# MASTER THESIS

## Frontier anaerobic digestion technologies for renewable energy recovery

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## SAMENVATTING

Deze studie onderzocht de geschiktheid van het afvalwater van de start-up Wastech voor behandeling met anaerobe vergisting, met name UASB-reactoren, voor duurzame energierecuperatie en verbetering van de circulariteit van hun proces. Het onderzoek begon met een uitgebreide karakterisering van het afvalwater. Vervolgens werden twee UASB-reactoren gebruikt, geïnoculeerd met korrelig slib: één voor ruw afvalwater en de andere voor gefilterd afvalwater. De reactoren werkten continu gedurende 60 dagen bij mesofiele temperaturen. De prestaties van de reactoren werden geëvalueerd op basis van organische stofverwijdering, biogasproductie en stabiliteit van vluchtige vetzuren (VFA) en ion concentraties. Daarnaast werd een economische analyse uitgevoerd op basis van het potentiële methaanrendement om de haalbaarheid van gasbenuttingstechnologieën, zoals een biogasketel en een gecombineerde warmte- en krachtcentrale (CHP), te beoordelen.

De karakterisering van het afvalwater onthulde verschillende uitdagingen voor behandeling met UASB-reactoren, waaronder lage pH, geen alkaliniteit, lage kationconcentraties en een verhoogde C/N-verhouding. Behandeling van gefilterd afvalwater met UASB-reactoren toonde superieure prestaties in methaanrendement en processtabiliteit, met veerkracht tegen pH-verstoringen, in vergelijking met de behandeling van ruw afvalwater. Uit de economische analyse bleek dat een investering in een CHP-installatie de voorkeur had, met een positieve netto contante waarde (NPV), terwijl de warmteketel een negatieve NPV vertoonde. Deze bevindingen benadrukken het potentieel van afvalwaterverwerking om Wastech's doelen op het gebied van duurzaam afvalbeheer, winstgevendheid en energie-autonomie te ondersteunen. Echter, verdere strategische verbeteringen en uitgebreide evaluaties van de prestaties en stabiliteit van de UASB-reactoren zijn noodzakelijk voor grootschalige implementatie.

## ABSTRACT

This study investigated the suitability of Wastech's leachate for its treatment with anaerobic digestion, particularly UASB reactors, for sustainable energy recovery and enhancing circularity of their process. Extensive characterization of the leachate was conducted. Followed by the continuous operation of two UASB reactors inoculated with granular sludge—one for processing raw leachate and the other for filtered leachate. The reactors were operated for 60 days at mesophilic temperatures. The performance in terms of organic matter removal and biogas production, and the stability in terms of volatile fatty acid (VFA) and ion concentration, were evaluated for both reactor set-ups. Based on the potential methane yield, an economic analysis was conducted to establish the economic feasibility of gas utilization technologies, such as a biogas boiler and combined heat and power unit (CHP).

The results of the characterization of the leachate revealed many challenges for its treatment with UASB reactors, such as low pH, zero alkalinity, low cation concentrations and elevated C/N ratio. Treating filtered leachate via UASB reactors showcased superior performance in methane yield and process stability—manifesting resilience against pH perturbations—compared to the reactor treating the raw leachate. The economic analysis favored investment in a CHP unit, reflecting a positive net present value (NPV), whereas the heat boiler exhibited a negative NPV. These findings underscore the potential of leachate treatment to advance Wastech's objectives in sustainable waste management, profitability, and attainment of energy autonomy. Nonetheless, further strategic refinements and comprehensive evaluations of reactor performance and stability are imperative preludes to the industrial-scale deployment of UASB reactors.

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| ABR | Anaerobic Baffled Reactor |
| AD | Anaerobic Digestion |
| AF | Anaerobic Filter |
| AnMBR | Anaerobic Membrane Bioreactor |
| ATP | Adenosine Triphosphate |
| BOD | Biological Oxygen Demand |
| BMP | Biomethane Potential |
| BSFL | Black Soldier Fly Larvae |
| CHP | Combined Heat and Power |
| COD | Chemical Oxygen Demand |
| CSTR | Continuous Stirred Tank Reactor |
| DIET | Direct Interspecies Electron Transfer |
| EC | Electrical Conductivity |
| EET | Extracellular Electron Transfer |
| EGSB | Expanded Granular Sludge Bed |
| EPM | Extracellular Polymeric Matrix |
| EU | European Union |
| FLI | Food Loss Index |
| FPI | Food Processing Industries |
| FVW | Fruit and Vegetables Waste |
| F2F | Farm-to-Fork |
| GC | Gas Chromatography |
| GHG | Greenhouse Gasses |
| HPLC | High Performance Liquid Chromatography |

| | |
|---|---|
| HRT | Hydraulic Retention Time |
| IC | Ion Chromatography |
| IRR | Internal Rate of Return |
| LCFA | Long Chain Fatty Acids |
| NPV | Net Present Value |
| OLR | Organic Loading Rate |
| PEC | Purchased Equipment Cost |
| SAB | Syntrophic acetogenic bacteria |
| SRB | Sulphate Reducing Bacteria |
| SRT | Sludge Retention Time |
| TCI | Total Capital Investment |
| TS | Total Solids |
| UASB | Upflow Anaerobic Sludge Bed |
| VFA | Volatile Fatty Acids |
| VS | Volatile Solids |
| WWTP | Wastewater Treatment Plant |

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# PART I. INTRODUCTION

Climate change, induced by human activity, is a significant environmental problem. On aspect of climate change is the proliferation of greenhouse gasses (GHG) trapping heat in the atmosphere. Therefore, reducing the amount of GHG emissions emitted into the atmosphere is vital in mitigating global warming and climate change. The European Union has committed - via the European Green Deal - to turning the EU into the first climate neutral continent by 2050 and pledges to reduce emissions by at least 55% by 2030, compared to 1990 levels (Delivering the European Green Deal - European Commission (n.d.)).

The energy industry accounts for roughly more than 75% of total GHG emissions globally (*Energy System Struggles in Face of Geopolitical and Environmental Crises | Energy Institute*, n.d.), emphasizing the need to shift to renewable energy and reduce energy consumption. The European Commission proposed to increase the binding target of renewable sources in the EU's energy mix to 32% (Directive 2018/2001). In addition, to reduce energy consumption, the Commission proposed to increase energy efficiency targets to achieve an overall reduction of 36-39% for final and primary energy consumption (*Delivering the European Green Deal - European Commission*, n.d.). In response to the difficulties in the global energy market caused by the Russian invasion of Ukraine, the European Commission presented the RepowerEU Plan, which focuses on energy savings, clean energy production and diversification on energy supplies. It aims to make Europe independent of Russian fossil fuels by 2030, by boosting the biomethane production, as well as increasing renewable energy targets from the current 40% to 45% (Calero et al., 2023).

Besides global warming, another emerging threat to the wellbeing of the entire biosphere is the chemical pollution of our ecosystems. Wastewater pollution makes up for the largest percentage of pollution worldwide. Globally an estimated 80% of wastewater, including human sewage, is discharged into the environment without treatment releasing an array of harmful contaminants into all water bodies, causing direct harm to people and the ecosystems. Moreover, the availability of safe fresh water sources is endangered, due to the untreated release of wastewater and global warming. Thus, wastewater treatment stands pivotal to protect water supplies to ensure availability of potable water and to protect ecosystems and human health (Anekwe et al., 2022). Wastewater treatment plants (WWTP) consume large amounts of energy, estimated at between 1% and 3% of global energy output (Gherghel et al., 2019). However, studies have shown that wastewater contains nearly five times the amount of energy that is needed for the process of treating it (Filmore, 2014). Wastewater treatment facilities therefore have the potential to produce energy (Eib, 2022).

The food production industry is a major contributor to climate change and chemical pollution, with 10 million hectares deforested every year for food production and one third of the food produced goes to waste before reaching the consumer. This waste holds potential for resource recovery and conversion into valuable commodities. For instance, black soldier fly larvae can efficiently convert plant-based organic food processing waste into a valuable protein source for local animal feed. By doing so, food waste is reduced and our reliance on soy-based animal feed decreases (Xu et al., 2018).

Anaerobic digestion (AD) serves as a crucial technology for managing diverse organic waste streams, including both liquid and solid substrates. Beyond waste management, AD holds the distinct capability of

recovering resources, such as energy and nutrients. Through this biochemical process, microorganisms break down organic substances in the absence of oxygen, producing biogas and digestate. Biogas, primarily composed of methane and carbon dioxide, can be used to produce either heat and electricity or refined into biomethane, a gas substitute. The digestate can be used as biofertilizer, offering a potential additional income for many biogas facilities across the world (Chozhavendhan et al., 2023).

The AD process can contribute to face the challenges posed by the energy industry, the food industry and the wastewater treatment industry. It can provide renewable energy based on biomass, it revalorizes the food waste that would otherwise go to landfill or be incinerated, and it treats wastewater, without consuming large amounts of energy. Compared to conventional WWTP, AD consumes less energy, since the biogas recovery by AD of the sludge can provide between 39% to 76% of the total energy consumed in the WWTP (Soares et al., 2017). Theoretically 1 $m^3$ of wastewater can produce 0.14 $m^3$ of methane at standard operating conditions, which is equivalent to 5.026 MJ of energy. On average 380 billion $m^3$ of wastewater is produced globally, thus if all this wastewater is treated via AD, it would generate 212.2 billion kWh of power and the WWTP would no longer require external energy input (J. Sun et al., 2023).

Anaerobic digestion technologies have a relevant role in transforming the linear economy model by integrating circularity (González et al., 2022). The AD of wastewater offers four important aspects in this road towards a more sustainable and circular society; it stabilizes wastewater, generates energy from a renewable resource, recovers nutrients and enables water reuse. Hence, AD plays a pivotal role within the framework of the European Green Deal.

# PART II. LITERATURE REVIEW

## 1. ANAEROBIC DIGESTION

### 1.1. INTRODUCTION

Anaerobic digestion (AD) is a biological process in which biomass in the form of solid fraction, slurries, or suspended material, is microbially converted to biogas in the absence of oxygen. It is a popular waste treatment practice since it combines pollution control and energy recovery. The AD offers many advantages such as biogas production, organic waste stabilization, low cell yield, high organic loading rates, limited nutrient demands and low costs for operation and maintenance (Y. Chen et al., 2008).

