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Enhancing Anaerobic Digestion of Food Waste: Investigating the Effects of Additives on Biogas Production and System Stability

Final Report Research Project

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Abstract

Anaerobic digestion (AD) is a process where organic materials are converted into biogas, it offers a sustainable solution for waste management and renewable energy production. However, the efficiency is often limited by system instability and low gas yields. This study investigates the effects of two additives, ANDY 1 and ANDY 2, on the stability and performance of AD using food waste as a substrate. Multiple experiments were conducted using small reactor setups, where gas production, pH variations, and VFA concentrations were monitored. The results demonstrated that the addition of ANDY 2 led to a significant increase in biogas production, exceeding the control by over 200%. The pH and VFA measurements also indicated that the ANDY 2 additive had the potential to improve the stability of the system. The second set of experiments, in which only the ANDY 2 additive was used, aimed to identify the specific stage of digestion improved by the additive. It also determined whether the feed rate could be increased without causing system acidification. The results demonstrated that ANDY 2 enhanced the methanogenesis step. Furthermore, the ANDY 2 additive allowed for a higher feed rate than the control before acidification occurred. Additionally, these experiments validated previous findings, having nearly double the gas production compared to the control, further validating the results of the initial experiments. The findings of this study highlight the potential of ANDY 2 as a promising additive for enhancing AD performance, offering practical implications for industrial-scale biogas production and sustainable waste management.

Contents

List of abbreviations

- AD: Anaerobic digestion
- VFA: Volatile fatty acid
- OLR: Organic loading rate
- VS: Volatile solids
- HRT: Hydraulic retention time
- FAN: free ammonium nitrogen
- TAN: Total ammonium nitrogen
- TE: Trace elements
- BMP: Biochemical Methane potential
- HPLC: High-Performance Liquid Chromatography
- sCOD: Soluble chemical oxygen demand

1 Introduction

Nowadays, fossil fuels continue to dominate global energy consumption, accounting for more than 80% of global energy use, burning of fossil fuels continues despite the known negative impact on our environment and human health [1]. To address these issues, the European Union aims to achieve climate neutrality by 2050, aiming for net-zero greenhouse gas emissions. [2]. To achieve this target a significant increase in the adoption and development of renewable energy sources is required. Among renewable energy sources, biogas represents a promising alternative, contributing approximately 7% of the EU's total gas consumption [3]. By 2050 this number should have increased to 61% [4].
A promising approach for biogas production is the utilization of organic waste, such as food waste, as a substrate in AD [5]. In the ENTRANCE building in Groningen, a pilot plant has been built to produce biogas using food waste. The facility consists of two reactors, each with a volume of 5 m^3.

Current biogas production is limited by a low biogas yield per kg of food waste added and a restricted substrate feed rate, beyond which system instability becomes a risk [6]. The AD process can be enhanced by the addition of additives, which may lead to higher yields and improved stability. Furthermore, it is important to determine which stage of the AD process is improved by these additives. This information is valuable when utilizing a two-phase system, where the four stages of AD are carried out in two separate reactors [7]. Understanding which stage is improved helps to determine in which reactor the additive should be added. If no significant improvements are observed, alternative co-digestion strategies using additional substrates will be explored to enhance biogas production.

This study aims to develop a method for increasing biogas production from food waste in a single-phase anaerobic digester by enhancing biogas yield and increasing the feed rate through the use of additives. Experiments will be conducted to assess the viability of using additives. If no improvements are observed with the additive, co-digestion with alternative substrates will be considered as another potential approach to achieve the same goal. To achieve the goal and successfully conduct the research, the following research questions will be addressed:

- Can additives enhance biogas production in AD of food waste by improving the yield?
- Which stage of the digestion process benefits most from the additive?
- Can system stability be improved when using an additive?

To investigate these questions, two additives produced by Paques, a biotechnology company [8], will be tested in lab-scale experiments. First, a control experiment without additives will be conducted to establish a baseline for biogas yields. Following this, the two additives, ANDY 1 and ANDY 2 will be tested under the same conditions to determine whether they actually enhance biogas production. This will serve as a go/no-go point—if no improvements are observed, co-digestion will be explored as an alternative method for increasing biogas production. However, if an improvement is noted, subsequent experiments will assess the specific phase of digestion that benefits from the best additive and evaluate the potential for increasing the feed rate.

2 Literature review

This chapter provides an overview of the essential information needed to understand the research. It begins with a description of AD, followed by a review of key parameters. Next, it covers food waste, alternative substrates, and commonly used additives. Lastly, it discusses microorganisms, single- and two-stage AD systems, foaming, and cost considerations.

2.1 Anaerobic digestion (AD)

AD is a biological process that breaks down complex organic matter into simpler chemical compounds without the presence of oxygen, resulting in the production of biogas. This biogas consists mainly of methane (CH_4), approximately 62. 5%, and carbon dioxide (CO_2), around 37.5% [9]. Besides biogas a solid digestate is formed, this is a waste product but it has applications in agriculture. The AD process consists of four different stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Each stage utilizes various types of bacteria, each playing a crucial role in the breakdown of organic material (see Figure 16) [5].

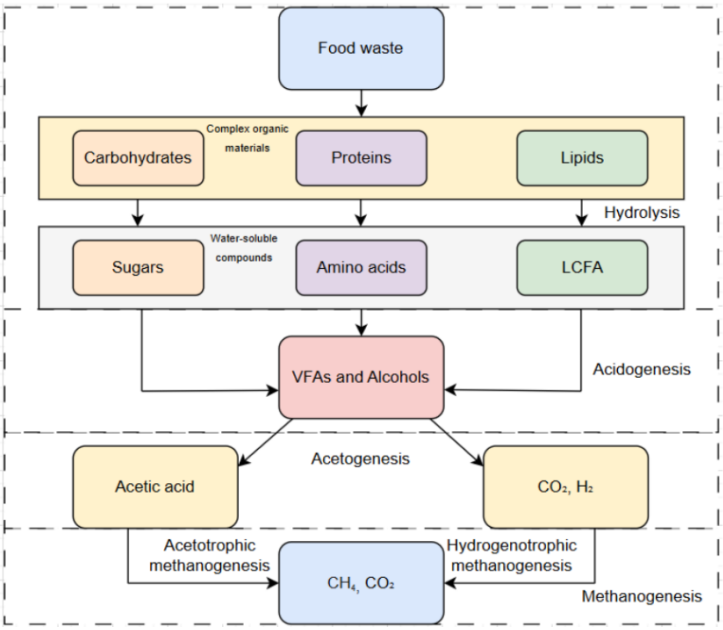

Figure 1: The AD pathway illustrating what happens in each stage of digestion [5]

Hydrolysis
The hydrolysis phase is the first stage in AD, here complex organic materials are broken down into smaller, water-soluble compounds. Carbohydrates, proteins, and fats are converted into soluble sugars, amino acids, and fatty acids, respectively. If the substrate consists of carbohydrates, hydrolysis can occur within hours, while protein and lipid-rich substrates take several days. When lignin or lignocellulose is present, the process can take even longer, and complete digestion may not occur [10]. It is important to note that hydrolysis is a relatively slow stage, and can therefore be the rate-limiting step in AD. The optimal pH for the microorganisms in this stage is 5.5 - 6 making it the lowest out of the 4 stages [5].

Acidogenesis
The next stage, acidogenesis, involves the further breakdown of the soluble compounds produced by the hydrolysis stage. The main products are volatile faty acids (VFAs) such as propionic, butyric, and acetic acids, as well as alcohols, CO_2, H_2, and NH_3[11]. Acidogenic bacteria function optimally at a pH range

from 6 to 7.[5]. VFAs are mainly produced at pH values bigger than 5, while alcohols are being produced when the pH is lower than 5 [12].

Acetogenesis
Acetogenesis is also called the dehydrogenation phase, through the oxidation of VFAs and alcohols, they produce more acetic acid, H_2, CO_2 [10]. dehydrogenation refers to this process, and during acetogenesis, VFAs and alcohols undergo oxidation, leading to the release of molecular H_2. For example, in the conversion of ethanol to acitic acid, ethanol reacts with water to form acetic acid, a proton, and hydrogen gas. Similarly, in the conversion of propionic acid to acitic acid, propionate reacts with water to produce acetate, bicarbonate, a proton, and hydrogen gas. These reactions demonstrate how hydrogen atoms are removed from organic compounds and released as H_2 gas [12]. The optimal pH for acetogenic bacteria is similar to that of the acidogenesis phase, typically ranging from 6 to 7 [5].

Methanogenesis
Methanogenesis is the last stage of anaerobic digestion and often the cause of system failure. Methanogenic bacteria are highly sensitive to changes in environmental conditions. Slight changes in, substrate type, temperature, pH, and feeding rate can significantly impact their activity. Overloading the digester, temperature fluctuations greater than 3°C, or the presence of excessive oxygen can disrupt the process and potentially halt methane production due to the vulnerability of methanogenic bacteria [10]. The sensitivity of the bacteria involved in the process makes the methanogenesis step a potential rate-limiting step [13]. The bacteria perform optimally at a higher pH, thriving within a pH range of 6.5 to 7.5 [5].

2.2 Critical parameters

In AD for biogas production, numerous parameters can potentially impact the system. These factors influence process efficiency, stability, and biogas yield. A thorough understanding of these parameters is essential for ensuring a successful and optimized process [14].

