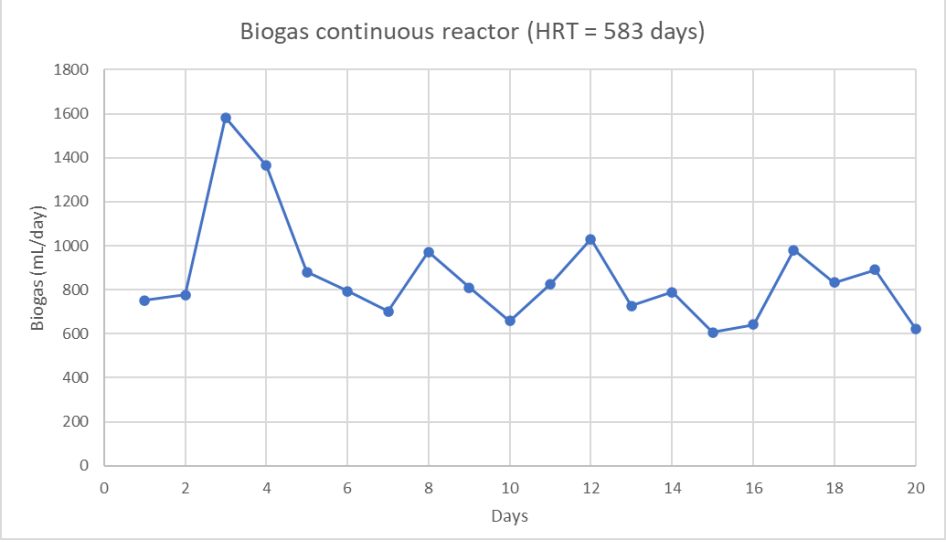

Figure 12 biogas production continuous experiment (HRT = 583 days)

On the day the reactor was stopped three samples were taken for analysis, see Table 5. From the table, it can be observed that this run does not indicate overloading (reactor sludge). The pH is the same as of the reactor sludge and the VFAs are relatively still low. The TAN is increased due to the low HRT of 292 and 583 days which results in high nitrogen income because of the high protein content of FW and low outflow of these TAN.

Table 5 values reactor sludge after continuous reactor experiment

Parameters	*Units*	*Anaerobic sludge Garmerwolde*	*Reactor sludge (end of the experiment)*
Total solids (TS)	%	1.8 ± 0.3	3.34 ± 0.20[a]
Volatile Solids (VS)	%	1.3 ± 0.2	2.16 ± 0.41[a]
VS/TS	%	71.2 ± 4.4	64.53 ± 8.41[a]
COD	mg/L	21213 ± 7947	33058 ± 541
TAN	mg/L	1837 ± 161	2709 ± 512
FAN	mg/L	158 ± 14	249 ± 47
pH	-	7.85 ± 0.02	7.88 ± 0.03
Formic acid	mg/L	0 ± 0	0 ± 0
Acetic acid	mg/L	33 ± 46	0 ± 0
Propionic acid	mg/L	21 ± 30	0 ± 0
Butyric acid	mg/L	0 ± 0	642 ± 1112

[a] Make use of two samples

The working of the digester with such a high HRT was expected since in a report by "Climenhaga & Banks, 2008" for a high HRT of 180 days and the same OLR of 1.45 g VS/L/day no digester failure is observed.

4.3. Continuous reactor experiments with TE addition

Since experiments with the addition of FW by a syringe result in mechanical issues over time and only high HRTs can be performed, leading to high TAN values, another set-up is used. In this setup, high OLRs and low HRTs of 24 days are executed. Different papers show a higher volumetric methane production (VMP) with an increase of OLR, not necessarily resulting in higher BMP [33], [15], [23]. VMP is of more interest since high biogas production per volume of reactor instead of per amount of FW (FW is readily available) is more favourable in this research project.

For both experiments, a reference reactor is run next to the reactor with the addition of TEs. TAN is not included in this chapter since low pH values are obtained. The remaining data like TAN can be found in appendix F.

4.3.1. Continuous reactor experiments; OLR = 10 g VS/L/day

The first experiments are run with an addition of FW with an OLR of 10 g VS/L/day. The addition of TEs is based on VS and is shown in Table 4. For this experiment, the trace elements are measured for the anaerobic sludge from the WWTP of Garmerwolde and the FW used for this experiment. A simple subtraction of the added TEs is not possible since it will result in negative values since the FW is not perfectly mixed. Observed is that without TE addition the FW will eventually experience low TE values.

Table 6 measured TE values in anaerobic sludge from Garmerwolde and diluted FW with TE addition

	Units	*Anaerobic sludge Garmerwolde*	*Diluted FW with TE addition*
Cu	mg/L	18.2 ± 4.3	2.7 ± 0.6
Fe	mg/L	1410.9 ± 408.2	204.7 ± 7.5
Co	mg/L	0.6 ± 0.2	1.5 ± 0.1
Mn	mg/L	12.5 ± 3.9	2.0 ± 0.1
Ni	mg/L	<0.2	-
Se	mg/L	<0.9	<0.6
Zn	mg/L	34.9	5.1 ± 0.1
W	mg/L	<1.9	<1.2
Mo	mg/L	0.3 ± 0.1	2.8 ± 0.1

The reactors with the OLR loadings of 10 g VS/L/day experienced issues with the FW addition. From Figure 13 there can be observed that biogas production fluctuates and is quite low. Expected is a biogas production of 8 L/day based on the experiments in previous chapters. In the red area, the FW supply faces some difficulties due to blockages in the inlet. However, the pH of the reactor was dramatically decreased and seemed acidified before mechanical issues appeared. After the supply problems are repaired the reactor will produce some biogas again. In the start, there is a bounce back to a biogas production of 1261 mL/day. However, the pH is already very low and the methane fraction measured on the last day is only 1 % (76% of CO_2). This is observed more often for reactors that are acidified, CH_4 content will decrease drastically [42], [23] and [15]. Probably because the reactor methanogens are already acidified, but some other microorganisms that can withstand these lower pH values produce CO_2 or other gasses like hydrogen.

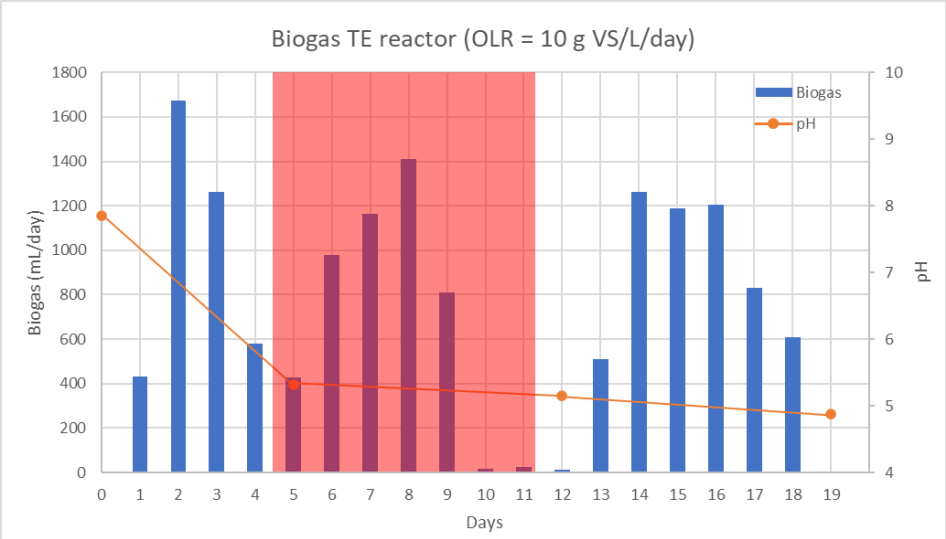

*Figure 13 biogas TE reactor (OLR = 10 g VS/L/ day) *red box indicates mechanical problems*

From Figure 14 is observed that COD and total VFA are increasing even if the supply of FW is not active. The rise of total VFA can be explained since the reactor has time to process all incoming FW in this period. Intermediate products like propionic and butyric acid will accumulate since methanogenesis does not work anymore. The acetic acid is decreasing in the stage where there was no addition of FW, which indicates that the aceticlastic methanogens are still working or some SAOB converts it to CO_2 and H_2.