Biogas, produced during AD, is a mixture of carbon dioxide (10-60%) and methane (40-75%), and is a renewable energy source. It is mainly used for the production of heat, steam and electricity because these approaches do not require any pre-treatment of the biogas. In general, 1 kg of carbohydrates generates 0,36 $m^3$ of methane (0.5 $m^3$ of biogas), which yields on average about 1 kWh of electrical energy (Pham et al., 2006). Biogas can also be used as a gas-substitute after being desulfurized and purified. The biogas can also be upgraded by reducing the concentration of carbon dioxide, increasing the energy content of the gas. This yields an increased level of methane and is called biomethane. Raw biogas usually contains between 50-75% methane and 25-45% carbon dioxide, whilst biomethane contains up to 99.9% methane and only 0.1-4% carbon dioxide.
Biogas is considered a renewable energy source, since the organic material used for the AD process is renewable. The methane can be combusted more cleanly than coal and provides the desired energy with lower levels of carbon dioxide in the atmosphere (Bharathiraja et al., 2018).

A second product that is produced during AD is called digestate. This can be used as a high-value fertilizer for crop cultivation, since it is rich in both macro- and micro nutrients, and can replace the common mineral fertilizers. The digestate has improved flow properties, and thus soil penetration increases which reduces the chance of nitrogen loss (Bharathiraja et al., 2018). However, digestate from wastewater treatment plants is not used for crop cultivation because of pathogen risks. Like biogas, the digestate needs to be treated before it can be applied. The direct discharge of digestate without proper treatment could be detrimental to the quality of the receiving environment and lead to GHG emissions. However, these extensive treatments can be costly and even though efforts have been made to recover the constituents in digestate to realize the circular economy, several studies have shown that the biorefinery process for digestate management is still underdeveloped (W. Wang & Lee, 2021).

### 1.2. STOICHIOMETRY

Theoretically, the amount of methane or biogas that can be produced from a given type of organic compound, is presented by the Buswell equation, assuming 100% material breakdown. For example, 1 kg of glucose can produce 373 L of methane theoretically.

$$C_nH_aO_bN_cS_d + \left(\frac{4n - a - 2b + 3c + 2d}{4}\right)H_2O$$
$$\rightarrow \left(\frac{4n + a - 2b - 3c - 2d}{8}\right)CH_4 + \left(\frac{4n - a + 2b + 3c + 2d}{8}\right)CO_2 + cNH_3 + dH_2S$$
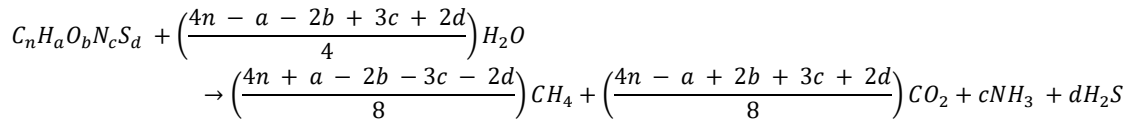
However, in practice, this is more complex since part of the organic matter is not biodegradable under the conditions or the time period of the anaerobic digester. The waste streams that are treated with AD processes are also complex, variable and heterogeneous, making it nearly impossible to determine the exact composition at any point in time. Moreover, other factors, such as inhibitors, might be present in the reactor influencing the conversion process (Banks, 2009).

## 1.3. MICROBIAL PROCESSES

The AD process mainly consists of four successive stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Different organisms carry out the different stages and the digestion process is dependent on the interactions between the diverse microorganisms (Figure 1).
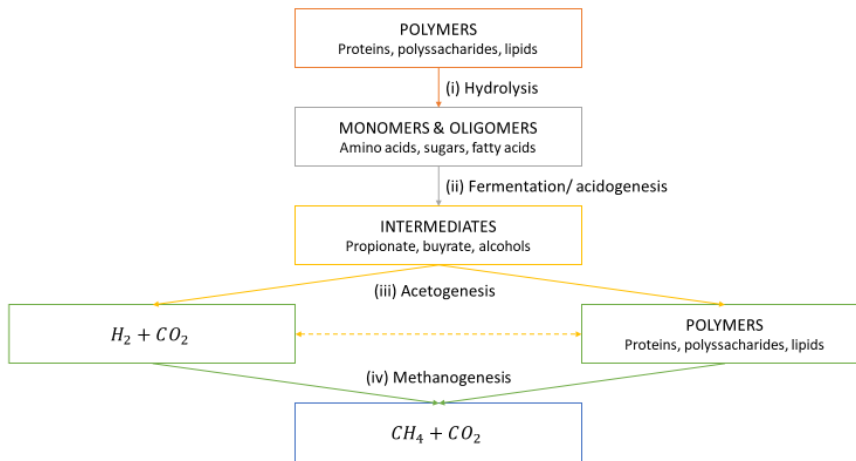


**Figure 1.** Four microbial processes of anaerobic digestion

### 1.3.1. HYDROLYSIS

The first step is hydrolysis  during which complex polymers are broken down into monomers and oligomers. Hydrolytic bacteria (such as *Clostridium* or *Bacteroides*) are able to secrete extracellular enzymes that can convert complex polymers, such as carbohydrates, lipids, proteins etc. into sugars, long chain fatty acids and amino acids. The breakdown of the polymeric substances occurs through hydroxylation or hydrogenation in which the glycosidic bond is broken. Energy, in the form of ATP, is consumed during this step. After the enzymatic cleavage, the monomers and oligomers are able to diffuse through the cell membranes of acidogenic microorganisms (Y. Li et al., n.d.; L. Lin et al., 2010).

Some substrates, such as lignin are hard to break down and can be inaccessible to microbes, due to their complex structures. Thus, hydrolysis is often the rate-limiting step of AD. Several solutions exist to increase the speed of hydrolysis, such as adding enzymes to the reactor, implementing a pretreatment step, or

increasing pressure or temperature of the reactor. In general, the optimal temperature for hydrolytic bacteria is 30-50°C with a pH optimum of 5-7 (Luo et al., 2012; Ma et al., 2013; Meegoda et al., 2018).

### 1.2.2. FERMENTATION/ ACIDOGENESIS

Next the fermentation or acidogenesis occurs, where the formed monomers and oligomers are converted into intermediates by acidogenic bacteria (For example *Bacteroides, Clostridium* or *Escherichia*). These products from the hydrolysis steps are able to diffuse through the cell membranes of acidogenic microorganisms, which are able to produce volatile fatty acids (VFA) and other products. The VFA are a class of organics containing acetates (C2) and larger organic acids such as propionate (C3) or butyrate (C4). During the acidogenesis step energy is generated through substrate level phosphorylation (ATP synthesis), which allows microorganisms to grow. Acidogenesis is believed to be the fastest step of the four AD stages, with acidogenic bacteria having a regeneration time of fewer than 36h (Meegoda et al., 2018).

### 1.2.3. ACETOGENESIS

With the production of acetate during acidogenesis, a part of the original substrate has already been converted into a substrate suitable for methanogenesis. However, the other products from acidogenesis need to be further broken down to become available for methanogens. Acetogenesis is the process during which these VFA and other intermediates are converted into acetate (C2) and hydrogen by syntrophic acetogenic bacteria (SAB). It is not always possible to draw a clear distinction between acetogenic and acidogenic reactions, since hydrogen and acetate are produced during both steps and both of them are substrates for methanogenic archaea (Bajpai, 2017; Meegoda et al., 2018).

The hydrogen, the electron transfer, produced during this step is responsible for an interesting syntrophic relationship present in AD between the acetogens and methanogens. However, acetogenesis is inhibited by the accumulation of hydrogen. Thus, the hydrogen partial pressure should be low enough so that the thermodynamics become favorable for conversion of volatile acids and alcohols to acetate (Dinopoulou et al., 1988; Fournier & Gogarten, 2008).

### 1.2.4. METHANOGENESIS

The last step is methanogenesis where a gas mixture of methane and carbon dioxide is produced, known as biogas, by methanogens. Three key pathways occur during methanogenesis; hydrogenotrophic, acetoclastic and methylotrophic, during which different substrates are consumed, namely hydrogen and $CO_2$, acetate and methanol respectively. The methanogenic step is the point at which the organic pollution load, expressed in chemical oxygen demand (COD) or biological oxygen demand (BOD) is significantly reduced since in the preceding stages the different organic molecules are merely converted from one form to another (Bajpai, 2017; Ferry, 2010; Meegoda et al., 2018).

Methanogenic microorganisms represent a group of obligate anaerobic archaea and can only assimilate a narrow array of simple substrates. They are slow growing, are extremely sensitive to the changes in the environment and are vulnerable to even small amounts of oxygen (Kiener & Leisinger, 1983). There are two key genera of methanogens, namely the *Methanosarcina* and *Methanotrix*. The first genus has a spherical shape, consumes hydrogen, acetate and C1-donor (such as methanol or methylamine) and is quite tolerant

to pH changes (optimal pH between 5-8). The second genus has a filamentous shape, consumes acetate as electron donor and is quite sensitive to pH (optimal pH between 6.5-8.5). They also have different growth strategies. The *Methanosarcina* are so-called r-strategists; they have a high maximum specific growth rate and a high half-saturation coefficient. On the other hand, the *Methanotrix* are K-strategists meaning they have a low maximum specific growth rate and a low half-saturation coefficient. This results in a *Methanosarcina* dominance at high acetate levels and unstable conditions, and a *Methanotrix* dominance at low acetate levels and stable conditions (Conklin et al., 2006).

## 1.3. OPERATIONAL CONDITIONS

The success of the AD process is very dependent on the operational conditions of the reactor and can suffer from process instability, which could limit the wider application of the process for energy production. For instance, a study of 20 centralized biogas plants in Denmark showed frequent periods of instability that lasted for several weeks or months, during which biogas production was reduced by 20-30%. It is important to understand how the different operational conditions influence the process and help to achieve stable and effective biogas production (D. Wu et al., 2019).

### 1.3.1. SUBSTRATES

Different substrates can be used to convert organic pollutants to biogas. These different substrates significantly influence the reactor configuration, both design and operational considerations, and have an influence on the microbial physiology. For instance, lignin degradation is hardly noticeable under anaerobic conditions and cellulose breakdown is very slow, lasting several weeks. Hemicellulose, fat, and proteins are degraded in a couple of days, whilst low molecular sugars, VFA's and alcohols show rapid degradation, lasting only a few hours.

Moreover, the substrate influences the quality of the biogas, quality of the effluent and the necessity of pre- or post-treatment. The quality of the substrates can influence the economic viability of the AD installation because the substrate may need expensive pre-treatment or may produce low quality biogas or digestate needing expensive post-treatment (Steffen et al., 1998).