Organic loading rate (OLR)
The organic loading rate (OLR) represents the amount of volatile solids (VS) fed into the digester per unit volume per day. It plays a crucial role in the stability and performance of AD. Increasing the OLR can enhance biogas production up to a certain threshold, after this imbalances in the AD may occur, leading to process instability. Excessively high OLRs can cause irreversible acidification and system failure. In general, single-stage AD systems remain stable at OLRs up to 4.0 kg VS/m^3/day, while OLRs exceeding 5.0 kg VS/m^3/day often result in instability due to acidification [6].

Temperature
Temperature is one of the most important parameters in AD, it significantly affects microbial activity and methane production. AD can occur under psychrophilic, mesophilic, or thermophilic conditions, with mesophilic (30–40°C) and thermophilic (50–60°C) ranges being the most commonly used. Thermophilic digestion, while offering faster microbial activity and better pathogen destruction, is more sensitive to environmental conditions and requires higher energy inputs. Mesophilic conditions, on the other hand, provide more stable operation and are generally more energy-efficient for long-term continuous processes [15]. When comparing thermophilic and mesophilic digestion of food waste, mesophilic conditions show better system stability, mainly due to lower VFA concentrations. Although thermophilic digestion yields more biogas per kilogram of substrate, its lower OLR results in reduced overall biogas production. Therefore, mesophilic conditions are generally more suitable for food waste digestion [16].

pH
The pH is closely linked to the VFA accumulation and they play a crucial role in the performance and stability of AD systems. The optimal pH varies across different stages of the process[5]. VFAs are produced during the acidogenesis and acetogenesis phase [10]. At higher OLR, VFAs continue to accumulate. When VFA concentrations become too high and the pH drops below 6.5, methanogenesis

is inhibited which ultimately leads to system failure [5]. In contrast, at moderate OLR levels, VFAs remained stable, indicating better process control [17]. In AD Two natural buffering systems typically exist in digesters, helping to maintain pH within the neutral range. One is the ammonia/ammonium equilibrium, which will be discussed in more detail later in this chapter. The other is the naturally occurring carbonic acid/bicarbonate/carbonate equilibrium, shown below[6]:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \rightleftharpoons CO_3^{2-} + 2H^+$$

Retention time

Retention time refers to the time required for the complete degradation of organic matter within a digester or the duration that organic matter remains in the system. there is hydraulic retention time (HRT), which represents the average time the liquid substrate stays in the reactor, and solid retention time (SRT), which indicates how long the microbial biomass remains in the reactor [18]. A longer retention time allows for more complete degradation of organic material, leading to higher VS reduction and greater process stability. However, it also requires a larger digester volume and higher investment costs. On the other hand, shorter retention times reduce digester size and cost but may result in incomplete digestion and lower biogas yields. The optimal retention time depends on factors such as OLR, feedstock composition, temperature, and digester type, typically ranging from 10 to 60 days [5] [6]. Lower OLRs with longer HRTs can reduce buffer capacity and slow down biogas production, while higher OLRs with shorter HRTs may lead to biomass washout and system instability [7]. The HRT is determined by the ratio of the reactor's total volume to the volume of feed added per day [19].

C/N ratio

The C/N ratio reflects the balance between the amount of carbon and nitrogen in the substrate. An optimal C/N ratio is vital for a successful AD process [15]. The C/N ratio not only maintains a favorable environment but also ensures a proper nutrient balance for microbial growth. If the nitrogen content in the substrate is too low, the microbial population may increase slowly, leading to longer degradation times and reduced CH_4 yield. On the other hand, excessive nitrogen can lead to ammonia inhibition, hindering microbial growth and disrupting the process [5]. Microorganisms consume carbon roughly 25-30 times faster than nitrogen, making the ideal C/N ratio approximately 25-30:1 [20].

Ammonia (NH_3)

Ammonia plays a crucial role in AD. The biological breakdown of nitrogen-rich organic compounds, including proteins and urea, leads to the accumulation of total ammonium nitrogen (TAN), which exists as free ammonium nitrogen (NH_3) (FAN) and ionized ammonium nitrogen (NH_4+). The balance between these forms is influenced by pH, OLR and temperature during AD. At the right concentrations, ammonium nitrogen serves as a nutrient and provides buffering capacity for AD systems [21] [15]. Although NH_3 helps to buffer acidification by increasing pH and alkalinity, studies have shown that biogas production at pH 7 is 41.3% higher than at pH 8 [22] [23]. The following equilibrium reaction occurs shifting to the left at higher pH values and temperatures [6]:

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

Excessive ammonia concentrations can inhibit microbial activity and potentially cause AD failure. AD operates effectively at TAN concentrations between 600 mg/L and 800 mg/L. Higher TAN levels can suppress methanogenic bacteria and reduce biogas production [21]. Even more concerning, FAN acts as a strong inhibitor in anaerobic digestion when exceeding threshold concentrations [24], especially at elevated pH and temperatures. Since free ammonia can diffuse across the cell membrane, it disrupts cellular function by interfering with potassium and proton balance within the cell. Ammonia inhibition can be managed by controlling the OLR, co-digesting with low-nitrogen substrates, or adjusting the pH to maintain optimal conditions for methanogenic bacteria [15].

Nutrients and trace elements (TE)

Nutrients and trace elements (TE) such as phosphorus, sulfur, and metals like iron, cobalt, and zinc are essential for maintaining microbial health. A shortage of these nutrients can limit microbial activity,

particularly for methanogenic bacteria, while excessive concentrations can lead to toxicity. Proper supplementation of TE is crucial to maintain a stable microbial population [6].

Moisture content
The moisture content of the feedstock is an important factor in wet AD systems. Food waste typically has a high moisture content (60–90%), which helps microbial activity and mass transfer. However, maintaining the right balance between solid and liquid fractions is essential to avoid overloading the system [6].

Particle size
The size of the substrate particles affects the AD, smaller sizes increase the available surface area for enzyme activity, enhancing the degradation process and improving biogas production. Size reduction benefits the AD process by increasing biogas yields and reducing the digestion time. Particle size reduction is a commonly recommended pre-treatment for AD. However, excessive size reduction can lead to VFA accumulation and reduced methane yields. In solid-state AD, small particles can cause foaming and process failure, making it important to select particle size based on the digester type to ensure successful digestion [6].

Mixing
Mixing in digesters helps to provide microorganisms with the necessary nutrients, prevent foaming, reduce temperature gradients, and eliminate floating and sinking layers. However, improper agitation can disrupt the balance between acetogenic and methanogenic microorganisms, damage microbial cells, and increase operational costs. Slow and controlled mixing can enhance biogas production [6].

2.3 Food waste

Food waste is a substrate that can vary vastly in composition depending on a number of factors. For example, the first is where the food waste is sampled from, but also the country of origin and season. Figure 2 displays the various types of food found at two different food waste collection locations. On average food waste has a biochemical methane potential (BMP) of 0.440–0.480 m^3/kg of VS [25]. Food waste as a feedstock offers several advantages, such as lower operational costs due to minimal collection and transportation needs, high biodegradability, and a high BMP [26] [27].

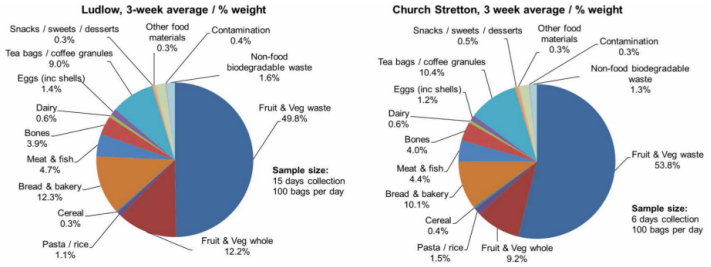

Figure 2: Food waste composition from two collection points over a 3 week period [28]

Food waste consists of around 25 percent of VS [29]. The VS primarily consisted of carbohydrates (111.7 g/L), proteins (32.9 g/L), and lipids (23.3 g/L). Compared to other substrates, food waste has lower concentrations of essential TEs, including cobalt, copper, iron, manganese, molybdenum, nickel, and zinc [30]. Food waste is high in VS and organic matter, which can make it prone to microbial instability. The rapid degradation of VS in food waste leads to quick acidification and the accumulation of VFAs. Low pH and high acidity inhibits methanogenic bacteria, reducing the overall digestion process. The high biodegradability of the organic matter in food waste limits AD to low OLRs, to avoid process instability [25] [29]. Food waste also has a high lipid content, largely due to the presence of animal fats and oils

from the cooking process. Lipids theoretically produce significantly higher methane yields compared to proteins and carbohydrates [31]. However, the breakdown of lipids can lead to long-chain fatty acids (LCFA), which inhibit microbial activity by forming oil flocs and binding to microbial cells, limiting the degradation of organic matter and reducing biogas production [25]. Lipids also have low solubility and density, leading to limited bioavailability and a tendency to float within the digester. As a result, their digestion is often associated with process instabilities, including foaming and sludge flotation.[32]. Food waste has a varying C/N ratio, with C/N ratios ranging from as low as 3.0 to as high as 54.9 [33]. The pH is typically around 5.5 [25].