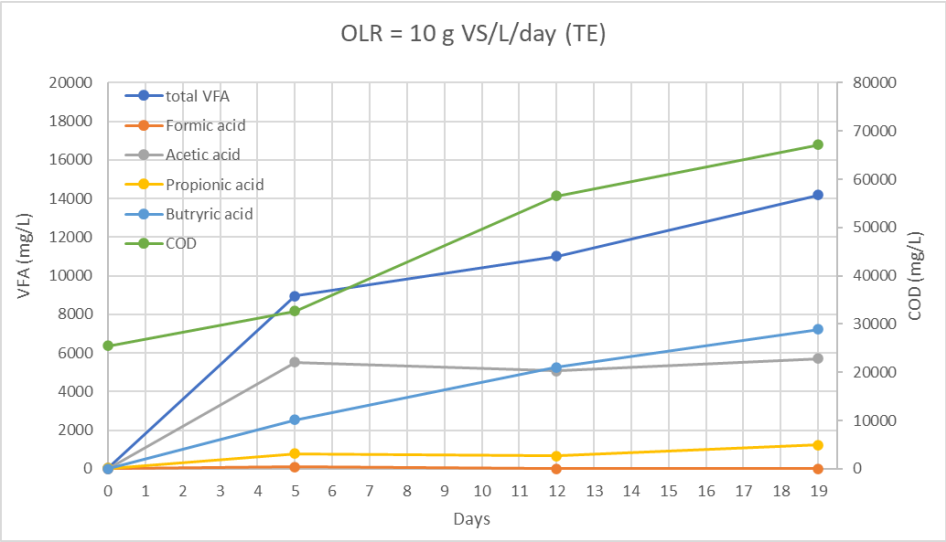

Figure 14 VFA and COD continuous TE reactor (OLR = 10 g VS/L/ day)

This reactor was also not able to recover after a few weeks of no addition of FW and the addition of 0.1 M NaOH to increase the pH to a level between 6.5 and 7.5. However, a propionic acid concentration of 1237 mg/L should result in biogas production within ten days [8]. The difference is that this research has more VFAs like butyric acid that prevent the digester from recovering.

The reference reactor is used to observe if the TE-supplementation makes any difference, the only difference is in the TE-supplementation.

In Figure 15 the gas production per 24h is observed. However, due to food waste loading problems, the behaviour of this reactor is more like the batch reactors discussed in chapter 4.1. The FW loading in the first stage was high resulting in a lower pH, but recovery after is observed. The reactor was cancelled after ten days since the TE reactor showed no perspective for a long run with a working FW supplementation since it was already acidified in 5 days. The methane fraction on the last day was 81% (31% of CO_2). The total composition of biogas is above 100%, probably because the peaks of nitrogen and methane are very close to each other in the GC analysis and some nitrogen is detected as methane.

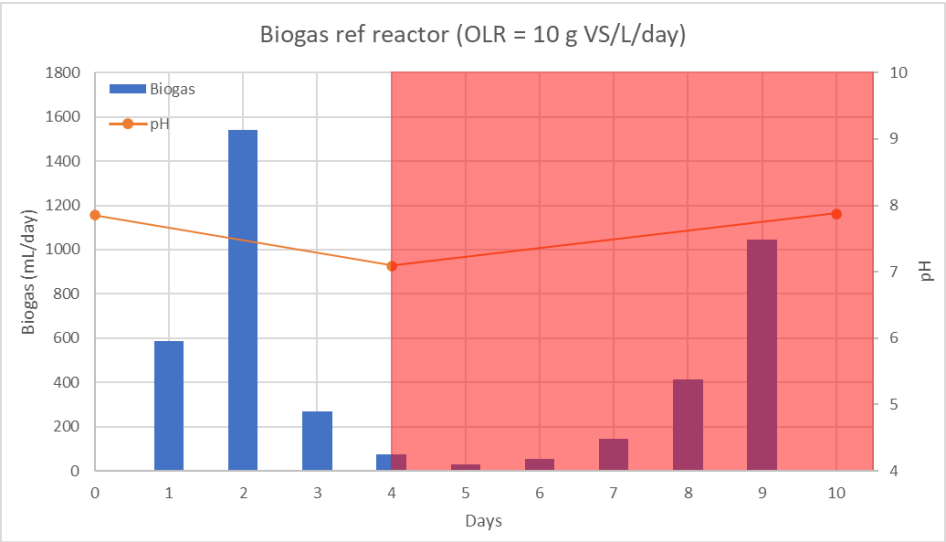

*Figure 15 biogas reference reactor (OLR = 10 g VS/L/day) *red box indicates mechanical problems*

The batch-like behaviour of the reactor is also observed in Figure 16, the reactor recovered after a food waste loading of 4 days. The COD is going back to its initial value and the same applies to the total VFA content (mainly composed of acetate).

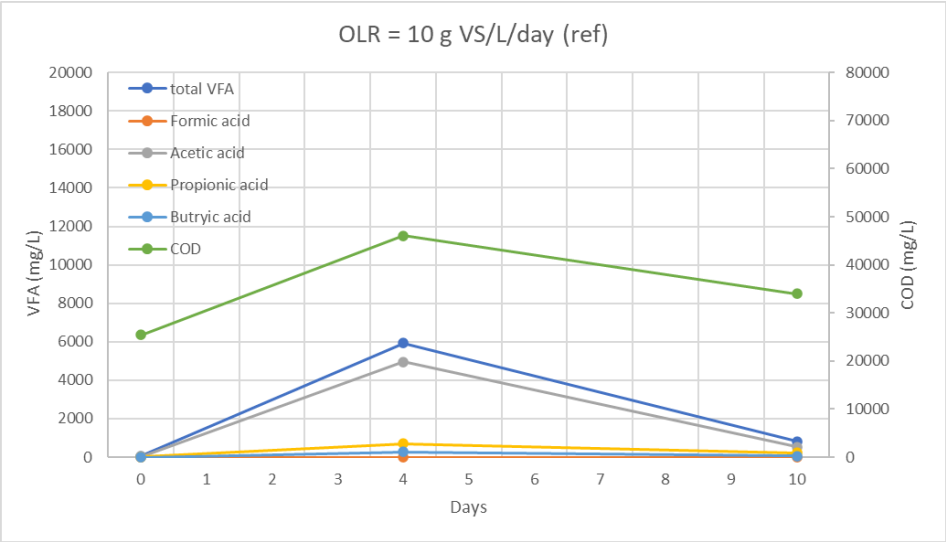

Figure 16 COD and VFA continuous reference reactor (OLR = 10 g VS/L/day)

4.3.2. Continuous reactor experiments; OLR = 5 g VS/L/day

Since the OLR of 10 g VS/L/day with a HRT of 24 days turns out in an acidification of the reactors the OLR is decreased by 50%. This diluted FW resulted in fewer mechanical problems with the addition of FW. The outflow of the reactor was blocked sometimes, when the reactors were opened big clay-like pieces in the reactor were observed which could explain the blockages. These bigger particles could FW that is not degraded and are clumped together.