### 1.3.2. PH AND ALKALINITY

The AD process is the result of joint work between several groups of microorganisms. However, two groups of these microorganisms have different optimal pH ranges making the process very sensitive to fluctuations in pH. The best pH range for acidogenic bacteria is 5.5-6.5, and for methanogenic archaea this is 7.8-8.2. Therefore, it is necessary to keep the reactor pH close to neutral. Low pH will inhibit the methanogens, and will reduce biogas production, and cause accumulation of hydrogen and VFA in the reactor. The latter will cause the drop in pH even further, causing the reactor to fail.

If the pH increases beyond 7.5, the microorganisms still grow, but the degree of metabolism seems to be reduced. This might be due to the fact that the key nutrients or trace metals are precipitated as the pH increases limiting metabolism or an increased concentration of free ammonia at high pH is present, which is discussed further below (Meegoda et al., 2018).

The AD process possesses natural alkalinity, since all weak acid/base systems present in the digesters contribute to the buffer capacity. This commonly includes carbonate, ammonium, phosphate, VFA's and sulfide subsystems. Generally, the carbonate subsystem has the most influence on the cumulative buffer intensity of AD due to its high concentration. Degradation of the organic matter results in the release of $CO_2$. The $CO_2$ can subsequently react with ammonium, which is released from protein degradation, to produce ammonium bicarbonate as alkalinity. Salts of organic acids release cations when the acids are metabolized, which also can react with $CO_2$ to produce bicarbonates. Sulfite and sulfate reduction can also produce alkalinity (Nguyen et al., 2015; H. Sun et al., 2016). Appels et al. (2008) reported that the relationship between VFA and $HCO_3$ concentrations of a well buffered digester should be at least 1.4:1 (molar ratio) of $HCO_3$/VFA or a buffering capacity of 70 meq $CaCO_3$/L. Zhang et al. (2014) reported that methane production was inhibited completely when the concentrations of VFA in the reactor exceeded 5800 mg/L.

To ensure the stability of the AD process, pH should be monitored throughout the whole process. If the natural alkalinity of the system is not enough to buffer the system pH, external buffers can be added. Chemical buffers help in maintaining optimal pH levels within the reactor, which ensures the stability of the reactor. However, relying solely on chemical buffers for pH control may create dependency on external inputs, leading to a lack of resilience in the reactor. Chemical buffers are also expensive which increases to the operational costs of anaerobic digesters specially for large-scale operations. Chemical buffers can interreact with other components in the AD process affecting microbial activity or the composition of biogas produced (Meegoda et al., 2018; H. Sun et al., 2016). Different buffers have been applied in AD reactors, such as a sodium bicarbonate buffer (J. Wu et al., 2020), calcium carbonate buffer (Salek et al., 2015), and phosphate buffer (Loganath & Mazumder, 2018).

### 1.3.3. TEMPERATURE

Temperature is a crucial environmental parameter influencing the rate of the AD process. The AD process has three known operating temperature ranges, namely psychrophilic (5-15°C), mesophilic (35-40°C) and thermophilic (50-55°C). During the digestion process it is important to keep a constant temperature, as temperature fluctuations will negatively influence the biogas production (Moset et al., 2015).

Operating at thermophilic temperatures allows a higher rate of hydrolysis and is associated with the inhibition of pathogens present in the waste. High temperatures reduce retention times, making the process faster and more efficient. However, at temperatures between 40-50°C, methanogens can be inhibited, which results in a decrease in biogas production and acidification of the reactor. Other disadvantages during thermophilic AD include decreased stability, low-quality effluent, increased toxicity and susceptibility to environmental conditions, larger investments and higher net energy input (Hartmann & Ahring, 2006).

Operating at mesophilic temperature results in lower reaction rates than at thermophilic temperature, however the mesophilic systems exhibit better process stability and higher richness in bacteria. Mesophilic systems are also more attractive because of their lower heater energy costs compared to thermophilic

temperatures. Therefore, most of the industrial scale AD processes operate in mesophilic temperatures (Mao et al., 2015).

Operating at psychrophilic temperatures is very challenging; The low temperature leads to a decreased bacterial metabolism and bacterial reaction kinetics. The psychrophilic AD process is unstable and the biogas production is low (B. R. Tiwari et al., 2021).

### 1.3.4. C/N RATIO

The C/N ratio reflects the nutrient levels of a certain feedstock. A high C/N ratio induces a low solubilization rate and leads to low total ammonia nitrogen and low free ammonia concentrations in the digester. If the C/N ratio is too high, there is insufficient nitrogen to maintain biomass, reducing biogas production. When the C/N ratio is too low, the risk of ammonia inhibition is increased, which is toxic to methanogens. The optimal C/N ratio for AD is between 20-35, with the ratio of 25 the most commonly used (Mao et al., 2015; Puñal et al., 2000).

### 1.3.5. ORGANIC LOADING RATE (OLR)

The OLR is the amount of volatile solids fed into the digester per day under continuous feeding. With an increasing OLR, the biogas production will also increase to an extent, until an equilibrium is reached. Bacterial inhibition can occur when the OLR is too high, leading to a higher hydrolysis/ acidogenesis activity and therefore increased VFA production. If the methanogens cannot consume this excess of VFA, the VFA will accumulate in the reactor, leading to a decrease in pH, which further inhibits the AD process (Mao et al., 2015).

The AD processes at industrial scale are often run at suboptimal OLR levels to avoid the overload inhibition. This results in significant oversizing of reactors and low biogas yield with corresponding high costs, compromising the economic viability of the technology (D. Wu et al., 2019). To address this issue, thermophilic operating conditions coupled with effluent regeneration have been explored as potential solutions to relieve the overload inhibition (Mao et al., 2015).

### 1.3.6. SOLIDS RETENTION TIME (SRT) AND HYDRAULIC RETENTION TIME (HRT)

The SRT indicates the average time the solids (bacteria) spent in the digester. A decrease in SRT decreases the extent of the reactions and vice versa. Each time sludge leaves the reactor through the effluent, a fraction of the bacterial population is removed which should be compensated by the cell growth to avoid process failure (Nges & Liu, 2010).

The HRT indicates the average time the liquid sludge is held in the digester. The retention time is the time required to complete the degradation of organic matter. Obtaining an effective HRT depends on the feedstock composition and OLR, where typically 15-30 days are necessary. Decreasing HRT usually leads to VFA accumulation, whereas a longer than optimal HRT results in insufficient utilization of digester components (Appels et al., 2008; Mao et al., 2015).

## 1.4. INHIBITORY FACTORS

A wide variety of substances have been reported to be inhibitory to the AD processes. A material is defined as inhibitory when it causes an adverse shift in the microbial population or inhibition of microbial growth. Inhibition is usually indicated by a decrease of the steady-state rate of methane gas production and accumulation of organic acids, decreasing the pH (Y. Chen et al., 2008).

### 1.4.1. AMMONIA

Ammonia is produced during the biological degradation of nitrogenous matter, mostly in the form of proteins and urea. Several mechanisms for ammonia inhibition have been reported such as the change in intracellular pH, increase of maintenance energy requirement, and inhibition of specific enzymatic activity. Ammonium ion ($NH_4^+$) and free ammonia ($NH_3$) are the most common forms of ammonia in the AD reactor (Wittmann et al., 1995).

The $NH_3$ is membrane permeable and can diffuse passively into the cell, causing proton imbalance and potassium deficiency. It has been reported that among all AD microorganisms, the methanogens are the most sensitive to ammonia inhibition . Concentrations of ammonia should not surpass 50 mg $NH_3$-N/L. An increasing pH also affects the ammonia inhibition, since at high pH the ammonium (pKa = 9.25) will be mostly present in the form of $NH_3$, which is the actual toxic agent.  An increased process temperature also results in a higher concentration of $NH_3$ (Y. Chen et al., 2008).

### 1.4.2. SULFATE AND SULFIDE

Many industrial wastewaters are rich in sulfate, which is reduced to sulfide during the AD process by sulfate reducing bacteria (SRB). The SRB consist of incomplete oxidizers, which reduce compounds such as lactate to acetate and $CO_2$, and complete oxidizers, which completely convert acetate to $CO_2$ and $HCO_3^-$. Two kinds of inhibition exist as a result of sulfate reduction namely, primary and secondary inhibition (Hilton & Oleszkiewicz, 1988; Koster & Lettinga, 1984).

Primary inhibition is due to competition for common organic and inorganic substrates from SRB, which suppress methane production. SRB compete with all different types of microorganisms in the AD process, namely the methanogens, acetogens, and fermentative microorganisms for available acetate, hydrogen, propionate and butyrate. Thus, sulfate reduction by SRB consume electrons, meaning these electrons won't be available to produce methane (Harada et al., 1994).

Secondary inhibition is due to the toxicity of sulfide to various bacteria groups. $H_2S$ is the most toxic form of sulfide, because, like $NH_3$, it can freely diffuse through the membrane into the cell. Once inside the cell, $H_2S$ is responsible for denaturing native proteins, by forming sulfide and disulfide cross-links between polypeptide chains, interfering with the various coenzyme sulfide linkages, and interfering with the assimilatory metabolism of sulfur. Moreover, the production of $H_2S$ leads to the precipitation of metals causing potential nutrient deficiency. When $H_2S$ is present in biogas, it may cause corrosion of the equipment (Y. Chen et al., 2008).

### 1.4.3. ORGANIC MICROPOLLUTANTS

Many different organic compounds can inhibit AD, such as benzenes, phenols, alcohols, ethers, aldehydes, ketones, acrylates, carboxylic acids, nitriles, amides, pyridine and some long chain fatty acids (LCFA). Organic chemicals are poorly soluble in water or are adsorbed to the surface of sludge or solids and may therefore accumulate to high levels in AD reactors. The accumulation of apolar toxicants in bacterial membranes causes the membrane to swell and leak, disrupting ion gradients and eventually causing cell lysis (Heipieper et al., 1994; Sikkema et al., 1994).

The inhibition concentration ranges vary widely for specific toxicants and the toxicity of the compounds can depend on toxicant concentration, biomass concentration, toxicant exposure time, cell age, feeding pattern, acclimation and temperature. In general, the higher the biomass concentration, the more robust the reactors are in the presence of toxic shocks. Acclimation is important, where microorganisms can adapt to the presence of toxic chemicals, through induction of specific enzymes, genetic engineering etc. (Y. Chen et al., 2008).

### 1.4.4. LIGHT AND HEAVY METALS

Both light metals, including Na, K, Mg, Ca and Al, and heavy metals, including Cr, Fe, Co, Cu, Cd and Ni, can cause toxicity in AD reactors. Both groups are required for microbial growth, and affect specific growth rate, however when they are present in excessive concentrations they slow down growth and can eventually cause severe inhibition (Y. Chen et al., 2008).