The maximum OLR for single-stage wet AD of food waste is an important factor in determining the efficiency and stability of the process. On average single-stage wet AD systems typically operate effectively at OLRs ranging from 1 to 4 kg-VS/m^3·day, but with innovations in reactor design and operational strategies, OLRs can be increased significantly. A single-stage reactor with a working volume of 3000 mL was maintained at an average mesophilic temperature of 37 ± 1 °C. Mixing was performed using a blade-operated system running at 60 rpm in cycles of 1 minute on and 10 minutes off. In this reactor, stable biogas production and VS reduction were observed at an OLR of 9.2 kg-VS/m^3·day, which is significantly higher than traditional OLRs. The system achieved a methane yield of 455 mL CH_4/g-VS and a VS reduction of over 90%, highlighting the potential of single-stage wet AD for handling higher organic loads. However, at an OLR of 12.9 kg-VS/m^3·day, process instability occurred, characterized by reduced methane yields and increased VFA accumulation, indicating the system's operational limits. It is assumed that the maximum OLR for single-stage wet AD of food waste, without compromising stability, could reach 10.5 kg-VS/m^3·day, provided that proper precautionary measures are taken[34].

2.4 Other substrates

Besides food waste, various other substrates can be used for AD to produce biogas, each with specific compositions influencing AD parameters. Agricultural waste arises from farming activities and is a growing concern due to its environmental impact and greenhouse gas emissions. The EU alone generates approximately 90 million tons of agricultural waste annually [35]. Agricultural waste varies significantly in composition, with fodder beet having a BMP of 0.5 m^3/kg.VS CH_4, while cattle manure yields only 0.2 m^3/kg.VS [10]. Additionally, cattle manure has a higher C/N ratio compared to less nitrogen-rich agricultural waste [36]. Municipal solid waste (MSW) consists of organic household waste, food scraps, and garden waste. Globally, MSW generation is estimated at 1.3 billion tons annually and is expected to rise to 2.2 billion tons shortly. Like agricultural waste, MSW differs significantly in composition. When used as a co-substrate with animal manure or sewage sludge, MSW can enhance methane yield during AD. Organic waste, which constitutes a significant portion of MSW, is a promising feedstock for biogas production if challenges like high solid content and heterogeneity are managed effectively. Industrial processes produce large quantities of by-products and residues, such as pulp and paper sludge and petrochemical waste. These wastes are rich in organic content and can boost methane production in AD systems. However, variability in composition, impurities, and potential inhibitors like heavy metals pose challenges. Pretreatment and co-digestion with other substrates can improve methane yields [10].

As mentioned earlier, multiple substrates can be used in AD. Many AD plants currently rely on mono-digestion, where a single feedstock is processed. However, mono-digestion has several drawbacks, including digester instability, heavy metal accumulation, and low biogas yields. For instance, highly biodegradable organic wastes, such as certain food wastes, can lead to the rapid accumulation of VFAs, inhibiting methanogens and reducing overall efficiency. Anaerobic co-digestion, which involves processing two or more feedstocks simultaneously, offers a solution to these challenges. Co-digestion provides several benefits, including enhanced system stability and increased methane yields due to the combination of substrate characteristics. These benefits include diversifying the microbial community, optimizing the C/N ratio, supplementing essential trace elements, improving buffering capacity, and diluting toxic compounds like heavy metals. Despite these advantages, co-digestion also presents potential risks. Incorrect feedstock mixing ratios may lead to negative effects such as organic overloading, acidification, and system failure.

Therefore, proper feedstock selection and ratio optimization are essential to fully obtain the benefits of co-digestion while minimizing potential risks [37].

2.5 Additives

The use of additives in AD is a promising method to enhance biogas production and process stability. Several categories of additives have been identified, each with specific roles and benefits:

1. **Metal-based additives**: Metals like nickel , iron, cobalt, and zinc are commonly added to improve microbial activity. Metal-based additives can enhance AD performance by stimulating methanogens and increasing CH_4 production [38]. The addition of trace metals (TM) plays a crucial role in supporting methanogen growth during enzymatic synthesis [39]. Many enzymes rely on transition metals as catalytic centers at active sites or as co-factors in electron transport. Trace metals can be introduced into the AD process in different forms, such as chloride salts, metal oxides, and metal nanoparticles [40].

2. **Carbon-based additives**: The use of carbon-based materials such as graphene, activated carbon, and biochar in AD has gained growing interest due to their great physical and chemical properties, including adsorption capacity and high electrical conductivity. These materials act as carbon-based accelerants, creating a favorable environment for microbial growth [41]. Their properties, such as fine pore structure, high porosity, large surface area, and good electrical conductivity, contribute to direct interspecies electron transfer (DIET). For example, activated carbon, with a highly porous structure, facilitates microbial colonization, thereby accelerating the startup of methanogenesis [42].

3. **Biological additives**: The addition of enzymes can also improve AD, as enzymatic activity directly influences both the biomethane production rate and yield. The application of enzymes in AD enables low-energy in-situ treatment, improving CH_4 production from lignocellulosic materials. Specific enzymes, such as laccase and peroxidase, facilitate lignin degradation, making cellulose more accessible to cellulolytic enzymes. Cellulase, in particular, accelerates straw decomposition, enhancing biomass conversion efficiency. The addition of cellulolytic enzymes and other polysaccharases effectively degrades cellulose and polysaccharides during AD [43].

4. **Alkaline additives**: The addition of alkali to AD systems can enhance CH_4 yield and production rate. NaOH is particularly effective in removing lignin and hemicellulose, improving substrate degradability. Other caustic salts, such as lime ($Ca(OH)_2$) and KOH, offer advantages due to their easy recovery and potential use as fertilizers. However, NaOH is often preferred due to its lower cost and environmental friendliness compared to KOH [43].

Food waste is rich in carbohydrates, proteins, and lipids. However, most organic matter in food waste exists as suspended solids, leading to slow hydrolysis and low methane production efficiency. While various pre-treatment methods can address this issue, they often come with disadvantages. In contrast, the use of bio-additives is a clean, efficient, and environmentally friendly approach. These additives function similarly to enhanced fermentative bacteria, improving hydrolysis. Carbohydrates, particularly starch, make up 55–65% of the total organic solids in food waste and play a vital role in methane production. Starch is first converted into glucose, which is subsequently broken down into methane and carbon dioxide. Proteins are another important component in food waste. Protease enzymes have been shown to break peptide bonds, facilitating protein degradation during hydrolysis. The resulting protein hydrolysate, which contains FAN, is further broken down into organic acids and ammonia by fermentative bacteria through deamination. The use of protease additives improves hydrolysis rates and enhances methane production. Additionally, proteins serve as the primary nitrogen source for methanogens in AD. A significant portion of protein-derived nitrogen is converted into ammonia, which is vital for pH self-regulation in AD systems treating food waste. Lipids have a high theoretical methane potential and can significantly increase biogas production. However, they are also the main contributors to slow process speed due to their hydrolysis rate. The addition of lipase enzymes during hydrolysis has been shown to

increase methane production by 37.0 - 40.7% over a digestion period of 10 - 40 days [44].

TEs are essential for maintaining microbial activity and ensuring the efficient operation of metabolic pathways in AD. However, during long-term AD of food waste, an unstable phase often occurs, leading to methanogenesis decline and an accumulation of VFAs. This issue can be effectively addressed by supplementing specific trace metals TMs, which help restore or even enhance methane production. Key TMs such as iron, cobalt, nickel, selenium, and molybdenum are important components that regulate acetogenesis and methanogenesis. For example, hydrogenase enzymes facilitate electron transfer from H_2, while formate dehydrogenase plays a crucial role in electron transfer from formic acid (HCOOH). While conventional elements like potassium, calcium, and magnesium are typically abundant in food waste, the limited availability of essential TMs can hinder microbial activity. Supplementing these deficient TEs ensures process stability, prevents VFA accumulation, and supports sustained methane production in AD systems [44].

2.6 Microorgansism

Microorganisms are present in al the stages of AD. In the AD process, hydrogen-producing, acid-producing bacteria, and methanogens are well-characterized. Hydrogen-producing bacteria are categorized into strict anaerobes and facultative anaerobes, with *Clostridium sp., Enterobacterium sp., and Citrobacter sp.* being notable examples. Efficient hydrogen-producing bacteria are essential for AD and hydrogen production from food waste. Hydrogen- and acid-producing bacteria often exhibit mutualistic relationships and are sometimes referred to as "specialized mutual bacteria." Methanogens are strictly anaerobic microorganisms and studies on methanogen isolation from food waste are limited. Typically, methanogens in AD systems are sourced from inocula, such as activated sludge. Methanogens possess unique enzymes that facilitate methane production, with the mcrA gene playing a vital role. This gene is essential for methane production via both the H_2/CO_2 and acetic acid pathways [45].

2.7 Systems and foaming

Systems
Anaerobic digesters can be designed as single-phase or two-phase systems to produce biogas. Figure 3 shows the process flow diagram for both systems. In a single-phase digester, all steps of anaerobic digestion hydrolysis, acidogenesis, acetogenesis, and methanogenesis occur in one reactor. These systems are used due to their simplicity and lower capital costs. However, VFAs produced during acidogenesis can inhibit methanogenesis if not properly managed. Single-phase digesters can operate in batch or continuous modes [7].

Two-phase digesters separate the acidogenesis and methanogenesis steps into distinct reactors. The acidification reactor produces VFAs, which are then fed into the methanogenesis reactor, enabling faster and more controlled methane production. This configuration is particularly effective for substrates with high biodegradability, such as food waste. Two-phase systems provide distinct environments for different microbial consortia, enhancing process efficiency [7].