From Figure 17, a decrease in biogas production over time is observed, with a biogas production that is already very low compared to what is expected (4 L biogas/day). Methane fractions are fluctuating; 17% on day 4 (CO_2 = 64%), 26% on day 11 (CO_2 = 54%) and 14% on day 19 (CO_2 = 36%) and again the sum of the fractions is not 100% and the methane fraction is as low as observed in chapter 4.3.1. pH is not as dramatically decreased as for the last experiments with the high OLR of 10 g VS/L/day and is at the lower pH value after 2 weeks instead of 4 days.

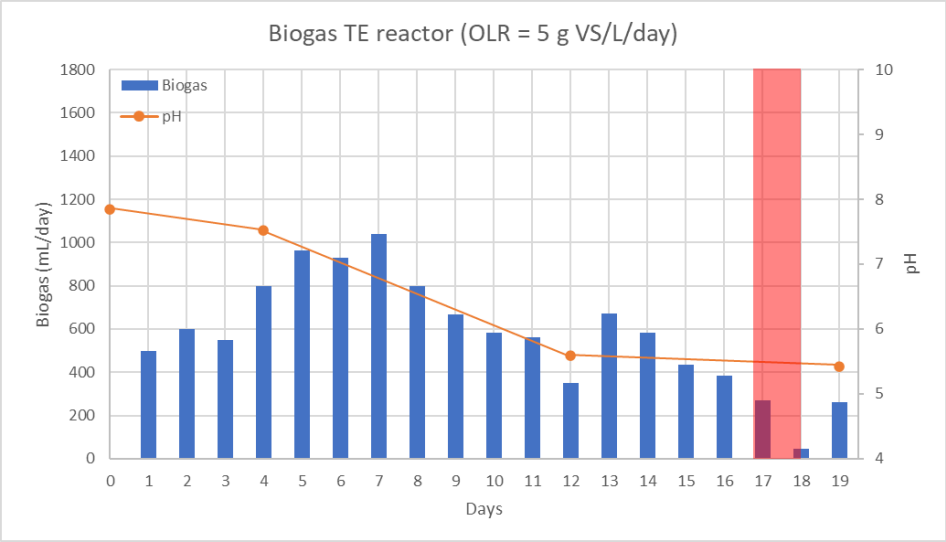

*Figure 17 biogas TE reactor (OLR = 5 g VS/L/day) *red box indicates mechanical problems*

Figure 18 gives more perspective on the AD of FW with TE supplementation. After 12 days the COD shows a constant of ± 65000 mg/L which indicates that the reactor can convert all incoming organic matter to biogas. However, the clay-like material (probably FW that is clumped together) could also be the reason for the constant COD. Also, the total VFA is decreasing which indicates that the methanogen and acetogens can handle the amount of VFA. This is majorly observed in the decrease of acetic acid within the reactor. However, for the HPLC diagram also another peak was observed after the peak for butyric acid. This could probably indicate that there was also produced some valeric acid. That is normal in a good working anaerobic digester [11]. Also, an increase in biogas is expected since the COD and total VFA are not increasing anymore. However, this is not observed yet which could be because the reactor probably has just changed to producing VFAs and the higher biogas rates will just start increasing. There is observed a small increase on day 19 compared to day 18 which could as be through the addition of FW that is working on day 19 and not on day 18.

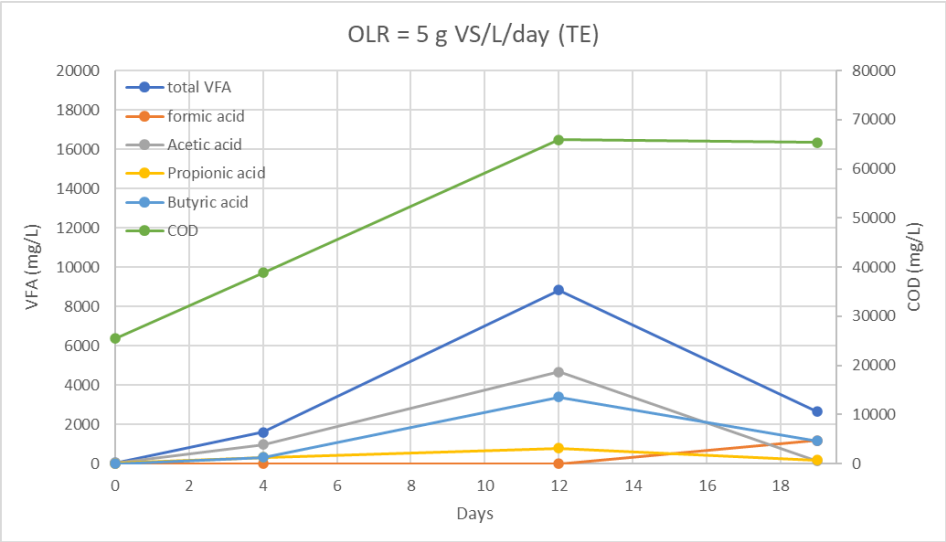

Figure 18 COD and VFA continuous TE reactor (OLR = 5 g VS/L/day)

In other papers, there was also first observed a peak in total VFA whereafter it decreases [23]. So it could be the start of a working AD with an OLR of 5 g VS/L/day. However, the biogas production does not match the decrease in total VFA and the constant COD.

What stands out for the reference reactor is that between days 7 and 12 there was no production of gas. The gas measuring device was not connected due to the probable occurrence of foaming. It may be assumed that the biogas decreases following the blue line. The biogas production fluctuates very similarly to the TE reactor with only a small decrease in biogas production. Methane fraction is also fluctuating again; 20% on day 4 (CO_2 = 74%), 27% on day 11 (CO_2 = 27%) and 21% on day 18 (CO_2 = 53%). Also in the same pattern as for the TE reactor. The decrease in pH is observed to be lower than that for the higher OLRs. However, the pH decreases continuously after 12 days and that is not the case for the TE reactor which was almost constant.