High salt concentrations cause bacterial cells to dehydrate due to osmotic pressure. These salts may be released by the breakdown of organic matter or added as pH adjustment chemicals (buffers). Heavy metals can cause the disruption of enzyme function and structure binding of the metals with thiol and other groups on protein molecules or by replacing naturally occurring metals in enzyme prosthetic groups (Y. Chen et al., 2008).

## 1.5. REACTOR TYPES FOR LIQUID STREAMS

The AD process can take place in many different reactor configurations. The configuration that is chosen often depends on the composition of the feedstock, microbial requirements and environmental temperature (Uddin & Wright, 2023).

The **continuous stirred tank reactor** (CSTR), shown in Figure 2, is a simple system without biomass recirculation. Mixing is very important in this type of reactor and the amount of mixing determines its success or failure. Mixing guarantees the distribution of nutrients and microorganisms, inoculation of fresh feed, homogenization of the material, removal of end products and ensuring a constant temperature throughout the entire reactor. However, too much mixing can disturb syntrophic interactions and foam formation. A typical OLR for a CSTR is between 3-10 kg COD/$m^3$*day. Here, the HRT is equal to the SRT, between 20-60 days (Loganath & Senophiyah-Mary, 2020).
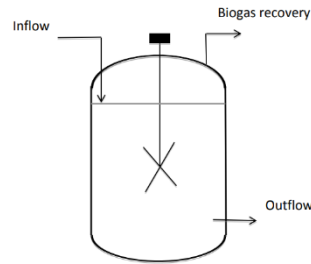
**Figure 2.** Continuous Stirred Tank Reactor model (Merlin & Boileau, 2013)

Decoupling HRT and SRT is one of the main advantages of the following reactors, where HRT is lower than SRT. This avoids the washout of slow growing anaerobes and still allows the treatment of high amounts of high strength feedstock in less time (Loganath & Senophiyah-Mary, 2020).

The **upflow anaerobic sludge bed** (UASB) reactor (Figure 3) is a vertical configuration in which three phases are separated, namely liquid, biogas and biomass. The reactor operates in an upflow mode and there is formation of microbial granules. The anaerobic granules are structures in which the concentric microorganisms live together in different layers. The most sensitive microorganisms, namely methanogens reside in the inner core of the granules. Acetogens are generally more prevalent in the middle section. In the outer layer, more aerotolerant microorganisms such as fermentative microorganisms can be found. The granule structures offer protection for anaerobic microorganisms that are essential to the AD process. Both salinity and excessive presence of solids in the reactor can negatively affect granular formation (Abbasi & Abbasi, 2012; Lettinga, 1995; J. Li et al., 2014).

The upflow velocity is usually not more than 1 m/h and baffles are located at the top of the reactor to avoid sludge washout. The UASB reactor is able to digest higher OLR rates, namely 15-20 kg COD/m$^3$*day than the CSTR (Ghangrekar et al., 2005; Lettinga & Hulshoff Pol, 1991).
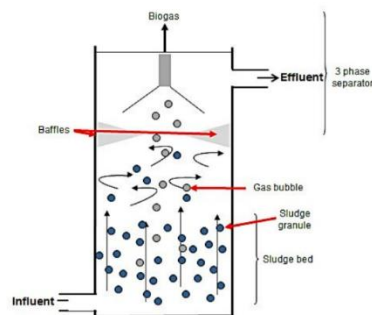


**Figure 3.** Upflow Anaerobic Sludge Bed reactor model (Anijiofor-Ike et al., 2017)

The **expanded granular sludge bed** (EGSB) reactor (Figure 4) is a modification of the UASB reactor. The EGSB is longer, more extended, contains more biomass, can operate at lower temperature (10°C) and can deal with higher OLR than the UASB reactor, up to 30 kg COD/m$^3$*d. The EGSB doesn't contain baffles as the UASB but has a broader top to decrease the velocity and avoid granule washout. The EGSB has a recycling line that stimulates the expansion of the sludge bed. Thanks to the recycling line, the upflow

velocity is higher with 6-15 m/h. To avoid sludge washout, membranes can be introduced (Chu et al., 2005; Lettinga, 1995).
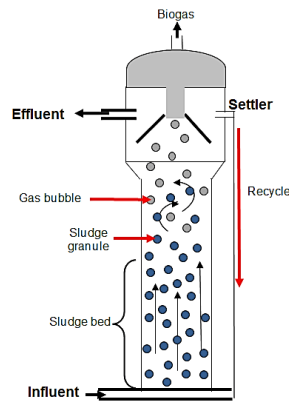


**Figure 4.** Expanded Granular Sludge Bed reactor model (Anijiofor-Ike et al., 2017)

The **internal circulation** (IC) reactor (Figure 5) has evolved from the UASB and EGSB. It is a long vertical reactor, like the EGSB, and has internal structures to decrease the velocity and avoid granule washout, like the UASB. The IC has two internal structures to limit granule washout, one in the middle of the reactor and one at the top, separating the reactor into two zones; The first zone is the high intensity zone with high loading rates and the second zone is the polishing zone where the loading rates are lower and the biogas is efficiently separated. The OLR of an IC reactor is usually between 15-30 kg COD/m$^3$*d, with an upflow velocity of 20-30 m/h (Mutombo, 2004 ; Pereboom & Vereijken, 1994).
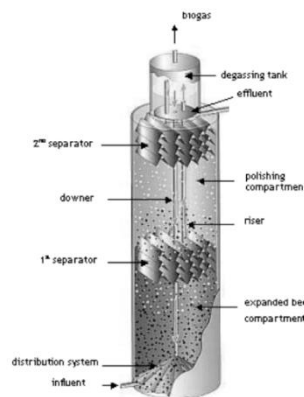


**Figure 5.** Internal Circulation reactor model (Mutombo, 2004)

The **anaerobic filter** (AF) is a reactor that contains a biofilm where the biomass is immobilized. The biofilm grows on an inert material like polyethylene or polypropylene with a high specific surface area. The higher the specific surface area, the higher treatment capacity, however this material is quite expensive. Different feeding modes are possible, namely Upflow Anaerobic Filter (UAF), Downflow Anaerobic Filter (DAF) and Multi flow Anaerobic filter (MAF). This configuration cannot treat high OLR, between 5-10 kg COD/m$^3$*day (Karadag et al., 2015; Loganath & Senophiyah-Mary, 2020; Rajinikanth et al., 2009).

The **anaerobic baffled reactor** (ABR) is a reactor that is divided into 4 horizontal stages. Biomass is retained by physical separation in the different stages. This configuration allows the separation of hydrolysis/ acidogenesis, the first two stages, and acetogenesis/ methanogenesis, last two stages. The first stages can therefore act as a buffer against inhibitory components and acidification due to overloading, protecting the sensitive methanogens. This configuration can withstand shock loads and the OLR can be up to 36 kg COD/ $m^3$*day (Bachmann et al., 1985; Barber & Stuckey, 1999).

The **anaerobic membrane reactor** (AnMBR) is the combination between anaerobic biological treatment and membrane separation. The membranes prevent biomass washout of slow growing microorganisms particularly the methanogens. The membranes can be either external or internal submerged membranes. This allows a mediocre OLR, up to 10 kg/ $m^3$*day, high mixing intensities, increased COD removal, improved biogas production and reduced system footprint. However, the membrane fouling requires regular cleaning (H. Lin et al., 2013).

## 1.6. GRANULATION PROCESS IN UASB REACTOR

### 1.6.1. INTRODUCTION

The granulation process, *i.e.,* the formation of microbial aggregates in AD reactors has allowed the successful introduction of the UASB reactor as a form of anaerobic treatment of industrial wastewaters. These microbial aggregates have the same composition as the traditional biofilm, but develop without carrier material (Lettinga, 1995).

The formation of aggregates has proven to be essential to the stable operation of biological systems. It has allowed much higher loading rates in UASB reactors, far beyond common loading rates applied thus far in conventional activated sludge processes. This has resulted in a reduction in reactor size and required area for the treatment, decreasing investment and operational costs (Hulshoff Pol et al., 2004; Yan & Tay, 1997).

The success of the granulation process is mainly due to these two factors (Hulshoff Pol et al., 2004);

- Granules have superior settling characteristics. The settling velocity of granular sludge is approximately 60 m/h, whilst the upflow velocity of a UASB reactor is usually 1 m/h. This allows extreme decoupling of the HRT from the SRT.

- Granular sludge shows high methanogenic activity. Studies on the micromorphology of the granules demonstrated that acetogenic bacteria are closely linked to hydrogenotrophic methanogenic archaea allowing efficient interspecies hydrogen transfer, resulting in high degradation rates.

The microbial aggregates formed during the granulation process can differ considerably due to the complexity of microbiological and physico-chemical interactions: as yet there is still no consensus about the determining mechanism triggering granulation. Multiple theories exist on anaerobic sludge granulation, mostly organized in three groups; physical, biological and thermodynamic (Hulshoff Pol et al., 2004).

Individual theories to describe anaerobic granulation, such as the Spaghetti theory (1987), the Selection Pressure theory (1983), and Multi-Layer Model, are not able to conclusively depict the entire granulation

process. Based on existing mechanisms for the formation of granules, a four-based general model for anaerobic digestion has been proposed by Liu et al. (2003);

The first step involves physical movement to initiate interbacterial and bacteria- to-archaea contact or bacterial attachment onto nuclei, with the contribution of multiple forces such as hydrodynamic force, diffusion force, gravity force, thermodynamic forces (e.g., Brownian movement), and cell mobility.

This is followed by the second step, which focuses on initial attractive forces to maintain stable multicellular contacts, including physical forces like Van der Waals forces and opposite charge attraction, as well as chemical forces such as hydrogen liaison and biochemical forces like cellular surface dehydration. The hydrophobicity of bacterial surfaces is highlighted as crucial for biofilm and anaerobic granule initiation.

Step three outlines microbial forces that mature cell aggregation, involving the production of extracellular polymers (namely Extracellular Polymeric Matrix, EPM), growth of cellular clusters, and metabolic changes induced by the environment.

Step four describes the formation of a steady-state three-dimensional structure of microbial aggregates shaped by hydrodynamic shear forces, where the outer shape and size are determined by the interactive strength between aggregates and hydrodynamic shear force, microbial species, and substrate loading rate. This four-step model aims to comprehensively address the understanding of anaerobic granulation, acknowledging the challenge of identifying molecular or genetic events and emphasizing the complexity of community development.