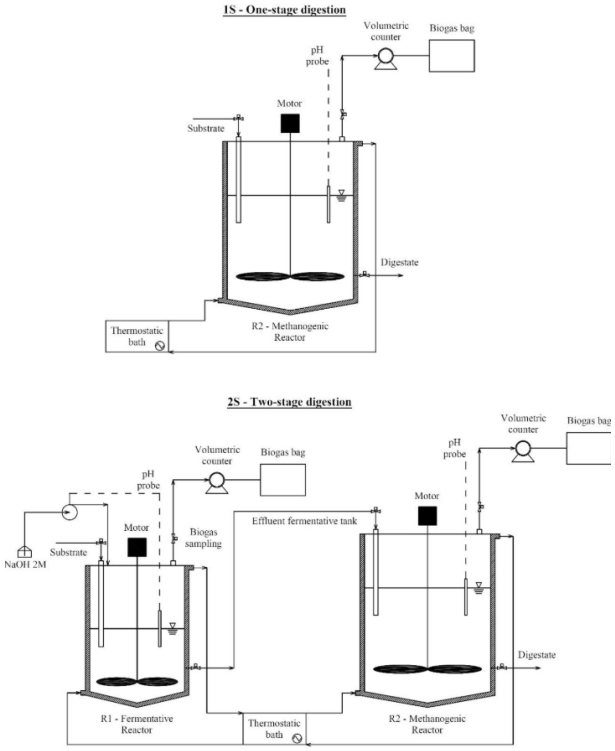

Figure 3: Schematic diagrams of one-stage and two-stage systems [46]

Foaming
Foaming in AD is a complex process influenced by surface-active materials or surfactants, both solid and soluble present in the substrate, the liquid within the digester, and the biogas produced. When foaming occurs, biogas becomes trapped in the liquid phase instead of being released into the gas phase, leading to an increase in digestate volume as gas production continues. Foaming is often triggered by a sudden gas release. The biogas in the digester primarily consists of CH_4, CO_2 with smaller amounts of hydrogen sulfide, ammonia, and other compounds. Due to the difference in solubility between methane and carbon dioxide, most methane forms gas bubbles, whereas a significant portion of carbon dioxide dissolves in the liquid phase as carbonic acid and bicarbonate (HCO_2-), depending on temperature and pH. Sudden changes in conditions, such as a temperature increase or pH decrease, can cause excessive foaming by releasing large volumes of gas, primarily CO_2. Various strategies have been developed to control foaming. The use of antifoam additives is a common and effective short-term solution, particularly for temporary foaming caused by sudden gas releases. However, for digesters experiencing persistent foaming, relying on commercial antifoam agents can be costly. Instead, operational adjustments and proper management often provide more sustainable solutions. Foaming due to overfeeding can be mitigated by carefully controlling the daily OLR, especially when using highly digestible substrates like food waste. Additional strategies to reduce foaming include avoiding foam-forming substrates such as oils and installing mechanical foam-breaking equipment [47].

2.8 Applications and costs

In European households, heating accounts for nearly 80% of energy consumption. Biogas can be used for both heating and the generation of electricity, making it a sustainable solution for residential, commercial, and industrial heating needs. Biomethane, which is almost pure methane, is compatible with existing gas heating systems and district heating networks, allowing buildings such as hospitals, offices, and retail spaces to transition away from non-renewable energy sources. Additionally, biomethane acts as a feedstock for chemical synthesis, supporting the manufacturing of pharmaceuticals, plastics, and

chemicals like ethanol and hydrogen. It also plays an important role in fertilizer production, particularly for ammonia synthesis. Biomethane is one of the few viable alternatives to fossil fuels for long-distance heavy-duty transport. Its use in compressed (bio-CNG) or liquefied (bio-LNG) forms has proven effective in reducing emissions from coaches, trucks, and the maritime sector [48].

The price of feedstock for biogas from food waste is often zero or even negative, as gate fees can be charged for waste removal. However, food waste requires relatively high pre-treatment costs. The CAPEX costs for a smaller biogas production plant (500 - 1300 m^3 biogas/h) using public waste are around €3,800 - €5,500 per MW electricity, which is higher than for plants using non-public waste due to the additional pre-treatment steps required. The operating costs can vary significantly, ranging from €20 - €120 per MW electricity [49]. Currently, one 1 m^3 of biomethane is sold for €0.89 [50].

3 Methods

This section will explain the various experiments, including the research objectives, experimental setup, analytical methods, and the procedures followed for each task.

3.1 Biogas yield without additives

Determining the current methane yield is important to act as a control baseline for evaluating the effectiveness of additives. From the literature review insights will be gained into the anaerobic digestion of food waste, enhancing the understanding of the process, identifying key parameters, and shaping the experimental design. The parameters will need to be the same as the pilot plant at the entrance buildings, these values are obtained from Prof. Euverink.

3.1.1 Experimental setup

The experiments will take place in the laboratory at the University of Groningen, using available equipment. A 500 ml flask will be used where the bubbles generated will result in stirring, mimicking the effects of a CSTR [51]. It will be filled with the inoculum sampled from the pilot-scale reactor at the ENTRANCE building at Zernike. The digestion process will operate in a single-phase mode, allowing all four stages of digestion to occur simultaneously in the same reactor [52]. The temperature will be maintained at 38°C using a water bath, and no pH adjustments will be made. To maintain anaerobic conditions, the headspace will be flushed with nitrogen gas [53]. A cork with two needles positioned on opposite sides is placed on top of the bottle, allowing nitrogen to flow in through one needle and escape through the other. After two minutes at maximum nitrogen pressure, the needles are sealed, ensuring that the majority of oxygen is removed. The substrate input will consist of 5 grams of VS per liter per day. At a VS percentage of 25%, this will result in [29]:

$$\begin{aligned}
\text{Total VS} &= \text{Loading rate} \times \text{Reactor volume} && (1)\\
&= 5 \text{ g VS/L/day} \times 0.4 \text{ L} && (2)\\
&= 2 \text{ g VS/day} && (3)\\
\text{Total Substrate} &= \frac{\text{Total VS}}{\text{VS Percentage}} && (4)\\
&= \frac{2 \text{ g}}{0.25} && (5)\\
&= 8 \text{ g substrate/day} && (6)
\end{aligned}$$

The substrate will be added from Monday to Thursday, resulting in an OLR of 2.857, as 20 grams of VS are added over 7 days. Food waste will also be collected from the ENTRANCE building. To maintain a constant working volume, an equivalent volume will be removed for each addition of food waste. The primary goals of the setup are to measure biogas production and to monitor the process stability. The HRT is calculated as the ratio of the daily volume added to the reactor volume. Since the food waste is mostly water, it is assumed to have the same density. The HRT will be approximately 140 days, as shown below, though the reactor can be stopped earlier [19].

$$\frac{400}{2.857} = 140 \tag{7}$$

On day 0, a tank is filled with 400 mL of inoculum collected from the ENTRANCE building. An initial 8 grams of food waste is added. For the first 4 days, the system is left undisturbed to allow the microorganism to adjust to the initial feeding. After this acclimation period, the daily procedure begins: 8 grams of food waste is added, the headspace is flushed, and the system is monitored for another 4 days. Following this, the sample is left untouched for 3 days to prevent acidification of the system. After the

resting phase, the experiment continues for 4 additional days with daily feeding of 8 grams of food waste, ensuring the working volume remains at 400 mL by removing an equivalent amount of liquid each day. Picture 4 shows the setup that was used.

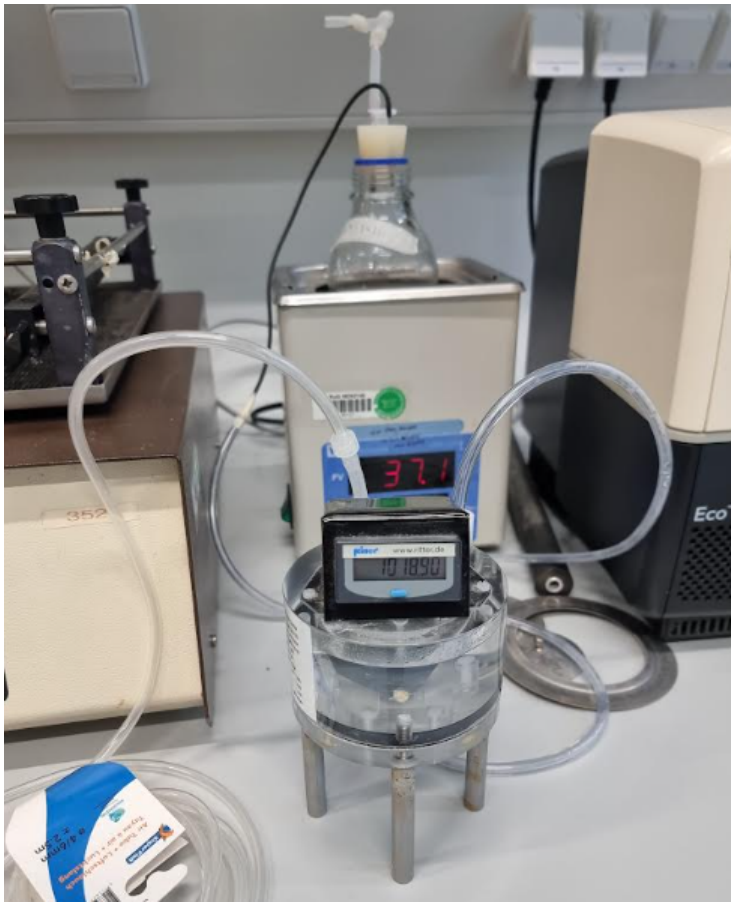

Figure 4: Experimental setup

3.1.2 Analysing methods

The primary goal of this experiment is to measure gas production without an additive. The main focus will be on quantifying the gas produced, as well as monitoring pH and VFA levels daily to assess the stability of the system under these conditions [54].