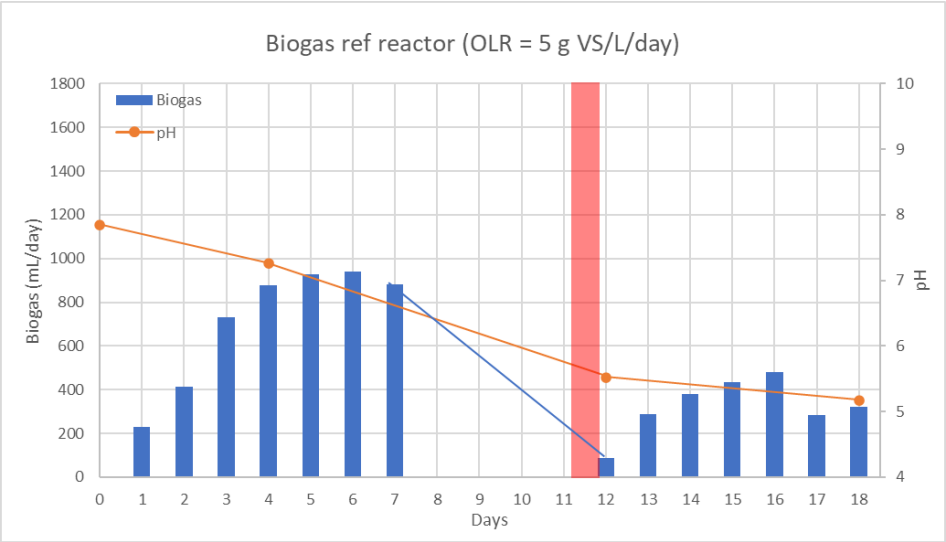

*Figure 19 biogas reference reactor (OLR = 5 g VS/L/day) *red box indicates mechanical problems*

The COD is still increasing after 12 days but not in a linear way as before. This indicates that the reactor cannot handle the amount of organic matter added, but seems to come to a maximum. However, the total VFA is still increasing linearly. Acetic acid is not rising linearly anymore but is still mostly available, which indicates that acetoclastic methanogens cannot handle the high loading. Eventually, the amount of butyric acid seems to overcome the concentrations of acetic acid which also happened for the higher OLR loading.

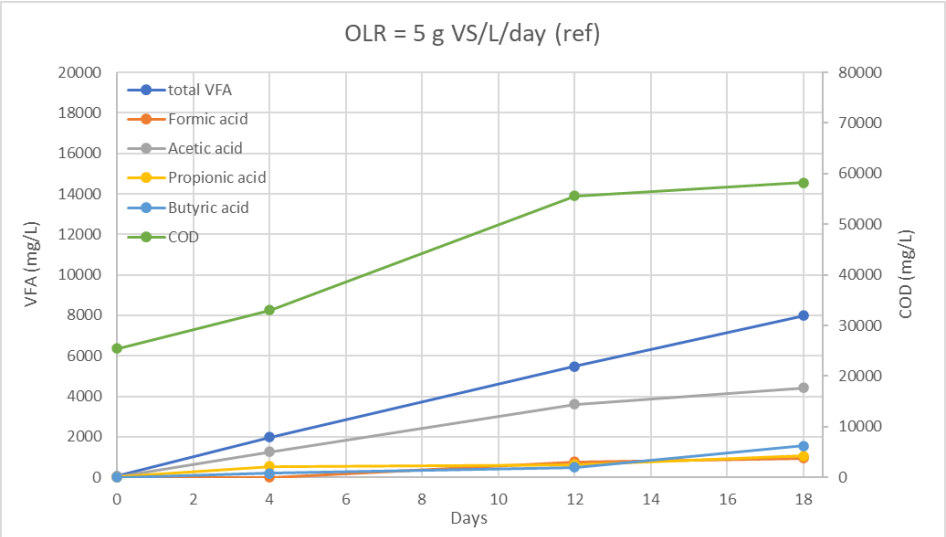

Figure 20 COD and VFA continuous reference reactor (OLR = 5 g VS/L/day)

4.3.3. General discussion of continuous reactor experiments with TE addition

Former research on the AD with high OLRs (5-6.6 g VS/L/day) low HRTs (15-38 days) and TE addition results in constant biogas production with a VMP of 2.2-3.2 m^3 CH_4/m^3/day [33], [15], [23]. However, experiments in this research result in process acidification within 3 weeks. A possible reason for this is that the OLR in other experiments is gradually increased while the HRT is kept constant. This results in time for the microorganisms to adapt to the substrate [11]. An example of this is shown in a report by "Banks, Zhang, Jiang & Heaven, 2012" where there is a gradual increase in OLR in 220 days to an OLR of 5 g VS/L/day (steps are 1.6, 2, 3 and 4 g VS/L/day). But also other reports use a slow build-up of OLR [23], [15]. However, the addition of TEs is necessary from the beginning since low OLRs of 2.19 g VS/L/day and HRT of 30 days also show inhibition over time [23]. Next to the gradual increase of OLR, the semicontinuous experiments in other reports are also in contrast to experiments in this report. The FW will be for multiple days at room temperature in this report. The possibility that the FW will change in characteristics is therefore possible. Other papers only describe semi-continuous or batch and do use cooled FW that is thawed at a max of 14 days before addition day [33], [15] and [23]. However, for these experiments, inhibition of the degraded FW cannot be conceived.

With the low HRT that is used in these experiments, the TAN cannot reach values that can be toxic for microorganisms. A HRT of 38 days and OLR of 5 g VS/L/day results in TAN up to 5000 mg/L which still works for long-term digestion of 500 days [33]. A possible increase in HRT will result in an even higher TAN but more time to convert the organic substrates into biogas since maximum BMP is not optimized (352 mL vs 439 mL CH_4/g VS) [23]. The addition of TEs are also positive for the reduction in TAN since there is more biological fixed nitrogen which results in more microbial biomass [33].

Biogas values below 100% are observed for GC analysis throughout the report, what they have in common is a low biogas production rate. Probably due to the lower rate of biogas, there is a chance for air to come into the gas bag during sampling. In other papers, there is also observed an amount of water contributed to the biogas and is not measured with the GC in this paper [28].

4.4. Economic analysis

Capital costs for building an AD plant are not implemented since there is already a plant available at the ENTEG. The operating of AD without TE addition is no option since it will result in digester failure over time.

Based on the results and literature used in chapter 4.3. there is chosen to make use of a VBP of 3.75 m^3 biogas/m^3/day (58% methane) obtained by a report of "Banks, Zhang, Jiang, & Heaven, 2012" with an OLR of 5 g VS/L/day and a HRT of 38 days. The characteristics of AD are given in chapter 3.4. These process settings will result in total production of 13687.5 m^3 of biogas per year and total methane production of 7938.75 m^3. To come to these production rates an amount of 18,250 kg of VS is needed on a yearly bases, which comes down to an additional approximate addition of 50 tons of FW per year.

4.4.1 Operating costs AD with TE addition

First, an overview of the operating costs of the AD of FW to biogas will be given. Operating costs include costs associated with staff, insurance, transportation, annual licenses, pollution abatement and control, maintenance, chemicals, water and heat [25].

Transportation costs are the costs that are very dependent on the number of rides for transportation. In this overview, there is assumed that there is needed a ride every month because of the degradation of FW before it even enters the reactor. The distance from Europa park to Zernike is 10 km and a truck cost approximately 2.60 €/km [43]. This brings the transportation costs to €624 per year. Assumed is that the transportation of the digestate is compensated with gate fees since normally companies get money out of processing waste (in this case the FW) [25].

For TE supplementation, the addition of TE used in our report (Table 4) are have been used with the same compounds used. The total costs for TEs are €628 [44], [45], [46].

Since the FW needs to be diluted an amount of 51 m^3 of water has to be added to the FW to come to the process characteristics. The water price of drink water is used which is not necessary to use €0.87/m^3 [47].