## 1.6.2. GENERAL CHARACTERISTICS OF GRANULES

Researchers have been trying to visualize the surface and internal structure of UASB granules by scanning electron microscopy and transmission electron microscopy respectively. In 1992, the multi-Layer structural model (Figure 6) was proposed by MacLeod and Guiot, suggesting that the granule is composed of different layers with each their own microbial activity (Guiot et al., 1992; MacLeod et al., 1990). The inner layer mainly consists of methanogens that may act as nucleation centers necessary for the initiation of granule development. Hydrogen-producing and -consuming bacteria are dominant species in the middle layer, and a mixed species including rods, cocci, and filamentous bacteria take predominant position in the outermost layer. This positioning of the different microorganisms in different layers allows the conversion of target organics into methane (Liu et al., 2002, 2003).
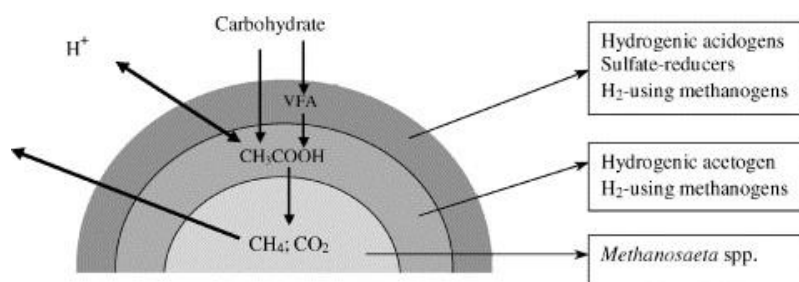


**Figure 6**. Schematic representation of the multi-layer model (Guiot et al., 1992)

The metabolic activity of anaerobic granules can be represented by the methanogenic activity and can be used to evaluate the performance of a system or used as an indicator of toxic inhibitory effects on anaerobic

granules. The methanogenic activity depends on operational conditions such as the OLR and the wastewater type (Liu et al., 2002).

In general, anaerobic granules have a brownish or blackish color. The color of the granules depends on the OLR and upflow liquid velocity, namely at low OLR and liquid upflow velocity, granules become lighter (gray or even white) with a hollow core. The texture of light-colored granules is soft and very weak mechanical stress can break up the wall of the granules. At high OLR and upflow liquid velocity, granules remain dark black and have a dense structure. Thus, changes in granule color reflect changes in metabolism and composition of granules (Kosaric et al., 1990; Liu et al., 2002).

The density of anaerobic granules represent the compactness of the microbial community.  The size of granules has a dual effect on the performance of a UASB reactor; If the size of the granule is too small, the probability of washout would increase, decreasing the stability of the system.  In general, higher density leads to faster settling velocity of sludge. When the size of large granules increases further, the efficiency of mass transfer inside the granules decreases. The size and density of anaerobic granules depend on many factors such as hydrodynamic conditions, OLR, and microbial species present. In industrial practice, a narrow size distribution of granules is preferred with medium-size granules with a diameter of 1.0-2.0 mm. In UASB reactors, granules can grow up to 2-5 mm or even bigger (J. Chen & Lun, 1993; Liu et al., 2002; Pereboom & Vereijken, 1994).

The strength of granules influences the stability of granules, with a higher strength reflecting a more compact and stable structure of anaerobic granules. Research to quantify the mechanical strength of anaerobic granules was done by Quarmby & Foster (1995), who stated that a high OLR results in lower-strength  anaerobic granules. The strength of anaerobic granules depends on multiple factors such as microbial diversity, OLR, surface charges of granules, feed properties, ECP production, hydrodynamic shear forces and presence of polymer or positive divalent/ trivalent ions (Liu et al., 2002; Quarmby & Forster, 1995).

### 1.6.3. ELECTRON TRANSPORT IN GRANULES

In general, chemotrophic organisms obtain their energy from chemical energy, namely from coupled oxidation-reduction reactions. In these reactions, ATP is formed by substrate-level phosphorylation and electron transport phosphorylation. Nitrate, sulfate, carbon dioxide and oxidized metal ions can be used as electron acceptors for anaerobic respiration. In methanogenic environments, bicarbonate and protons act as terminal electron acceptors (Stams et al., 2006).

Methanogenic archaea only convert simple compounds such as hydrogen gas and carbon dioxide, formate, methylamines, and acetate to methane, so most organic monomers must be further metabolized down to acetate prior to methanogenesis. This typically requires oxidation, the removal and disposal of electrons. The different electron transport pathways (Figure 7) are important to understand, as modeling them or manipulating them can enhance biogas production (Blaut, 1994).
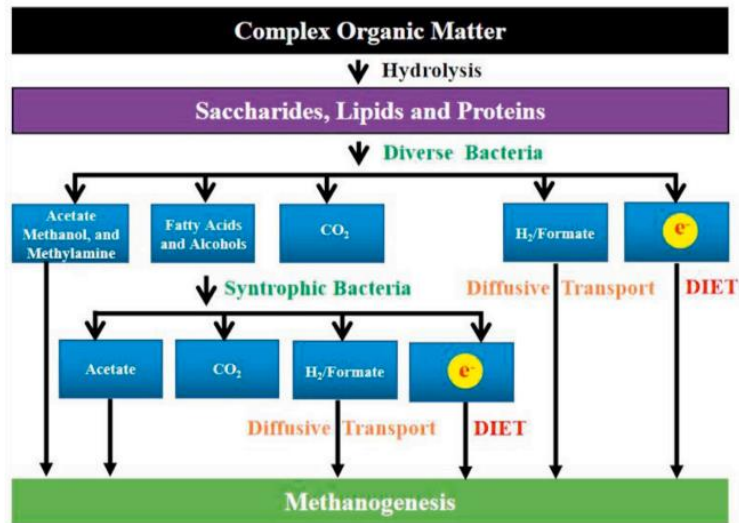
**Figure 7.** Schematic overview of methane formation in anaerobic digestion (Zhao et al., 2020)

The first electron transport mechanism in methanogenic communities that was discovered by Winogradsky in 1890, is called the **interspecies hydrogen transfer**. As stated above, anaerobic digesters convert organic matter into methane and $CO_2$ through a series of steps involving various intermediates, with volatile fatty acids (VFA) being key among them, including acetate, propionate, and butyrate. However, VFA are not ideal substrates for anaerobes due to their unfavorable oxidation properties, which require low $H_2$ partial pressures or formate concentrations. To facilitate VFA oxidation, a syntrophic association between VFA-oxidizing bacteria (syntrophs) and methanogenic archaea is necessary, with methanogens utilizing $H_2$ produced by syntrophs. Close physical contact between syntrophs and methanogens is crucial for efficient interspecies electron transfer, as suggested by researchers (Ishii et al., 2005).

Besides hydrogen, formate can be used by methanogens for the reduction of carbon dioxide to methane. This pathway is called the **interspecies formate transfer.** Formate and $H_2$ gas pools are interconnected by reversible microbial formate:hydrogen lyases. Thus, the control of syntrophic electron flow during methanogenesis could theoretically be achieved by either an interspecies formate transfer or an interspecies $H_2$ transfer coupled process (Thiele & Zeikus, 1988).

Recently, **direct interspecies electron transfer** (DIET) in AD environments has been suggested as an alternative electron transfer pathway. The DIET is energetically more advantageous, because it does not need hydrogen or formate to be produced for use as an electron carrier. This phenomenon was first reported by Summers et al. (2010) in co-cultures of *Geobacter metallireducens* and *Geobacter sulfurreducens*. They observed that DIET can be realized between different species through biological electrical connections by using the multiheme c-type cytochrome OmcS and electrically conductive pili. The DIET has been suggested to be faster and energetically more efficient than indirect interspecies electron transfer via hydrogen or formate as electron carriers; therefore, methanogenic performance can potentially be enhanced by promoting DIET in AD.

As mentioned above, interspecies hydrogen & formate transfer is a driving force for complete biodegradation in methanogenic environments. Many organic compounds are degraded by obligatory syntrophic consortia of proton-reducing acetogens and hydrogen- or formate-consuming methanogens. However, anaerobic organisms that use insoluble electron acceptors for growth, such as iron- or manganese oxide as well as inert graphite electrodes in microbial fuel cells, also transfer electrons extracellularly. This is called **extracellular electron transfer** (EET), which can occur direct or indirect, and organisms capable of doing EET are called exoelectrogens or electrochemical active species (Logan, 2009; Stams et al., 2006). Extracellular electron transfer reportedly occurs through three different mechanisms: (1) soluble redox shuttles, (2) direct contact between the electron acceptor and the redox-active protein on the outer-membrane surface, or (3) conductive filamentous structures. Biological DIET includes the second and third mechanisms in the form of cell-to-cell electron transfer via biological components like c-type cytochromes and conductive pili.

Direct EET occurs when microorganisms utilize metal-containing redox proteins, such as *c*-type cytochromes and rusticyanin, to electrically connect intracellular respiratory chains and extracellular solid materials. Furthermore, *G. sulfurreducens* and *S. oneidensis* were reported to produce conductive filamentous apparatus (pili and outer membrane extensions, respectively) that are specifically termed as 'nanowires'. These microorganisms have the ability to transfer electrons to or from distantly located solid materials using the filaments as 'electric wire' (Gorby et al., 2006; Kato, 2016; Reguera et al., 2005).

Some microorganisms are also capable of transferring electrons indirectly to or form solid compounds using different redox chemicals, namely electron mediators. These electron mediators are oxidized or reduced by intracellular electron mediators, after which the oxidized or reduced mediators diffuse out of the cell to solid surfaces and donate or accept electrons, and then the oxidized or reduced mediators return back into the cells and are again utilized as respiratory substrates. Examples of electron mediators are low molecular weight organic compounds, including phenazine compounds and flavin derivatives, naturally occurring (*e.g.* humic substances) and artificial (*e.g.* quinone derivatives) redox chemicals (Jiang & Kappler, 2008; Kato, 2016; Marsili et al., 2008; Nishio et al., 2013; Rabaey et al., 2004; Watanabe et al., 2009).

## 2. FOOD PROCESSING INDUSTRY AND FOOD WASTE

### 2.1. INTRODUCTION

The food processing industries (FPI) are essential to the global economy; They convert raw materials into finished food and drinks that are suitable for human consumption, with a global market size of USD 169.39 billion in 2022 which is expected to be expanded to USD 301.09 billion by 2031. As climate change becomes a more and more pressing challenge in our society, we find it more and more important that the FPI provides us with healthy and accessible food and drinks for the entire world population produced in a sustainable way. The *Farm to Fork (F2F) Strategy* is at the heart of the EU Green Deal aiming to make food systems fair, healthy, and environmentally-friendly. The F2F Strategy aims to accelerate the transition towards a sustainable food system that should encompass the four major themes presented in Figure 8 (*Farm to Fork Strategy - European Commission*, n.d.).

**Figure 8.** Four major themes of the *Farm to Fork Strategy* part of the EU Green Deal (*Farm to Fork Strategy - European Commission*, n.d.)