Gass production
The flask is sealed with a cork, allowing the produced gas to flow through a needle and a pipe into a RITTER Milligascounter [55], where the volume (in mL) is recorded. Measurements are taken daily at 10:00 to maintain consistent time intervals. After each measurement, the meter resets to zero, and new readings are recorded after 24 hours. The gas is then vented and removed through ventilation.

pH Measurements
The pH will be measured using the VOS-70002 pH meter [56]. The pH meter is calibrated using two buffers. Once calibrated, the pH of the sampled liquid from the reactors is measured. After the experiment is finished, the biogas yield and process stability without additives will be established through analysis of the collected data [34].

VFA Measurements
The VFAs are measured using High-Performance Liquid Chromatography (HPLC) [57]. Daily measurements are taken, and samples are prepared by centrifuging for 10 minutes at 14,680 rpm to ensure the removal of solids. The HPLC runs at the following settings:

- Temperature: 60 °C
- Pressure: <7 MPa
- Time: 15 minutes
- Mobile phase: 2.5 mM H_2SO_4
- Flow: 1 mL/min

The prominent VFAs are identified by the area under the curve at different retention times. The area under the curve (mAU.s) is proportional to the concentration of the VFA in the sample [58]. Acetic acid appears around 9.3 minutes, propionic acid around 10.9 minutes, and butyric acid around 12.5 minutes. Slight variations in retention times are expected, and impurities will appear on the chromatogram during the initial minutes of the run.

3.2 Biogas yield with additives

To address this question, similar experiments were performed as described in 3.1. The primary difference, however, will be the addition of ANDY 1 and ANDY 2 additives from Paques [8]. The previous experiment without additives will serve as a control, establishing the baseline performance without intervention. The results of this experiment will influence how research will continue, if no improvements are observed over the control, alternative methods, such as co-digestion of multiple feedstocks, must be explored to enhance overall biogas production. If improvements are found, further research will focus on the most effective additive.

3.2.1 Experimental set up

In the experiment, a constant OLR is maintained, biogas production and process stability with the additive will be compared to those of the control. Two different additives from Paques will be added, labeled as ANDY 1 and ANDY 2. The experimental setup described in 3.1.1 will be followed but for both additives 2 mL/L will be added on day 0, accounting to 0.8 ml . Gas, pH and VFA measurements will be performed as described in 3.1.2.

3.3 Effects of ANDY 2 on stages and stability of AD

This section builds upon the findings presented in Section 4.1. The goal of investigating the effects of ANDY 2 on the various stages of anaerobic digestion is to determine what reactor the additive should be added in a potential two-stage system, where digestion stages occur separately. Research suggests that such a system can achieve higher biogas yields with smaller reactor volumes, making this information valuable for future applications [59]. To answer the first question, literature research is required to see what substances are formed in each of the stages of digestion, in hydrolysis carbohydrates, proteins, and fats are converted into simpler soluble sugars, amino acids, and fatty acids, respectively. The next stage, acidogenesis, involves the breakdown of these hydrolyzed products into VFAs such as propionic, butyric, and acetic acids, as well as alcohols, CO_2, H_2, and NH_3. Acetogenins convert VFAs and alcohols into more acetic acid, H_2, and CO_2. Finally methanogenesis converts acetic acid, H_2, and CO_2 into CH_4 and additional CO_2 [5] [10]. The goal is to identify how much of these products are present in the reactor, to get a good understanding of what phase of digestion is increased by adding the additive. The goal of answering the second question is to determine if the OLR can be increased with the addition of additive 2, a higher OLR could increase biogas production, however, multiple issues can occur, the main one being

reactor acidification due to an accumulation of VFAs, the pH will rise since the methanogenesis can't process the acids fast enough [60]. Further process instability and foaming can also occur [61]. Since the initial experiments did not indicate high ammonia levels or inhibition, as evidenced by the increasing gas production and decreasing pH, no further analysis on FAN and TAN was conducted.

3.3.1 Experimental setup

For the first two weeks, the experiment will follow the procedure outlined in Section 3.1.1, with one reactor serving as the control (without additives) and the other receiving 0.8 mL of the additive ANDY 2. After this two-week period, the feed rate will be increased by 2 grams of VS each time the reactor is fed, which is equivalent to 3.2 grams of extra food waste per day [29]. To minimize additive loss, the daily liquid removal will remain constant at approximately 8 mL. Since the feed rate will not be constant, a clear HRT value cannot be estimated. The increased feed will start on Monday of week 3, and visual observations, including foam formation, will be recorded. The experiment will continue until one or both reactors show signs of acidification or other irregularities that cause a complete halt in gas production. Throughout the experiment, samples will be analyzed to assess the impact of the additive on different stages of digestion under both lower and higher feeding rates. The increased feed rates are outlined in Table 1.

Day	**Feed rate (g VS/L/day)**
Week 3	
Monday	7
Tuesday	9
Wednesday	11
Thursday	13
Week 4	
Monday	15
Tuesday	17
Wednesday	19
Thursday	21
Week 5	
Monday	23
Tuesday	25
Wednesday	27
Thursday	29

Table 1: Increased feeding rates over time

3.3.2 Analysing methods

To evaluate hydrolysis efficiency, measuring soluble chemical oxygen demand (sCOD) is essential, as it indicates the amount of dissolved organic material, including sugars, amino acids, and fatty acids. The sCOD concentration reflects the level of soluble organic compounds. In the AD process, hydrolysis is typically assessed by measuring the amount of sCOD released from biomass [62]. However, to fully assess the process, it is also important to analyze VFA production and methane yield to ensure effective conversion of hydrolysis products into biogas [63]. Especially the VFA concentration as they can make up around 30% of the sCOD [64]. Acidogenesis is assessed by indicating the amount of VFAs, as these are the main compounds that are produced and are present in the liquid [65]. Since alcohols primarily form at pH values below 5, this is not expected to have a significant presence in this experiment. Similar to acidogenesis, acetogenesis will be measured by determining the concentration of acetic acid in the reactor. It is often challenging to distinctly separate acidogenic and acetogenic reactions, as both involve the production of H_2 and acetic acid, which serve as substrates for methanogenic bacteria [12]. VFAs

can be further assessed through daily pH measurements, as the two are linked, a potential VFA accumulation will likely coincide with a pH decrease [66]. Lastly, methanogenesis is measured by investigating the amount of gas that is formed, as the products formed are CO_2 and CH_4 [10]. The maximum feed rate is determined through daily measurements of gas production and pH to assess process stability and efficiency. Visual observations, such as foam formation, are also taken into account. The results from the initial set of experiments indicated a low ammonia concentration, attributed to the continuous increase in gas production and the relatively rapid pH decrease at low OLR. As a result, no TAN or FAN measurements were taken during this experiment, as they were not necessary for assessing process stability [21].

sCOD measurements
The sCOD is measured every Monday and Wednesday, here a sample is taken from the reactors and it is centrifuged at 14680 rpm for 10 minutes, after this the solids and liquids are separated ensuring only the soluble parts are measured. It will be measured using the LCK-014 testing kit from Hach [67], being suitable for this application since it measures 1000–10.000 mg/L O_2 and the expected value for anaerobic digestion of food waste is around 5000 mg/L O_2 [68]. 0.5 mL of liquid is added to the testing solution, inverting the bottle, and placing it in the standard program of the HT 200 S for 15 minutes [69]. After cooling to room temperature, the sample is analyzed using the Hach DR 3900 [70], which measures sCOD in mg/L.

VFA measurements
The VFAs are measured using HPLC, with measurements performed as outlined in section 3.1.2. Daily measurements will be taken during the first two weeks, after which they will be conducted only on Mondays and Wednesdays.

pH, gas production, and visual observations
Gas production and pH measurements will be taken daily, following the methods outlined in section 3.1.2. Visual observations will also be recorded whenever signs of system instability are observed.

4 Results

Various experiments on the anaerobic digestion of food waste with additives have been conducted. The initial focus was on evaluating the feasibility of additive use, forming the basis for further research on its impact on different AD stages and the potential for increasing the feed rate. This section will discuss the results.