For the heat, there has been made use of the specific heat of water which is 4.2 J/g/°C and a temperature rise from 25 C° to 37 C°. With the assumption that FW has the same specific heat of water, an energy loss of 50% by tank isolation and an energy transfer effectivity of 58% [32],. If all these assumptions are taken 17.4 GJ/year is needed with a price of €1.132 per m^3 natural gas (bare gas price wholesale market, ICE Endex) this gives a total of €560 [48], [49].

Fixed operating costs that contain costs associated with staff, insurance, annual licenses, pollution abatement and control, annual licenses and maintenance are not yet taken into this report.

Table 7 total operating costs AD (TE supplementation)

	Cost Annual (EUR)
Transportation FW	624
TE supplementation	628
Water supplementation	44
Heat	560
Total operating costs:	**1856**

4.4.2. Biogas upgrading

For calculations, there have been made use of values that are normally applied for bigger installations. The costs are therefore lower than probably expected.

4.4.2.1. Heat and electricity

First, the upgrading of biogas to heat and electricity by CHP is considered. The used CHP unit has a conversion efficiency of 38% for electricity and 55% for heat [25], [50]. For the determination of the initial investment costs, there have been made use of the installation costs given of 400 €/ kWe [50]. With a potential of 5.77 kWh/m^3 of biogas, this results in 9.0 kW and eventually in initial investment costs of €4218 [51]. The economic lifetime is based on other machines and is estimated at 20 years [2].

For the revenue, there has made use an of electricity price of 0.17 €/kWh, which results in total revenue of electricity of €5040 [52]. However, thermal energy is not (yet) considered as an extra revenue while this is still an energy amount of 142 GJ/year. Due to the heat produced the energy costs for heating the AD are omitted. Operating costs are assumed to be 2.5% of the investment costs and are mainly maintenance costs.

4.4.2.2. Green gas

Green gas can be obtained by different methods discussed; PSA, MS, WS and CA. Based on an economical review by "Ardolino, et al., 2021" there are made some calculations regarding the upgrading of biogas. In that review, biogas was generated with 51% CH_4 and 46% of CO_2 through different upgrading methods to green gas. The functional unit of the report was 500 m^3 biogas/h, a huge difference compared to the 1.6 m^3 biogas/h used in this report. This is probably the main reason why the operating costs and investment costs are low [28].

The investment costs of the different methodologies are based on the total investment costs for an installation dived by the gas production of that plant (500 m^3 biogas/h) multiplied by the gas production of this plant (1.6 m^3 biogas/h) and are also used in the report itself. The investment costs for MS, WS, CA and PSA are €1,200,000; €1,700,000; €1,500,000 and €1,150,000. An economic lifetime of 20 years is expected. The same is applied to operating costs that contain maintenance, consumption and energy costs. [2].

De price of green gas is determined by determining the gas price per calorific heat value [28]. Comparing the lower heating values (LHV) obtained by the different methods and assuming that the price of gas is per potential energy of the gas. The price of natural gas is €1.132 /m^3 with a LHV of 31.65 MJ/m^3 [53]. The LHV of the green gas is between 34.89 and 35.32 MJ/m^3 and is assumed to have a price between €1.25 and €1.26 per m^3.

4.4.2.3. proteins

Proteins can be produced by ammonia and biogas generated by the AD of FW. This process is done in multiple steps since the input of these reactors includes H_2, CO_2 and NH_3. This case is compared with a report by "Oesterholt, et al., 2015" where this process is done by ammonia stripping, SMR, and PSA and is eventually converted in a reactor to proteins. The total protein production is limited by ammonia in the digestate. The total production of proteins is 2009 kg/year based on an efficiency of the stripper of 82% [32].

Starting with the ammonia that is produced by an ammonia recovery plant. In the economic review of this process, there is assumed a TAN concentration of 5000 mg/L in the digestate. Since the concentration of the TAN is high compared to that of WWTP (± 2000 mg/L) the price of the stripping process reduces from €2.60 per kg TAN to €1,30 per kg TAN (no residual heating assumed), resulting in operating costs €512 (Banks, Zhang, Jiang, & Heaven, 2012 [35]. Based on a production of 394 kg of proteins per year the investment costs of this ammonia stripper are €1817. A saving of €906 per year is assumed since the digestate does not need a purification process for ammonia anymore [32].

For PSA there are assumed operating costs of €0.06 per m^3 of biogas resulting in operating costs of €821 [35]. The Investments costs are already determined for green gas production and are €3594.

The highest production costs are due to the SMR of biogas since high amounts of electricity are needed. Based on the total biogas production of the AD in this report (13687.5 m^3/year) the total production of hydrogen by this SMR is 1929 kg per year with total production costs of €4990 per year. The investment costs are assumed to be ± €3000. There is also produced a surplus of H_2 334 kg/year which can be sold for ± €10 per kg H_2, resulting in an extra revenue of €3340 per year [54].

The reactor for the protein conversion itself is assumed to be €605 with total operational costs of €402 per year [35]. The pricing of proteins is estimated at €2000 per kg resulting in revenue from the proteins of €4018 [35]. However, the pricing of proteins is expected to raise when the product can also be consumed by humans.

4.4.2.4. General discussion upgrading economic analysis

In Table 8 an economic comparison between the different methods is given. Upgrading to green gas is the best option when focussed on the profit of the processes. There is no big difference between the methods of upgrading within the upgrading of biogas to green gas. The extra benefit of green gas is easy storage within the gas grid.

The lower profit of upgrading to heat and electricity by CHP is mainly because the heat that is produced is not taken into full account which results in a loss of heat. If this extra energy is also sold there will be an increase in revenue.

For the proteins, there is even observed a loss over time since this method is still in development. Expected is that when higher protein contents and its legalised the price of these single-cell proteins can go up by an approximate factor of 5, resulting in a larger revenue [35]. The hydrogen that is now sold is also a big part of the revenue yet, a focus on the conversion of hydrogen would also be of interest. The processing costs are high compared to the other processes which are mainly due to the multiple processes that are included in upgrading to proteins. Since the CO_2 produced by AD is also needed in the conversion to proteins a focus on the potential of SMR without PSA in front could be interesting since it will reduce investment and operating costs.

Table 8 economical comparison between different methods for upgrading biogas

	Unit	*Heat and electricity*	*Green gas*				*Proteins*
Method	-	CHP	PSA	MS	WS	CA	PSA, air stripping, SMR and SCP-reactor
Initial investment costs	€	4218	3594	3750	5313	4687	9016
Economic lifetime	Year	20	20	20	20	20	20
Pay-off	€/year	211	180	188	266	234	451
Operating costs	€/year	105	560	750	680	545	6725
Operating costs AD	€/year	1296	1856	1856	1856	1856	1856
Total revenue	€/year	5040	8738	8848	8775	8905	8264
Profit	€/year	**3428**	**6142**	**6054**	**5973**	**6270**	**-768**

5. Conclusions and follow-up research

This report is a step to put the pilot-plant reactors at the ENTEG building in Groningen into operation. The conclusions and follow-up research are separated into two chapters to maintain the clarity of the report and make it easy to continue with this research.