Addressing food loss and waste is crucial for sustainability. In the EU, over 58 tons of food waste are generated annually, with an associated market value estimated at 132 billion euros. Globally, approximately one third of all food produced for human consumption is lost or wasted and the FAO's Food Loss Index (FLI) estimates that globally around 14% percent of food produced is lost from the post-harvest stage up to, but excluding, the retail stage. These losses have not only negative economic and social impacts, but also have a huge environmental impact accounting for 16% of the total GHG's emissions from the EU food system (*Food Waste - European Commission*, n.d.).

To tackle food waste and losses, the Food Recovery Hierarchy (Figure 9) was proposed in 2018 by the Waste Framework Directive, which prioritizes actions that organizations, individuals and families can take to prevent and divert wasted food. The most preferred solution to reduce food waste is source reduction, *i.e.,* reduced volume of surplus food generated. For example, FPI can use artificial intelligence to predict demand patterns and adjust their production volumes reducing the production of surplus food. Next, when the food is already produced and still fit for human consumption, the surplus food should be donated to feed the hungry people. When the surplus food is no longer suitable for human consumption, it should be used to feed animals. Next, as the surplus food is no longer suitable for human or animal consumption, it should be donated to industries that can use it to provide biofuels or renewable energy through, for example, anaerobic digestion. When AD is not possible, the surplus food should be composted. Composting creates a product that can be used to help improve soils, grow the next generation of crops and improve water quality. Lastly, if none of the options above are possible, surplus food goes to landfill, which is the least preferred option (Food Waste Measurement - European Commission, n.d.; The Food Recovery Hierarchy, n.d.).
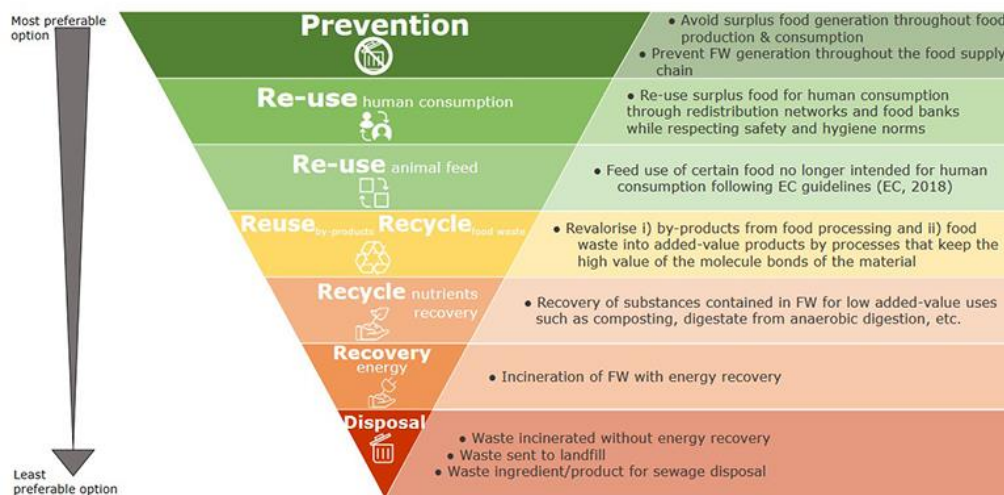
**Figure 9**. Food recovery hierarchy (*The Food Recovery Hierarchy*, n.d.)

## 2.2. ROLE OF ANAEROBIC DIGESTION IN FOOD LOSS AND WASTE PREVENTION

Anaerobic digestion emerges as one solution in combating the global food waste problem. With AD nutrients are recycled and energy is extracted from the waste streams. Compared to many other bioenergy technologies, AD can accommodate a much wider range of substrates, even those with high moisture contents and impurities. Food waste is a promising substrate for AD due to its high energy content, large quantity and wide availability. Moreover, AD reactors can be both large and small scale digesters and located at all geographical locations (Xu et al., 2018).

However, AD of food faces some technical, economic, and social challenges. One important challenge is the reactor instability coming from the rapid conversion of easily digestible food waste into volatile fatty acids (VFA) early in the digestion process. This can cause a sharp drop in pH if sufficient buffering capacity is lacking (C. J. Banks et al., 2011; Y. Chen et al., 2008; L. Zhang & Jahng, 2012). Also, significant technical challenge arises from the potential production of harmful intermediate compounds due to inadequate process control and optimization. This can undermine system stability, reduce methane yield, or lead to foaming issues (Grimberg et al., 2015). The high protein and lipid content in food waste can lead to elevated levels of inhibitory substances like ammonia, hydrogen sulfide, and long-chain fatty acids, as well as digester foaming (Y. Chen et al., 2008; Subramanian & Pagilla, 2015). Consequently, AD of food waste often operates at low organic loading rates (OLR) of 2–3 g/L*d of chemical oxygen demand (COD) to prevent process failure (Hecht & Griehl, 2009).

AD systems require substantial capital investment, with revenue primarily derived from organic waste collection fees and the sale of electricity and methane. Enhancing food waste loading and system stability is vital for the economic viability of AD (Xu et al., 2018).

# 3. CASE STUDY: WASTECH

## 3.1. INTRODUCTION

Founded in 2021, Wastech is an ambitious start-up focused on the eco-responsible and resilient re-industrialization of urban spaces. At the core of their project are "Black Soldier Fly" larvae (BSFL), allowing us to bridge the gap between organic waste management and value creation. Specifically, by cultivating BSFL from food processing waste, the company produces animal feed, offering a dual solution: valorizing food waste and reintegrating it into the food system through animal feed, thereby creating a circular process. Additionally, this approach reduces dependence on soy-based animal feed, a significant contributor to deforestation worldwide (*Wastech*, n.d.).

In November 2023, the start-up Wastech initiated their first large-scale project in collaboration with the retailer Delhaize aimed at combating food processing waste, more specifically fruit and vegetables waste (FVW), in supermarkets. Importantly, food processing waste is not "food waste" as it has not come into contact with the consumer. The objective of the project was to process 20 tons of food waste per month to 3 tons of larvae. The general idea of the process is shown in Figure 10 (VRT NWS: Nieuws, n.d.; Wastech, n.d.)
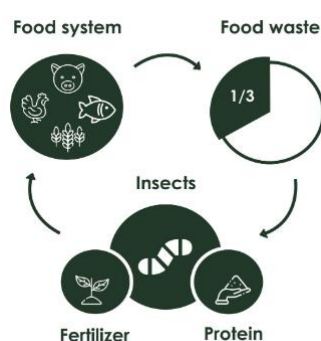


**Figure 10.** General overview of the Wastech process **(*Wastech*, n.d.)**

## 3.2. BLACK SOLDIER FLY LARVAE

Insect-based food waste treatment is gaining recognition worldwide for its eco-friendly resource recycling and cost-effective installation. Particularly black soldier fly larvae (BSFL), *Hermetia illucens,* belonging to the Diptera-family, are drawing significant attention for their efficient breakdown of various organic wastes. Under optimal conditions BSFL can pupate within 2 weeks, providing rapid composting that reduces bacterial growth and odor. Moreover, BSFL exhibit competition with disease-carrying houseflies and contain natural antibiotics that can modify harmful microorganisms in manure and food waste (Kim et al., 2021).

The BSFL have the ability to ingest about 80% of unsold food in less than 15 days. During this feeding period, the larvae accumulate good protein and fat, comprising up to 40% protein and 30% fat. At the end of their growth, the larvae become an ideal substitute for animal feed. The remaining 20% of unsold items will

become high-nutrient compost for agriculture. Their versatility in processing various organic wastes makes BSFL-mediated waste treatment environmentally, economically, and industrially advantageous. The BSFL production is not harmful to humans, since it does not feed on grain, invade human habitats, or carry pests and diseases (Kim et al., 2021).

## 3.3. WASTECH'S PROCESS

Wastech currently operates a system to convert food processing waste into two products: black soldier fly larvae (BSFL) and frass. A general overview of the process is given in the figure below, with the quantities of processed food waste and obtained larvae, leachate and frass (Figure 11).
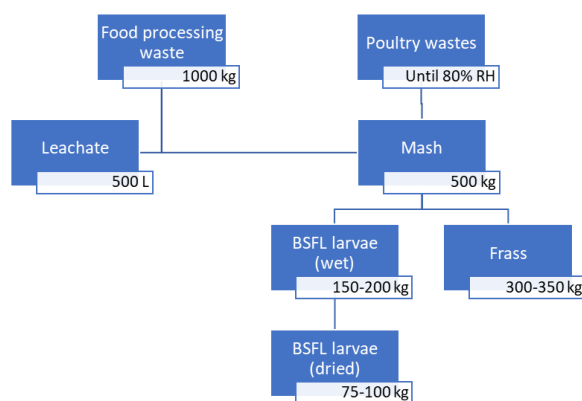
**Figure 11.** Overview of inputs and outputs of the process by Wastech

The first stage grinds the food waste into a pulp which is immediately passed into a centrifuge with a mesh size of 50 µm. The concentrate is processed to create a mash suitable for feeding BSFL. First, poultry wastes (mostly feathers) are mixed in to lower the water content to a total solids concentration of 80%. Then, lactic acid bacteria are added to render the mash more digestible for BSFL. The BSFL eggs are inoculated into the mash and grown over a one-week period. The remaining substrate – a mix of uneaten particles, BSFL faeces, and exoskeletons – is referred to as frass. This is a valuable fertilizer product but must be pasteurized before sale. The fully grown BSFL are collected and dried for sale. Both pasteurization and drying require heat.

The leachate is removed through the mesh and collected. There is currently no downstream process to revalorize this waste stream, and, as such, this is the target for this study. The goal will be to create methane through an UASB reactor that can be burned locally to heat the container unit, dry the BSFL, and pasteurize the frass.

## 3.4. LEACHATE AND THE ROLE OF ANAEROBIC DIGESTION

The leachate that is produced during the mashing of food processing waste contains very high amounts of COD, up to 125 g total COD/L and 115 g soluble COD/L. Treatment for such high COD concentrations is challenging and can be quite expensive. On the other hand, it contains a tremendous amount of energy that can be recovered through AD.

The leachate is primarily produced from fruit and vegetable waste (FVW). FVW is characterized by high moisture content, low total solids (TS), and high volatile solids (VS) making it a suitable substrate for AD. It generally has a low pH (3.7-4.2) and low lipid content (0.8-5.2% of TS). The methane yield of fruit and vegetable waste ranges between 0.16-0.35 L $CH_4$/g VS (Agrawal et al., 2022; Xu et al., 2018).