4.1 Feasibility of Additive Use

Gas Production
The overall gas production serves as the main criterion for evaluating the effectiveness of the additives ANDY 1 and ANDY 2. When examining the gas production results in Figure 5, it is evident that the addition of ANDY 2 resulted in significantly higher gas production compared to both ANDY 1 and the control. Over two weeks, the control produced a total of 1,689.75 mL of gas, ANDY 1 generated 2,001.00 mL, and ANDY 2 yielded 3,717.65 mL. The reactor with ANDY 2 produced more than twice the amount of gas within the same timeframe and with the same total feed. However, the composition of the gas remains unknown, and the methane percentage might be lower. Additionally, part of the reactor liquid used in the experiment with ANDY 2 was sampled at a later time. Although sourced from the same tank, this sample had a higher pH, leading to an increased initial pH. While this may have influenced gas production, less than 50 mL of the 400 mL reactor fluid was sampled at a later time, making it unlikely to have significantly contributed to the substantial increase observed with ANDY 2. Furthermore, in the experiment with ANDY 1, a higher initial pH was observed despite using the same reactor fluid as the control. The pH might have increased due to the presence of oxygen during the two-week storage period,

which could have also influenced the starting pH in the reactor with ANDY 2 [71].

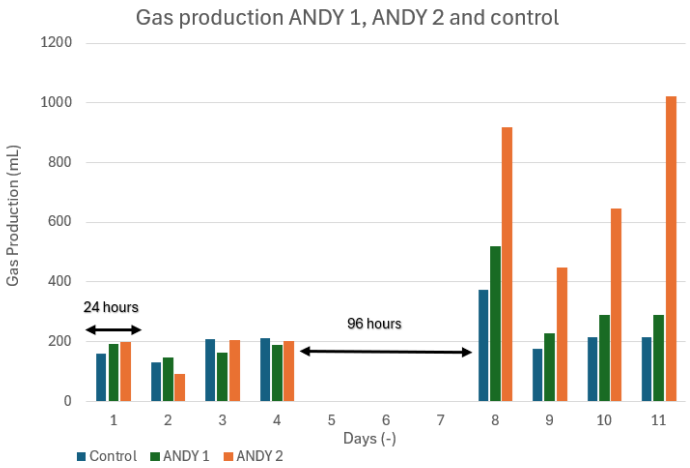

Figure 5: Gas production per measurement

pH

pH serves as an indicator of system stability. When the pH is too low, methanogenesis might be inhibited, resulting in lower biogas production or even system failure [60]. Figure 6 shows that the reactor with ANDY 2 had the highest pH after two weeks. However, since this is partially due to slight variations in reactor fluid, assessing the total pH drop over two weeks provides a clearer indication of the system's acidification. Over this period, the control reactor showed a pH drop of 0.62, the reactor with ANDY 1 dropped by 0.84, and ANDY 2 dropped by 0.47. Since all reactors remained within a stable pH range, drawing definitive conclusions about the additives' impact on stability is challenging. The pH drop in every reactor is significant considering the low OLR used in the experiments [6].

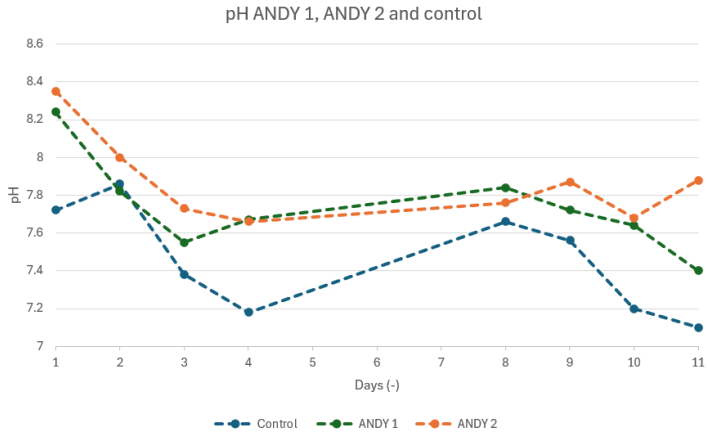

Figure 6: pH value over 2 weeks

VFAs

Figures 7, 8, and 9 indicate that the reactor with ANDY 2 had the lowest VFA accumulation, with significantly lower acetic acid concentrations compared to both the control reactor and the reactor with ANDY 1. Notably, elevated propionic acid concentrations were observed only on days 4 and 9, coinciding with the lowest pH recorded in the ANDY 1 reactor on day 4. However, after two weeks, VFA levels remained low. This minimal VFA buildup suggests strong system stability when using ANDY 2 [60]. Considering this, along with a more than 200% increase in gas production, further research will focus on the effects of ANDY 2. Future experiments will be conducted simultaneously with ANDY 2 and the control to ensure consistent conditions throughout.

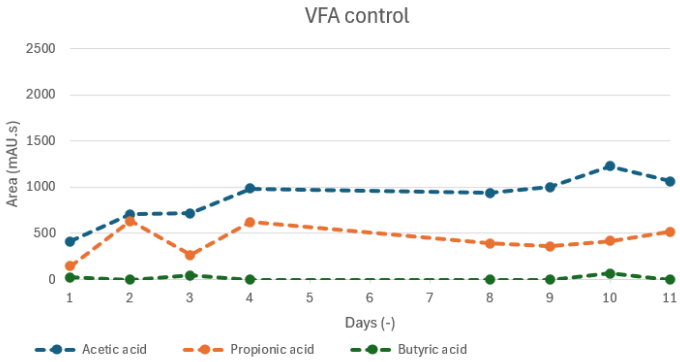

Figure 7: VFAs over 2 weeks in the control

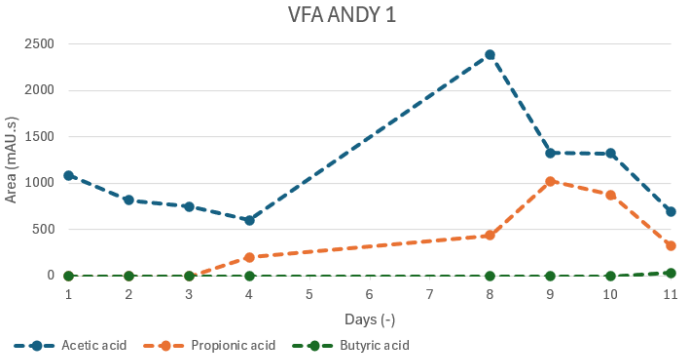

Figure 8: VFAs over 2 weeks with ANDY 1

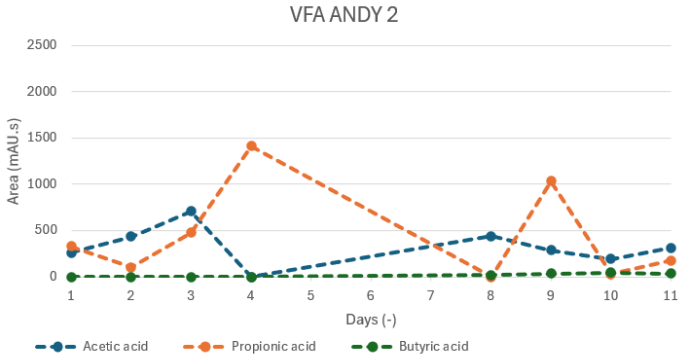

Figure 9: VFAs over 2 weeks with ANDY 2

4.2 Effects of ANDY 2 on Stages of Digestion

sCOD

When analysing sCOD values in Figure 10, no notable differences were observed in the beginning. However, from day 17 onward, a significant difference in sCOD emerged. At lower OLR levels, hydrolysis shows no noticeable variations, as sCOD remains stable and consistent in both reactors. However, after increasing the OLR, the samples without ANDY 2 exhibited higher concentrations of soluble organic materials [44]. This could indicate a negative effect of ANDY 2 on hydrolysis activity. However, it is

more probable that a later stage of digestion was enhanced in the reactor with ANDY 1, leading to a lower presence of soluble organic material. Since VFAs are included in the sCOD measurement, their concentrations should also be considered when evaluating hydrolysis efficiency based on sCOD [64]. On day 30, sCOD was very high in both samples. This likely indicates the microorganisms' inability to handle the amount of food waste that was added.

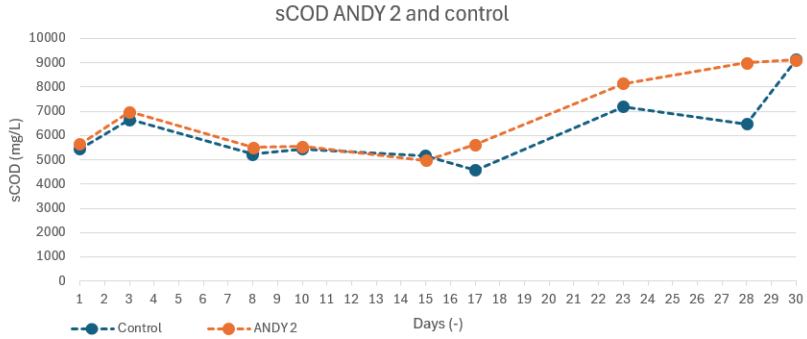

Figure 10: sCOD values over 30 days

VFAs

The primary products of the acidogenesis stage are VFAs, with acetic acid, propionic acid, and butyric acid being the most significant. During acetogenesis, additional acetic acid is produced alongside other byproducts [10]. Throughout the experiment, VFA concentrations remained low, which is reflected in the consistently high pH observed for most of the experiment. Almost no butyric acid or propionic acid was detected, as shown in the Figures in Appendix B. Significant propionic acid concentrations were measured only on day 17, however the values for the previous and following days were zero, this is assumed to be a measurement error. Acetic acid was the only notable VFA detected. It is important to note that multiple technical issues occurred with the HPLC device during the study. Due to the unavailability of the HPLC during the final week, no measurements were recorded for the last few days of the experiment. Despite this, there are indications of a higher acetic acid concentration in the reactor without ANDY 1 starting from day 16, as seen in Figure 11. This may be linked to increased methanogenic activity in the reactor with ANDY 1 [10]. This observation aligns with the sCOD results, where higher sCOD concentrations were recorded on days 17, 23, and 28, potentially due to acetic acid accumulation. Furthermore, the results of the first experiment support this trend, as Figure 9 shows a lower acetic acid concentration in the reactor with ANDY 2 compared to the control in Figure 7, suggesting enhanced methanogenesis.