5.1. Conclusions

From the experiments in this report, it can be concluded that for batch experiments an S/X ratio up to 0.89 g VS_{FW}/ $VS_{reactor}$ will not result in reactor acidification after the reactor is already adapted. However, digester foaming can occur which can be fixed by the addition of an anti-foaming agent. Experiments with the addition of FW by syringe pumps are mechanically impossible. TE addition to a reactor with an OLR of 5 g VS/L/day and a HRT of 24 days has some effect on the degradation of total VFA. However, a reactor that gradually increases in organic loading seems the only way to overcome fast digester acidification.

Upgrading of biogas in this report is reviewed for the upgrading to heat and electricity (by CHP), green gas (by PSA, MS, WS and CA) and proteins. From the economic analysis, it can be concluded that upgrading to green gas is the most profitable yet. The ease of green gas into the grid is an extra benefit of producing this biogas.

So this report concludes that upgrading FW to a green gas is the most efficient energy conversion technology based on an AD with TE addition, an OLR of 5 g VS/L/day and a HRT of 24 days.

5.2. Follow-up research

For follow-up research, there should be a focus on the operation of the pilot-plant reactor at the ENTEG building in Groningen.

For upgrading to pilot-plant scale processing, The parameters for an OLR of 5 g VS/L/day and a HRT between 24-38 days with the addition of TEs are recommended (Table 4). However, the organic loading should be gradually increased to overcome acidification at an early stage observed in chapter 4.3. (e.g. start with an OLR of 1 g VS/L/day and after a week start with an OLR of 2 g VS/L/day with keeping the HRT constant). The reactor can be controlled by maintaining the pH, T, FOS/TAC and VFA values in a save range.

The upgrading of biogas could be broadened by researching biogas conversion to hydrogen. Also, a focus on the usage of heat produced by CHP within the Zernike campus can be researched. Besides, an agreement with the “N.V. Nederlandse Gasunie” should be considered if the green gas is pumped into the gas grid.

Further recommendations for laboratory experiments are to lower TE-supplementation costs by checking the feasibility of TE-rich co-digestion material (see also chapter 2.2.4.). Measure Valeric acid since this can also be available in the amount of 2-30% (observed for the last experiment with FW) [11]. Test microorganism adaption to propionate by doing a continuous test with the addition of propionate. Build two reactors, one with the addition of 100 mg/L/day from the beginning on and one with the gradual increase to this concertation (so, 20 mg/L/day, 50 mg/L/day, 100 mg/L/day). Always use a blank reactor for testing since experiments are very substrate and inoculum dependent.

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Appendix A TE element addition

Table 9 characteristics of TEs in AD

Element	*Function*	*Source*
Fe	Cofactor of Carbon monoxide dehydrogenase (CODH); reduce H_2S concentration in biogas (detoxifying); widely needed for a lot of enzymes.	[5]; [23]
Co	Cofactor of CODH; an essential element for SAOB and hydrogenotrophic methanogens	[5]
Mo	Enhance reactor performance in combination with CO and Ni	[5]
Ni	Cofactor of methyltransferases, CODH and many hydrogenases; aceticlastic methanogenesis and acetogenic microorganisms	[5]; [23]
Se	Essential for propionate oxidation and syntropic hydrogenotrophic methanogenesis; an essential element for SAOB and hydrogenotrophic methanogens	[5]; [33]
W	-	
Zn	-	

Table 10 addition of TE in other reports

	Units	*[15]*	*[33][a]*	*[23]*
OLR	g VS/L/day	4-6	2-5	2.19-6.64
HRT	days	20-15	95-38	30-20
Fe	mg/L	100	5	100
Co	mg/L	1	1	2
Mo	mg/L	5	0,2	5
Ni	mg/L	5	1	10
Se	mg/L	-	0.2	-
W	mg/L	-	0.2	-

[a]Only initial addition of TEs, further it was based on the added amount of VS

Table 11 addition of TE elements for OLR = 10 g VS/L/day

Element	*Compound used*	*Element concentration (mg/L) (27-07-2022)*	*Element concentration (mg/L) (09-08-2022)*
Iron (Fe)	$FeCl_2*4H_2O$	250.5	248.6
Cobalt (Co)	$CoCl_2*6H_2O$	2.5	2.5
Selenium (Se)	Na_2SeO_3	1.0	1.0
Tungsten (W)	$Na_2WO_4*H_2O$	1.0	1.0
Nickel (Ni)	$NiCl_2*6H_2O$	12.4	12.3
Molybdenum (Mo)	$(NH_4)_6Mo_7O_{24}*4H_2O$	5.0	5.0

Table 12 addition of TE elements for OLR = 5 g VS/L/day

Element	*Compound used*	*Element concentration (mg/L) (09-01-2022)*
Iron (Fe)	$FeCl_2*4H_2O$	125.3
Cobalt (Co)	$CoCl_2*6H_2O$	1.3
Selenium (Se)	Na_2SeO_3	0.5
Tungsten (W)	$Na_2WO_4*H_2O$	0.5
Nickel (Ni)	$NiCl_2*6H_2O$	6.2
Molybdenum (Mo)	$(NH_4)_6Mo_7O_{24}*4H_2O$	2.5

The selection of compounds is based on a report by “C. Banks, Y. Zhang, Y. Jiang and S. Heaven, 2012”.

Appendix B protocol tests reactor sludge batch reactor

In this appendix the protocol for testing the reactor sludge is given which gives structure and reproducibility to the experiments, all samples are tested in triplicate.

Take 3 samples of 30 mL of reactor liquid out of the reactor and the fourth sample of 25 mL. Then put back the substrate with tap water. The food waste first needs to be blended, this can also be done with the water already added. And also add the fourth sample back.

VS, TS and ash content (following APHA standard methods [37])

- Put three small ceramic bowls in the 550 °C oven for 15 minutes and let them cool down to room temperature (first to below 200 °C than in the desiccator).
- Weigh and put 3 samples of +- 150 mg in the trays (shake the samples before) and dry in the 105 °C oven for 12+ hours. After this let it cool down in the desiccator to room temperature
- Weigh samples and put them in the 550 °C oven for 4 hours and let it cool down to room temperature afterwards (first to below 200 °C than in the desiccator).
- Weigh the samples for the last time (for weighing it is important to close the desiccator between the measurements and note the weight asap).

pH-meter

- Measure the pH of the three different samples that were taken with a VOS-70002 (calibration before is preferred).

Centrifugation samples

- Take from every Greiner tube a sample of +- 12 mL and put it in a 15 mL tube (weight accurate).
- Put the samples in the centrifuge at 12000 rpm, 30 minutes and 4 °C.
- From each of these centrifugated samples, 1.5 mL can be filtrated over a 45 µm filter with help of a 1 mL syringe.

COD

- In the meantime, the centrifugation of three samples for the COD measurement can be prepared.
- For this make use of a beaker with a magnetic stirrer and dilute to an expected value of +- 5000 mg/L with distilled water.
- Do the rest of the test following the protocol of test kit LCK514 (Hach).

Ammonia

- The filtrated sample can be used for ammonia testing.
- Since the expected value of ammonia is ± 2000 mg/L the dilution factor for the LCK 503 (Hach, US) test kit is 50. This is reached by adding 0.5 mL of the sample with a pipette and 24.5 mL of deionized water by a 50 mL measuring cylinder.
- This is mixed with a magnetic stirrer in a 50 mL beaker.
- Do the rest of the test following protocol.