Treating the leachate with an UASB reactor would allow Wastech to valorize that waste stream and recover energy through the production of biogas. As mentioned before, biogas yields thermal and electrical energy. This could decrease the operational costs of the installation significantly and make the entire project more profitable, which could lead to a large-scale implication in Belgian supermarkets, further decreasing food processing waste. It is also important to acknowledge the challenges that could arise during the treatment of the leachate with an UASB reactor. Namely, the high COD content could lead to VFA accumulation, which will inhibit the methanogenesis and decrease the pH of the reactor. However, if the system is well monitored and proper operational conditions are applied, such as lower OLR because of a higher HRT, VFA accumulation could be avoided.

## 3.5. HEAT AND ELECTRICITY PRODUCTION FOR WASTECH

During both the waste processing and insect farming steps of the Wastech process, a certain heat and electricity requirement is present. The basic power and consumption details of the waste processing and insect farming machinery are:

- Electric: 30 kW for waste processing and 24 kW for the rest of the machinery
- Gas: 7 kW for heating a water tank and the cultivation chamber

This adds up to 40 MWh for electric and 15 MWh of thermal energy consumed annually. Through cost simulation provided by Mega and Testaankoop, this heat and electricity consumption would cost up to 10,000€ per year (*Online Simulatie Gas- En Elektriciteitsverbruik | Mega*, n.d.), which is a significant operational cost for the start-up. Treating the leachate from the waste processing step with an UASB reactor would allow Wastech to valorize that waste stream and recover energy through the production of biogas. Biogas is a renewable energy that can be used in various pathways namely, using biogas in a Combined Heat Power (CHP) unit, using biogas directly on site, and upgrading biogas to a gas of a higher value (i.e. biomethane).

The simplest use of biogas is the direct use of biogas for heat generation through heat boilers. Hereby high quality hot water or steam is produced which can be used to heat to meet the heat requirements of Wastech. Heat boilers are 80-90% thermally efficient (I.Bitir & M.Tazerout and O.Le Corre, 2002). Using biogas in decentralized CHP units is the most common use of biogas. The biogas is dried, desulfurized and then used in a gas engine that drives a generator delivering power and heat. The electrical efficiency is usually between 30-40% and the corresponding thermal efficiency is 35-55% (Mertins & Wawer, n.d.).

# PART III. OBJECTIVES

In this study, the leachate from the start-up Wastech was studied and established whether anaerobic digestion, more specifically the UASB reactor inoculated with anaerobic granules, is suitable for the purposes of treating this waste leachate. Secondly, it was determined via two different UASB reactors whether raw leachate or filtered leachate was more suitable for the UASB reactor to obtain the best reactor performance and stability. Different organic loading rates (OLR) were tested to find a maximum OLR and optimal operating conditions. The main parameters to determine success of the reactors and which influent is more suitable for the UASB reactor were reactor performance in terms of organics removal efficiency, methane yield and reactor stability. Lastly, the biomethane potential of the leachate and its energy content was determined to assess the economic benefits of the system for Wastech. Two options for biogas utilization are explored, namely the direct use of biogas for heat generation with heat boilers and heat and electricity production through a CHP unit.

# PART IV. MATERIAL AND METHODS

## 1. EXPERIMENTAL SET-UP

### 1.1. REACTOR CONFIGURATIONS

Two laboratory-scale identical reactors were set up as shown in Figure 12 for 60 days (from February 2024 to April 2024). Each reactor was made of glass with a working volume of 2 L. They were set up with the help of a metal tripod in a basin to collect reactor fluid in case of a leakage. An airlock was located in the effluent PVC tubes, by creating a loop, to prevent oxygen from entering the reactors and biogas from exiting the reactors.

Each reactor was fed at the bottom of the reactor using a peristaltic pump (Watson Marlow blue - 323U). The pumps were calibrated before use. A marble was added in each reactor at the entrance of the influent line to prevent backflow of the granules through the bottom of the reactor. Two different influent streams were applied to the different reactors, namely one reactor was fed with raw leachate (total COD) and the other one with filtered leachate (soluble COD). The influent bottles (1 L) were manually refilled thrice a week. The raw leachate was stirred with a magnetic stirrer (Stirrer CR - big) at 1500 rpm to prevent the solids from settling. The effluent of each reactor was collected in separate 10 L plastic containers.

Each reactor had a recirculation line to avoid loss of the granular sludge via the effluent using a peristaltic pump (Watson Marlow blue – 323U). Both reactors were kept at a constant temperature of 34°C with a warm water bath and tubing around the reactor to maintain mesophilic temperatures.

The biogas formed during anaerobic digestion was also collected at the top using a rubber stopper pierced by a needle. This needle was connected to PVC tubing through which the gas was led to a sampling point. Two gas measurement methods were used. First, biogas produced was collected in gas columns (5 L), where the water level indicated the volume of gas generated. The gas columns were immersed in an acidic water bath (pH < 4.3) containing methyl orange to prevent $CO_2$ from dissolving, avoiding an overestimation of the methane fraction. Biogas samples were withdrawn daily using a 5 mL syringe.

A second method was used, since the biogas production was too much for the gas columns; The used gas counters were designed at Ghent University and measure gas using a U-shaped glass tube filled with oil. The incoming gas caused displacement of the oil in the tube. This displacement, after adding a certain amount of gas, depending on the amount of oil in the tube, triggered a sensor activated by the oil (clicking signal), after which the gas was led away from the tube through the outlet. Each click was displayed on the display and was a measure of the amount of biogas produced. By calibrating the gas counter beforehand, it could be determined how much gas was needed before the sensor was activated by contact with the oil, and thus how much gas was produced per click by the reactor. However, this method was abandoned, because the measurements were too inconsistent.
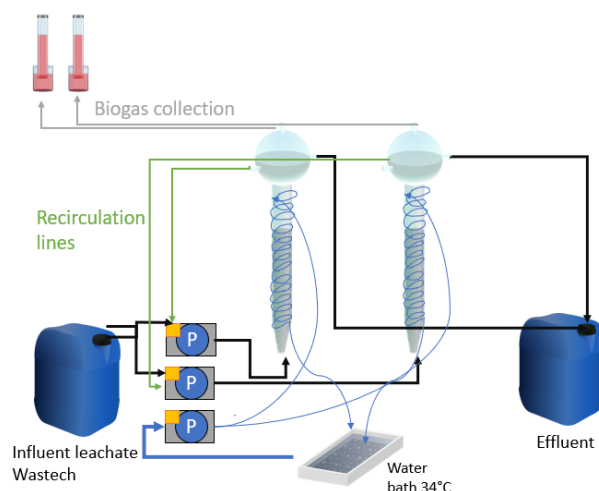
**Figure 12**. Schematic overview of the experimental set-up and reactor configuration with gas columns

## 1.2. REACTOR SET-UP

The leachate, the influent, was obtained from the start-up Wastech in January 2024 and was characterized. The characteristics of the leachate are shown in Table 2 (in Part IV. Results). In reactor 1, raw leachate (total COD) was treated and in reactor 2 filtered leachate (soluble COD) was treated. The filtered leachate influent was prepared by removing the solids with a centrifuge for 8 minutes at 4000 rpm (Centrifuge 5430/5430 R, Eppendorf™, Germany).

The reactor was inoculated with 1 L of inoculum to obtain 10 g VS/L in each reactor. The anaerobic granules, also collected in January 2024, were obtained from a local beer brewery Van Steenberge in Evergem, where a wastewater stream with OLR of 10 g COD/L*d is treated. For the start-up of the reactors, 40 mL of undiluted and unfiltered leachate was added to both reactors. The volume of the reactors was further filled with tap water. The inoculum was characterized (Table 2).

## 1.4. EXPERIMENTAL PROCEDURE

The reactors were run for a total of 60 days. The study was conducted in different experiments testing different OLRs. An overview of the different experiments is shown in Table 1.

In the first experiment, a timer was used to pump the influent for 2 minutes every 24 h. The influent was diluted to obtain the desired OLR of 10 g COD/L*d. Because of pressure build-up in the reactor, due to the biogas production or solids accumulation, the amount of leachate pumped in was not consistent, and, therefore, the exact applied OLR was unknown. The hydraulic retention time (HRT) was initially aimed to be at 12.5 days, but because of the inconsistent feeding the real HRT is not known. It was estimated that during this experiment the OLR was 4-5 g COD/L*d.

To overcome these feeding problems, it was decided to feed the reactors manually with influent pumped in every two days, on Monday, Wednesday and Friday, to obtain the desired OLR.

**Table 1**. Overview of the experiments of the study

| Experiment | Days | Target OLR [g COD/L*d] | Comments |
|---|---|---|---|
| I | 24 | 4-5 | During the initial start-up, a theoretical OLR of 10 g COD/L*d was applied. However, because of pressure build-up in the system, the amount of influent pumped in was inconsistent, leading to a lower actual OLR. Once the pressure build-up was removed, too much influent was pumped in and both reactors acidified.<br><br>During this experiment, the gas production was too much for the gas columns (more than 5 L per two days), so it was decided to switch to the gas counters on day 20. The gas counters were very inconsistent so no accurate biogas data (volume and composition) was collected from day 20 until the end of experiment II (day 31). |
| II | 7 | 10 | To prevent the inconsistent feeding of the reactors, due to pressure build-up, the reactors were fed manually every two days. In this way, the desired OLR is the actual applied OLR. The HRT during this phase was equal to 12.5 days.<br><br>The pH dropped below 6 after 7 days, and it was decided to re-inoculate.<br><br>During this experiment, gas counters were tried to collect data for biogas volume and composition. These gas counters were inconsistent, so no biogas data is available for this experiment. |
| III | 7<br>7<br>10 | 5<br>7<br>10 | For the third experiment a low start-up OLR was chosen and the OLR was slowly increased after one week of stable operation to reach the target OLR of 10 g COD/L*d.<br><br>Operation started at an OLR of 5 g COD/L*d and the HRT during this phase was equal to 25 days. It was increased to 7 g COD/L*d after 7 days of stable operation and During this phase the HRT was equal to 17 days. This was further increased to 10 g COD/L*d seven days later with an HRT of 12.5 days. The reactors failed at this OLR within 7 days. Feeding was stopped for 5 days to try to restabilize the reactors that acidified marking the end of experiment III.<br><br>For experiment III, it was decided to switch back to the gas columns to collect biogas data. |

## 1.5. MEASUREMENTS

The COD, TS & VS, VFA, cation and anion concentrations of the effluent (reactor 1 & 2) and influent (raw leachate & filtered leachate) were measured once a week, on Wednesday. The pH, biogas volume and biogas composition were measured 3 times a week, on Monday, Wednesday and Friday. The characterization of the leachates and inoculum was done once at the beginning of the study.