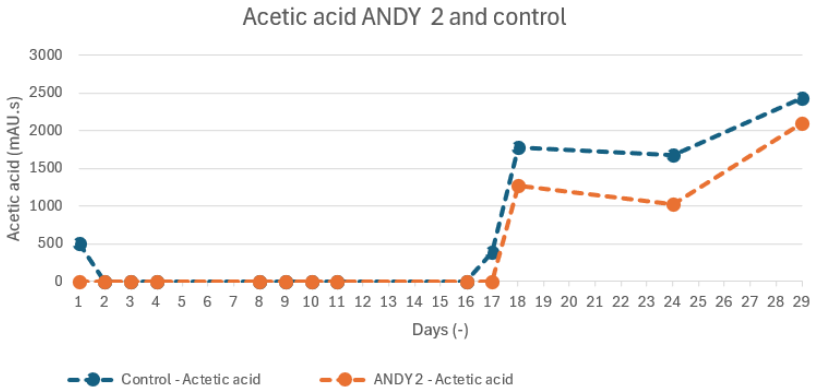

Figure 11: Acetic acid concentrations over 29 days

pH

Examining the pH values in Figure 12 largely supports the findings from the HPLC and VFA concentration analysis. The pH remained above 8 for the first 16 days and stayed relatively high for most of the experiment. Since the reactor liquid used in this experiment was taken from the ENTRANCE building

at a different time than in the first experiment, the lack of a significant pH drop could be due to an increase in alkalinity, likely caused by high concentrations of ammonia nitrogen or bicarbonate [23] [6]. From day 16 onward, pH values below 8 became more noticeable, possibly indicating an increase in acetic acid [60]. The continuous pH decline starting on day 29 likely suggests further accumulation of acetic acid especially in the reactor without ANDY 2.

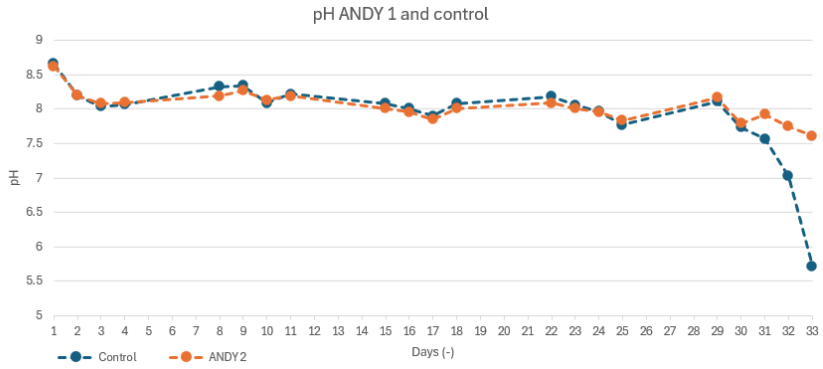

Figure 12: pH values over 33 days

Gas production

When comparing gas production, it is evident that the addition of ANDY 2 led to increased gas output compared to the reactor without the additive, as shown in Figure 13. The difference in gas production between the reactors became more pronounced as the OLR increased. This could be due to microorganisms in the later inoculum samples being able to fully digest the available materials within 24 hours, even without the additive. Another possibility is that the additive's effects are more effective at lower pH levels, at a higher pH more ammonia will be formed which inhibits the methanogenesis phase [19] [21]. Notably, after day 16 when the pH started to drop an increase in gas production was observed. The total ml of gas produced in the control reactor was 23,475.92 mL while this was almost double at 42,731.54 mL in the reactor with ANDY 2.

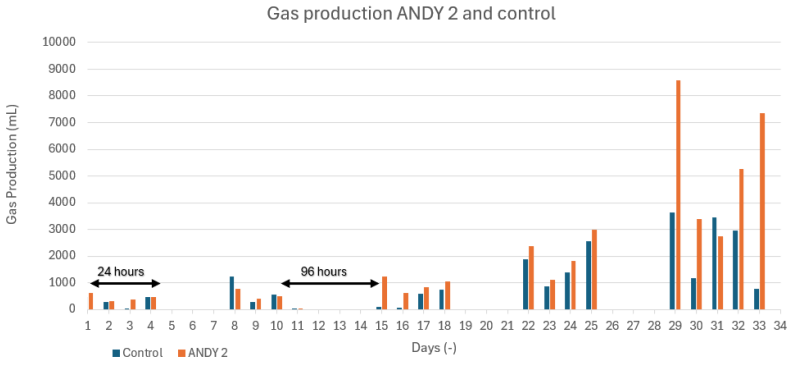

Figure 13: Gas production per measurement

4.3 Effects of ANDY 2 on an increased feed rate

When analysing pH evolution in Figure 12, both reactors exhibited similar trends until day 31, when the feed rate from the previous day reached 23 g VS/L. As the feed rate continued to increase to 29 g VS/L, a clear divergence became evident: the reactor with ANDY 2 maintained a stable pH of 7.61, while the control reactor experienced a decline to 5.72. This drop fell below the optimal range for methanogenesis, leading to a noticeable reduction in gas production starting from day 31. In contrast, gas production in the reactor with ANDY 2 continued to increase from that point onward, as shown in Figure 13[5]. While monitoring the reactors, foaming was observed. This began at a feed rate of 19 g VS/day, as shown in

Picture 14. Initially, the foaming was manageable, but it quickly escalated to nearly half of the reactor volume. As a result, a larger tank was introduced on Wednesday of week 5. This change may have contributed to the lower gas production observed on that day, as seen in Figure 13. The formation of foam indicated process instability, likely due to an excessively high feed rate in a small working volume [72]. Concerned that the foam might reach the top and clog the gas meter, feeding was halted once the reactor without ANDY 1 reached a pH of 5.71.

Figure 14: Foam layer on top of the reactor liquid

4.4 Economic analysis

The aim of this part is to evaluate the economic feasibility of the addition of additives for biogas production. The main focus of this report has been on biogas production therefore, biogas will be used instead of biomethane for this analysis. Furthermore, since energy generation is the primary application of the reactor at the ENTRANCE building, this will be considered. Since the facility already exists, no CAPEX costs are included, and the only notable difference in operating costs will be the addition of the additive. It is important to note that, besides biogas, digestate is also produced, but it is considered outside the scope of this study.

Various dosing strategies exist, such as continuous, pre-loading, and pulse dosing [73]. In this research, pre-loading was employed, where 2 ml/L of the additive was added before the start of digestion. After one month, the effects were still noticeable, but to ensure continuous positive effects, an additional 2 ml/L is assumed to be added every month. The results from the second experiment showed that adding the additive over one month resulted in a 1.82-fold increase in biogas production.

Literature indicates that biogas yields 450 mL methane/g VS without additives when using only food waste [74]. At the ENTRANCE building, which houses two tanks with a total volume of 10 m^3, the assumed OLR is 5 g VS/L/day. With methane comprising 62.5% of biogas, the daily biogas production is calculated as follows:

$$\text{Total VS input per day} = \text{OLR} \times \text{Total volume} \tag{8}$$
$$= 5 \text{ kg VS/m}^3\text{/day} \times 10 \text{ m}^3 \tag{9}$$
$$= 50 \text{ kg VS/day} \tag{10}$$
$$\text{Daily methane production} = \text{Methane yield} \times \text{VS input} \tag{11}$$
$$= 0.45 \text{ m}^3 \text{ CH}_4\text{/kg VS} \times 50 \tag{12}$$
$$= 22.5 \text{ m}^3 \text{ CH}_4\text{/day} \tag{13}$$
$$\text{Total daily biogas production} = \frac{22.5}{0.625} \tag{14}$$
$$= 35 \text{ m}^3\text{/day} \tag{15}$$

The energy content of each cubic meter of biogas is approximately 6 kWh, which translates to around 2 kWh of electricity [75]a. The price of electricity for businesses in the Netherlands is 0.508 €/kWh [76]. Knowing this, the annual revenue will be:

$$35 \times 365 = 12775 \text{ m}^3\text{/year} \tag{16}$$
$$12775 \times 2 \text{ kWh} = 25550 \text{ kWh/year} \tag{17}$$
$$25550 \times 0.508 = 12979.40 \tag{18}$$

With the ANDY 2 additive, a 1.82-fold increase is found, so:

$$12979.4 \times 1.82 = 23622.51 \tag{19}$$

That is an increase of 10643.11€ more than without an additive, so to be economically feasible, the annual additive cost should be below this amount.
The additive is assumed to consist of 1 g/L of $FeCl_2$ and 0.4 g/L of HCl, with the following costs:

- $FeCl_2$ price: 406 €/100 g → 4.06 €/g [77]
- HCl price: 219 €/2.5 L (37% solution) → 87.6 €/L [78]

Density of 37% HCl = 1.2 kg/L, meaning 1 L contains 444 g of pure HCl.