FOS/TAC

- Calibrate when the instrument gives the reminder to do so (AT1102 - Titralab AT1000-titrator (Hach, US)).
- Use the centrifugated but not filtrated sample for this and do it following protocol (make sure the sample is at room temperature

Appendix C protocol tests reactor sludge continuous reactor

In this appendix the protocol for testing the reactor sludge is given which gives structure and reproducibility to the experiments, all samples are tested in triplicate.

Defrost the samples from the freezer in a water bath for 100 minutes. Then shake the tubes.

VS, TS and ash content (following APHA standard methods [37])

- Put three small ceramic bowls in the oven, wait till it is 550 °C and then let it be at this T for 15 minutes. Open the oven and let it cool down for 40 minutes. Then put it for 20 minutes in the desiccator till room temperature is achieved.
- Weigh and put samples of +- 150 mg in the trays (shake the samples before) and dry them in the 105 °C oven for 12+ hours. After this let it cool down in the desiccator for 20 minutes to room temperature
- Weigh samples and put bowls in the oven wait till it is 550 °C and then let it be at this T for 4 hours. Open the oven and let it cool down for 40 minutes. Then put it for 20 minutes in the desiccator till room temperature is achieved. Weigh the samples for the last time (close the desiccator between the measurements and note the weight asap).

pH-meter

- Measure the pH of the three different samples that were taken with a VOS-70002 (calibration before is preferred).

Centrifugate samples

- Take from every Greiner tube a sample of +- 5 mL and put it in a 15 mL tube (weight accurate).
- Put the samples in the centrifuge at 12000 rpm, 30 minutes and 4 °C.
- From each of these centrifugated samples, 1.5 mL can be filtrated over a 45 µm filter with help of a 1 mL syringe.

COD

- In the meantime, the centrifugation samples for the COD measurement can be prepared.
- For this make use of a beaker with a magnetic stirrer and dilute to an expected value of +- 5000 mg/L with distilled water.
- Do the rest of the test following the protocol of test kit LCK514 (Hach).

Ammonia

- The filtrated sample can be used for ammonia testing.
- Since the expected value of ammonia is ± 2000 mg/L the dilution factor for the LCK 503 (Hach, US) test kit is 50. This is reached by adding 0.5 mL of the sample with a pipette and 24.5 mL of deionized water by in a 50 mL measuring cylinder.
- This is mixed with a magnetic stirrer in a 50 mL beaker.
- Do the rest of the test following protocol.

HPLC

- Do a HPLC measurement with the use of the stock solutions.

Appendix D reactor values batch experiments

Parameters	*Units*	*Reactor sludge (28-03-2022)*	*Reactor sludge (04-04-2022)*	*Reactor sludge (19-04-2022)*	*Reactor sludge (15-06-2022)*	*Reactor sludge (23-06-2022)*
Total solids (TS)	%	3.11 ± 0,47	2.77 ± 0.01	3.21 ± 0.20	2.56 ± 0.08	3.34 ± 0.39
Volatile Solids (VS)	%	1.45 ± 0.54	2.69 ± 0.15	2.34 ± 0.16	1.25 ± 0.16	3.03 ± 0.66
VS/TS	%	45.66 ± 12.11	96.97± 5.25	72.99 ± 3.62	48.92 ± 5.55	90.01 ± 8.75
COD	mg/L	38435 ± 485[a]	28888 ± 809	27823 ± 1753	26445 ± 1622	39197 ± 4071
FOS/TAC		0.117 ± 0.013[a]	0.104 ± 0.007	0.114 ± 0.015	0.108 ± 0.012	1.117 ± 0.044
TAN	mg/L	2306 ± 97[a]	2153 ± 107	2234 ± 164	2535 ± 997	2216 ± 351
FAN	mg/L	329 ± 14[a]	114 ± 6	90 ± 7	134 ± 53	59 ± 9
pH	-	8.08 ± 0.04[a]	7.63 ± 0.02	7.51 ± 0.01	7.63 ± 0.05	7.33 ± 0.07
Formic acid	mg/L	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Acetic acid	mg/L	0 ± 0	0 ± 0	0 ± 0	0 ± 0	3657 ± 56
Propionic acid	mg/L	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1483 ± 30
Butyric acid	mg/L	0 ± 0	0 ± 0	0 ± 0	0 ± 0	609 ± 50

Appendix E reactor values continuous experiments (syringe)

In the graphs below the whole runs with diluted FW with an OLR of 1.36 g VS/L/day are shown.

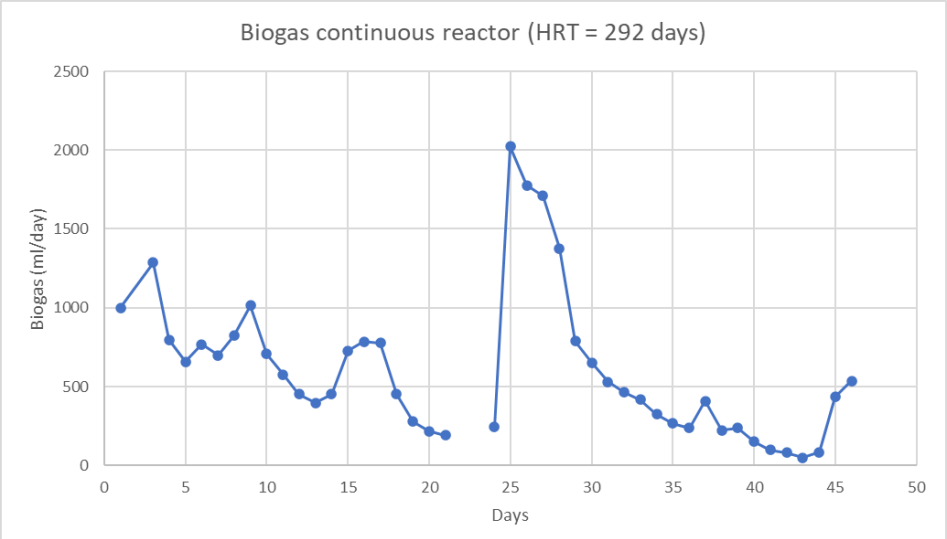

Figure 21 total biogas production continuous experiment (HRT = 292 days)

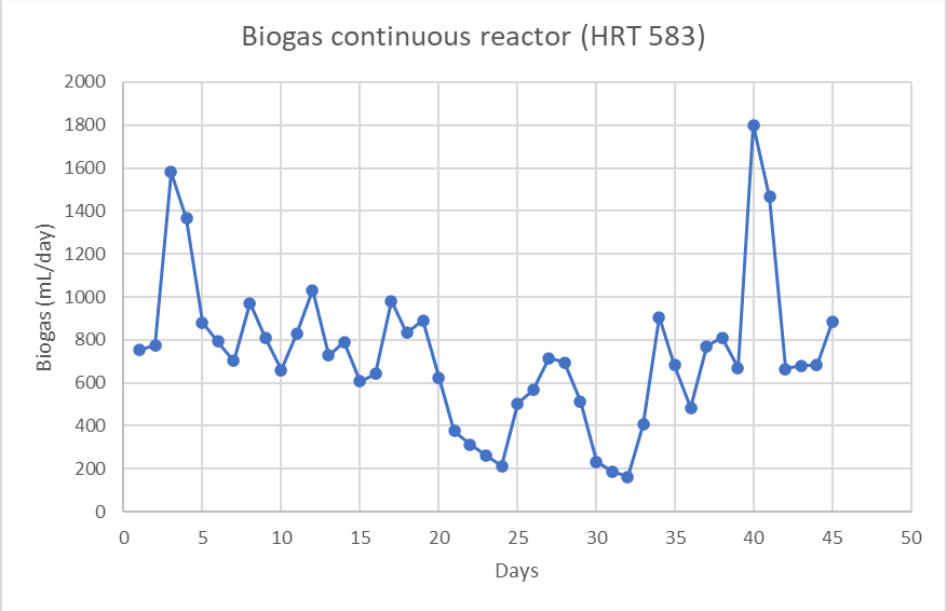

Figure 22 total biogas production continuous experiment (HRT = 583 days)

Appendix F reactor values continuous experiments (TE addition)

Table 13 reactor sludge values TE reactor (OLR 10 g VS/L/day)

Parameters	Units	Anaerobic sludge Garmerwolde (27-07-2022)	Day 5	Day 12	Day 19
Total solids (TS)	%	1.84 ± 0.34[d]	1.86 ± 0.41	2.38 ± 0.27	3.77 ±0.14
Volatile Solids (VS)	%	1.30 ± 0.16[d]	1.71 ± 0.45	2.28 ± 0.15	3.12 ± 0.19
VS/TS	%	71.19 ± 4.38[d]	91.97 ± 9.90	96.24 ± 5.31	82.72 ± 2.59
COD	mg/L	25460 ± 4257[d]	32735 ± 4109	56567 ± 5147	67168 ± 5608
TAN	mg/L	1837 ± 161	1870 ± 50[b]	1811 ± 34[a]	1761 ± 10[b]
FAN	mg/L	158 ± 14	0 ± 0[b]	0 ± 0[a]	0 ± 0[b]
pH	-	7.85	5.32 ± 0.03	5.15 ± 0.00	4.88 ± 0.01
Formic acid	mg/L	0 ± 0	92 ± 7	0 ± 0	0 ± 0
Acetic acid	mg/L	33 ± 46	5523 ± 125[c]	5079 ± 43	5700 ± 84[c]
Propionic acid	mg/L	21 ± 30	782 ± 48	663± 41	1237 ± 62
Butyric acid	mg/L	0 ± 0	2549 ± 129	5269 ± 64	7236 ±73

[a] For the second sample the double amount was added accidentaly, divided by two to get the correct value.
[b] Don't use one the three samples since there was spilled some reactor sludge.
[c] Take for stock solution the retention time of the one of that day but the area from the measurement 1 week later with the same stock solution (forgot to shake after preparing the solution)
[d] Skip the second measurement for the TS,VS and ash since it gives negative results. Also skip the COD since it gives low values that do not fit in the other two measurements.

Table 14 reactor sludge values reference reactor (OLR 10 g VS/L/day)

Parameters	Units	Anaerobic sludge Garmerwolde (27-07-2022)	Day 4	Day `10
Total solids (TS)	%	1.84 ± 0.34[a]	4.02 ± 0.25	3.23 ± 0.31
Volatile Solids (VS)	%	1.30 ± 0.16[a]	2.66 ± 0.24	2.03 ± 0.19
VS/TS	%	71.19 ± 4.38[a]	66.03 ± 1.81	62.78 ± 2.42
COD	mg/L	25460 ± 4257[a]	46018 ± 1293	34004 ± 1075
TAN	mg/L	1837 ± 161	2139 ± 39	1968 ± 375
FAN	mg/L	158 ± 14	32 ± 1	177 ± 34
pH	-	7.85	7.08 ± 0.08	7.87 ± 0.04
Formic acid	mg/L	0 ± 0	0 ± 0	0 ± 0
Acetic acid	mg/L	33 ± 46	4954 ± 331	536 ± 71
Propionic acid	mg/L	21 ± 30	707 ± 173	224 ± 46
Butyric acid	mg/L	0 ± 0	270 ± 119	53 ± 9

[a] Skip the second measurement for the TS,VS and ash since it gives negative results. Also skip the COD since it gives low values that do not fit in the other two measurements.

Table 15 reactor sludge values reference reactor (OLR = 5 g VS/L/day)

Parameters	*Units*	*Anaerobic sludge Garmerwolde (27-07-2022)*	*4 days*	*12 days*	*18 days*
Total solids (TS)	%	1.8 ± 0.3[a]	4.65 ± 0.4	4.0 ± 0.3	3.9 ± 0.1
Volatile Solids (VS)	%	1.3 ± 0.2[a]	3.1 ± 0.1	2.8 ± 0.1	2.7 ± 0.1
VS/TS	%	71.2 ± 4.4[a]	66.5 ± 5.45	69.5 ± 6.5	69.3 ± 0.7
COD	mg/L	25460 ± 4257[a]	33019 ± 2178	55615 ± 4929	58206 ± 2488
TAN	mg/L	1837 ± 161	2000 ± 23	2088 ± 40	1968 ± 22
FAN	mg/L	158 ± 14	46 ± 1	1 ± 0	0 ± 0
pH	-	7.85	7.26 ± 0.12	5.52 ± 0.14	5.17 ± 0.01
Formic acid	mg/L	0 ± 0	0 ± 0	769 ± 441	939 ± 583[b]
Acetic acid	mg/L	33 ± 46	1260 ± 57	3598 ± 42	4422 ± 2580[b]
Propionic acid	mg/L	21 ± 30	526 ± 159	632 ± 148	1066 ± 567[b]
Butyric acid	mg/L	0 ± 0	191 ± 76	486 ± 46	1558 ± 2009[b]

[a] Skip the second measurement for the TS,VS and ash since it gives negative results. Also skip the COD since it gives low values that do not fit in the other two measurements.
[b] Peaks were shifted

Table 16 reactor sludge values TE reactor (OLR = 5 g VS/L/day)

Parameters	*Units*	*Anaerobic sludge Garmerwolde (27-07-2022)*	*4 days*	*12 days*	*19 days*
Total solids (TS)	%	1.8 ± 0.3[a]	5.2 ± 0.1	4.5 ± 0.4	4.5 ± 0.0
Volatile Solids (VS)	%	1.3 ± 0.2[a]	3.1 ± 0.1	3.3 ± 0.5	3.1 ± 0.1
VS/TS	%	71.2 ± 4.4[a]	59.9 ± 3.1	73.1 ± 7.1	69.7 ± 1.4
COD	mg/L	25460 ± 4257[a]	38869 ± 1478	65948 ± 5855	65409 ± 3648
TAN	mg/L	1837 ± 161	2058 ± 12	2036 ± 36	2031 ± 51
FAN	mg/L	158 ± 14	85 ± 1	1 ± 0	1 ± 0
pH	-	7.85	7.52 ± 0.11	5.59 ± 0.01	5.42 ± 0.01
Formic acid	mg/L	0 ± 0	0 ± 0	0 ± 0	1179 ± 50
Acetic acid	mg/L	33 ± 46	967 ± 306	4660 ± 221	129 ± 52
Propionic acid	mg/L	21 ± 30	315 ± 138	783 ± 283	187 ± 25
Butyric acid	mg/L	0 ± 0	313 ± 64	3395 ± 26	1151 ± 247

[a] Skip the second measurement for the TS,VS and ash since it gives negative results. Also skip the COD since it gives low values that do not fit in the other two measurements.