Cost Calculation of 1 L of Additive:

$$\text{Cost of } FeCl_2 = 4.06 \tag{20}$$
$$\text{Cost per gram of pure HCl} = \frac{87.6}{444} = 0.1973\text{/g} \tag{21}$$
$$\text{Cost for 0.4 g of pure HCl} = 0.4 \times 0.1973 = 0.079 \tag{22}$$
$$\text{Total Cost of 1 L of Additive} = 4.06 + 0.079 = 4.14 \tag{23}$$

When adding 2 ml/L every month, the annual additive amount will be:

$$2 \text{ ml/L} \times 10 \text{ m}^3 \times 12 = 240 \text{ L} \tag{24}$$
$$240 \times 4.14 = 993.60\text{/year} \tag{25}$$

The annual cost increase due to the addition of ANDY 1 is €993.60. Since this is significantly lower than the annual revenue increase of €10,643.11, the addition of ANDY 1 is considered economically viable.

5 Conclusion and recommendations

Conclusion
The results indicate that the addition of ANDY 2 significantly improved biogas yield. Two separate experiments demonstrated an increase in gas production while all other parameters remained constant. In Experiment 1, a substantial increase was observed, particularly in the second week. Similarly, Experiment 2 showed a notable rise in gas production, which became more evedent as the feed rate increased and pH dropped. Both reactors with ANDY 2 exhibited nearly a 200% increase in gas production compared to the control.

Beyond yield improvement, another potential advantage of ANDY 2 was its ability to enhance system stability at higher feed rates. At a feed rate of 23 g VS/L, a difference in pH between the two reactors emerged, with the ANDY 2 reactor showing greater stability due to reduced acidification. At 29 g VS/L, the control reactor's pH dropped to 5.72, an inhibitory level for methanogenesis while the ANDY 2 reactor maintained a stable and relatively high pH of 7.61. Additionally, gas production in the control reactor began declining over the last three days, whereas the ANDY 2 reactor continued to show increasing gas output.

The evidence suggests that ANDY 2 primarily enhanced the methanogenesis phase of digestion. sCOD experiments indicated that after day 16, either hydrolysis slowed in the ANDY 2 reactor or the additive improved a later-stage process. This was further supported by VFA concentration trends, as acetic acid levels remained higher in the control reactor. Given that approximately 70% of biogas production by methanogenic bacteria is derived from acetic acid, the simultaneous increase in biogas output from day 16 onward strongly indicates that ANDY 2 enhances methanogenesis.

Recommendations
Since there is still limited information on the exact effects of ANDY 2, further research is recommended. Future research should focus on evaluating the impact and assessing the feasibility when scaling up. To better understand the impact of ANDY 2, an experiment with a constant OLR and daily feeding that gradually increases could help determine the reactor's capacity before acidification occurs. The feed rate used in the current study was too high for the reactor volume, and components in the hydrolysis stage often take multiple days to break down into water-soluble compounds. Gradually increasing the OLR on a weekly basis would allow the reactor to adapt to the feeding schedule and help establish the optimal OLR achievable with ANDY 2.

Additionally, while the observed 200% increase in gas production is promising, biogas typically consists of around 65% CH_4 and 35% CO_2. Without gas composition analysis, the actual improvement in methane yield remains uncertain. Further research using gas chromatography is necessary to confirm the true effectiveness of ANDY 2. Currently, there is no available information on the optimal dosing of the ANDY 2 additive. Paques provided a dosage of 2 mL per liter, however, the timing and frequency of application remain unknown.

Lastly, since these experiments were conducted on a lab scale, it is essential to assess whether similar results can be replicated in larger reactors with a capacity of $5m^3$.

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Appendix A: Results experiments in tabular form

Day	Measurement	Gas Produced (mL)	pH
Monday	After 3 days	160.25	7.72
Tuesday		131.10	7.86
Wednesday		207.00	7.38
Thursday		210.45	7.18
Next week			
Monday	After 3 days	374.90	7.66
Tuesday		174.80	7.56
Wednesday		216.20	7.20
Thursday		215.05	7.10

Table 2: Gas production and pH measurements control

Day	Measurement	Gas Produced (mL)	pH
Monday	After 3 days	192.05	8.24
Tuesday		146.05	7.82
Wednesday		161.00	7.55
Thursday		186.30	7.67
Next week			
Monday	After 3 days	516.35	7.84
Tuesday		225.40	7.72
Wednesday		287.50	7.64
Thursday		286.35	7.40

Table 3: Gas production and pH measurements ANDY 1

Day	**Measurement**	**Gas Produced (mL)**	**pH**
Monday	After 3 days	197.50	8.35
Tuesday		89.70	8.00
Wednesday		203.55	7.73
Thursday		201.25	7.66
Next week			
Monday	After 3 days	916.55	7.76
Tuesday		447.35	7.87
Wednesday		642.85	7.68
Thursday		1018.90	7.88

Table 4: Gas production and pH measurements ANDY 2

Day	Gas Produced (mL)	pH	sCOD (mg/L)
Monday (Day 1)	476.28	8.66	5635
Tuesday (Day 2)	291.06	8.20	-
Wednesday (Day 3)	19.60	8.04	6978
Thursday (Day 4)	449.82	8.07	-
Monday (Day 8)	1241.66	8.33	5501
Tuesday (Day 9)	273.42	8.34	-
Wednesday (Day 10)	565.48	8.09	5544
Thursday (Day 11)	8.82	8.22	-
Monday (Day 15)	81.34	8.08	4981
Tuesday (Day 16)	76.44	8.01	-
Wednesday (Day 17)	582.12	7.90	5626
Thursday (Day 18)	753.62	8.08	-
Monday (Day 22)	1886.50	8.18	-
Tuesday (Day 23)	869.26	8.06	-
Wednesday (Day 24)	1382.78	7.97	8148
Thursday (Day 25)	2542.12	7.77	-
Monday (Day 29)	3631.88	8.11	8993
Tuesday (Day 30)	1169.14	7.73	-
Wednesday (Day 31)	3452.54	7.57	9116
Thursday (Day 32)	2940.98	7.03	-
Friday (Day 33)	781.06	5.72	-

Table 5: Gas production,pH and sCOD control

Day	Gas Produced (mL)	pH	sCOD (mg/L)
Monday (Day 1)	618.52	8.62	5431
Tuesday (Day 2)	295.16	8.20	-
Wednesday (Day 3)	368.48	8.08	6650
Thursday (Day 4)	463.42	8.10	-
Monday (Day 8)	783.96	8.19	5225
Tuesday (Day 9)	391.04	8.27	-
Wednesday (Day 10)	494.44	8.13	5440
Thursday (Day 11)	21.62	8.19	-
Monday (Day 15)	1235.16	8.01	5181
Tuesday (Day 16)	602.54	7.96	-
Wednesday (Day 17)	842.24	7.85	4559
Thursday (Day 18)	1059.38	8.01	-
Monday (Day 22)	2362.22	8.09	-
Tuesday (Day 23)	1124.24	8.01	-
Wednesday (Day 24)	1802.92	7.96	7170
Thursday (Day 25)	2976.98	7.84	-
Monday (Day 29)	8578.44	8.17	6460
Tuesday (Day 30)	3375.54	7.79	-
Wednesday (Day 31)	2725.06	7.92	9108
Thursday (Day 32)	5256.48	7.75	-
Friday (Day 33)	7353.70	7.61	-

Table 6: Gas production,pH and sCOD ANDY 2

Appendix B: Propionic and butyric acid concentrations

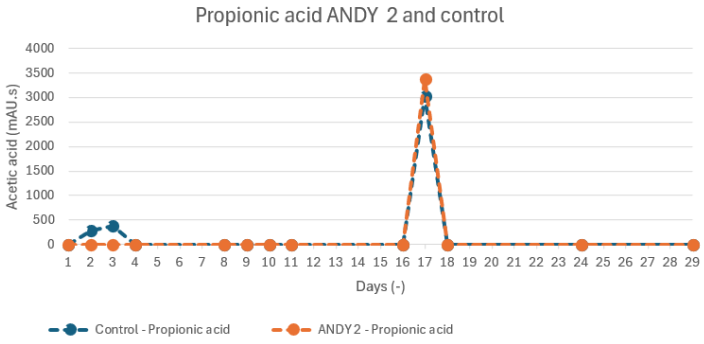

Figure 15: Propionic acid concentrations over 29 day

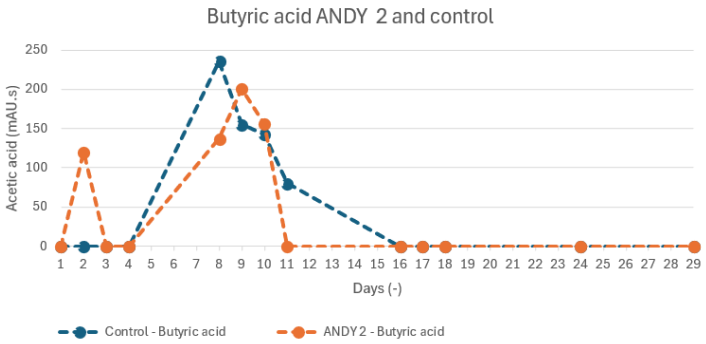

Figure 16: Butyric acid concentrations over 29 day