## 2. ANALYTICAL METHODS

### 2.1. CHEMICAL OXYGEN DEMAND (COD), TOTAL PHOSPHORUS AND TOTAL NITROGEN

The concentrations of COD were determined according to the Tube test NANOCOLOR COD HR 1500 (Macherey-Nagel, Germany); Here, the COD is determined by silver-catalyzed oxidation (increase in the oxidizability of aliphatic substances) with potassium dichromate/ sulfuric acid. The testing tubes are digested for 30 minutes at 160°C. The COD covers all oxidizable contents of the sample where the increase in yellow Cr(III) ion concentration is determined photometrically.

$$K_2Cr_2O_7 + 8H^+ \rightarrow 3 < O > + 2Cr^{3+} + 2K^+ + 4H_2O$$
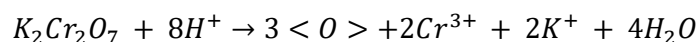
The concentrations of total nitrogen were determined according to the Tube test NANOCOLOR total Nitrogen TNb 22 (Macherey-Nagel, Germany). Here both organic and inorganic mineral nitrogen were oxidized to nitrate in an acidic medium. The nitrate formed 4-nitro-2,6-dimethylphenol in acidic medium after a reaction with 2,6-dimethylphenol. 4-nitro-2,6-dimethylphenol was determined photometrically.

Total phosphorus was determined according to the Tube test NANOCOLOR ortho- und total-Phosphate 5 (Macherey-Nagel, Germany). This measurement determines both ortho-phosphates and poly- and organophosphates in the sample. In the test, a reaction occurs between ortho phosphate and ammonium molybdate, resulting in the formation of phosphomolybdic acid. This was reduced to phosphorus molybdenum blue with the aid of a reducing agent.

Digestions were done using the heating block NANOCOLOR VARIO 4, double block and concentration measurements were performed with a Spectrophotometer NANOCOLOR Advance.

### 2.2. ALKALINITY, CONDUCTIVITY AND PH

The alkalinity of the leachate was measured with the titration test Visocolor HE Alkalinity AL 7 (Macherey-Nagel, Germany) with a range of 0.2–7.2 mmol/L OH⁻. The conductivity of the leachate and granules was measured with a Consort C3020 multi-parameter analyzer. The probe was calibrated weekly with KCl-solutions of 1, 0.1 and 0.01 M. The measured conductivity is expressed in mS/cm.

The pH of the influent and effluent samples was measured with a Consort C3020 multi-parameter analyzer. The probe was calibrated weekly with commercially available buffers with a pH of 4, 7 and 9.

### 2.3. CARBOHYDRATES AND SHORT-CHAIN ALCOHOLS

The quantitative analysis of carbohydrates and short-chain alcohols of the leachate was carried out using a high performance liquid chromatograph (Shimadzu HLPC LC-2030 series, detector A Shimadzu SPD-40V UV/Vis detector, detector B Shimadzu RID-20A Refractive index detector)). The HPLC is equipped with an Aminex HPX-87H column which separates compounds using a combination of size-exclusion and ion exchange mechanisms (ion-moderated chromatography technique). This particular column is packed with

a polystyrene-divinylbenzene co-polymer with bonded propyl benzenesulfonic acid groups presenting $H^+$ counter ions.

The concentration of all compounds must be below 2,000 mg/L. For the analysis of the carbohydrates and short-chain alcohols, the samples were filtered over a 0.22 µm filter and diluted 200 times with MilliQ-water.

## 2.4. VOLATILE FATTY ACIDS (VFA)

The quantitative analysis of volatile fatty acids was carried out using a capillary gas chromatograph, coupled with a flame ionization detector (FID) (GC-2014 with FID (Shimadzu) with autosampler). The method described below can be applied for all kind of liquid samples for the analysis of VFA in a range of 30 to 1,000 mg/L of the following compounds acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, isocaproic acid, caproic acid, heptanoic acid, and octanoic acid.

For the analysis of VFAs using a liquid-liquid extraction, 2 mL of the sample to be analyzed was transferred to 12 mL tubes. To this sample, 0.5 mL of $H_2SO_4$ (1:1) was added to obtain the organic acids in the sample in their less water-soluble undissociated form. Approximately 0.4 g of NaCl was then added to assist in separation. Next, 0.4 mL of internal standard was added. The internal standard (1.5 mL of 2-methyl caproic acid in 200 mL of 0.05 M NaOH) was added to compensate for ether evaporation and to account for variations in extraction efficiency during sample preparation. Finally, 2.0 mL of diethyl ether was added so that the VFA could migrate to such a hydrophobic component. This mixture was first mixed for 2 minutes using a rotator and then centrifuged for 3 minutes at 3000 rpm (Centrifuge Mega Star 600/600R, VWR, USA). The top layer (ether phase with the VFA) was transferred to a glass vial after centrifugation using a Pasteur pipette. These samples were eventually analyzed with the gas chromatograph to determine the concentrations.

## 2.5. ANIONS AND CATIONS

Once a week, an additional sample was taken from each reactor to measure the concentration of cations and anions. Such analysis was conducted to monitor the concentrations of $Na^+$, $NH_4^+$, $K^+$, $Ca^{2+}$, and $Mg^{2+}$ for the cations and $Cl^-$, $NO_2^-$, $NO_3^-$, $SO_4^{2-}$ and $PO_4^{3-}$ for the anions.

The samples were first filtered with a 0.22 µm filter. Subsequently, the samples were diluted in suitable vials with MilliQ water so that they fell within a concentration range of 1 to 100 mg ion/L.

The instrument used for the cation analyses was the 761 Compact IC, Metrohm with 837 Eluent Degasser. The cation column of this instrument used for separation consists of silica gel with carboxyl groups (Metrosep C6 – 250/4).

The instrument used for the anion analyses was the 930 Compact IC Flex (Metrohm) with chemical suppression and conductivity detector with 858 autosampler (Column: Metrosep A Supp 5- 150/4.0 (61006520)).

## 2.6. TOTAL SOLIDS (TS) AND VOLATILE SOLIDS (VS)

The measurements of TS and VS occurred by drying 5 mL of sample in the drying oven at 105°C (TS) and ashing in the muffler oven (at 450°C). The TS and VS were calculated by the following formula;

$$TS/VS\ [g/L] = \frac{m_{after} - m_{before}}{V_{sample}}$$

## 2.7. BIOGAS VOLUME AND COMPOSITION

A 2 mL syringe was used for $CH_4$ and $CO_2$ analysis (two syringes per treatment), with sampling taken from a gas sampling tube (Lenz, Germany). The gas phase composition was analyzed with a Compact GC (Global Analyser Solutions, Breda, Netherlands), equipped with a Molsieve 5 A pre-column and Porabond column ($CH_4$, $O_2$, $H_2$, and $N_2$) as well as a Rt-Q-bond pre-column and column ($CO_2$, $N_2O$, and $H_2S$). Concentrations of gases were determined by means of a thermal conductivity detector, with detection limits for each gas range from 0.05% v/v to 100% v/v. Anion and cation concentrations were measured using ion chromatography (Metrohm, Switzerland) using a Metrosep A Supp 5–150/4.0 (61006520) column. Detection limits for ions ranged between 0.05 and 100 mg ion/L.

From the GC results, the percentage $CH_4$ as part of the headspace gas composition was calculated by:

$$\%CH_4 = 100 * \frac{CH_4}{CO_2 + CH_4}$$

The volume of $CH_4$ produced per liter reactor at standard temperature and pressure was calculated by:

$$Volume\ _{CH_4} = \%CH_4 * Volume_{biogas,daily} \frac{273\ K}{301\ K}$$

The $CH_4$ yield was calculated from the volume of $CH_4$ produced per liter reactor divided by the volume of feed leachate added (L) multiplied by its VS or COD (g/L sludge) content. This results in the methane yield were related to the initial COD of the leachate (L $CH_4$ / g COD) or to volatile solids (L $CH_4$ / g VS).

$$CH4_{yield,VS} = \frac{Volume\ _{CH_4}}{\frac{g\ VS}{L\ sludge} * Volume_{feed}} \qquad CH4_{yield,COD} = \frac{Volume\ _{CH_4}}{\frac{g\ COD}{L\ sludge} * Volume_{feed}}$$

## 2.8. BIOMETHANE POTENTIAL TESTS (BMP)

Biomethane potential tests (BMP) were conducted for both unfiltered (TCOD), filtered (sCOD), ⅓ diluted unfiltered (⅓ TCOD), ⅓ diluted unfiltered leachates (⅓ sCOD), a negative control (only containing inoculum sludge) and a positive control (containing inoculum sludge and 0.4 g COD cellulose) in biological triplicates in the inoculum as shown in Figure 12. The BMP is defined as the maximum volume of methane produced per g of VS substrate provides an indication of the biodegradability of a substrate and its potential to produce methane *via* AD. The BMP test is a method of establishing a baseline for performance of AD.

Eighteen penicillin bottles, each with a total volume of 120 mL, were prepared by filling them with a pre-determined mixture of leachate and inoculum to achieve a final volume of 80 mL. These bottles were then

monitored for 17 days. The inoculum sludge was diluted with tap water until reaching a volatile solids (VS) concentration of 10 g VS/L. The anaerobic batch reactors were hermetically sealed with rubber stoppers and connected to a biogas capture system via syringe and PVC tubing. The bottles were submerged in a warm water bath to maintain a constant mesophilic temperature of 34°C. Biogas produced was collected in gas columns, where the water level indicated the volume of gas generated. The gas columns were immersed in an acidic water bath (pH < 4.3) containing methyl orange to prevent CO2 from dissolving, thus avoiding an overestimation of the methane fraction. Biogas samples were withdrawn using a 5 mL syringe. The BMP test concluded when there was a decrease of less than 3% in biogas production over three consecutive days, indicating complete conversion of all biodegradable organic material.
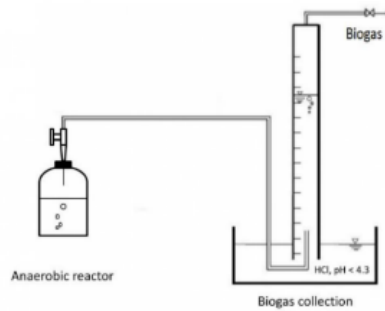


**Figure 13**. Schematic representation of the biomethane potential test set-up

## 2.9. ENERGY YIELD WASTECH

Based on the yield products, the annual energy and electricity production was estimated. Two options were considered for the utilization of biogas, namely the heat boiler and CHP unit. A methane to energy conversion of 1 $m^3$ $CH_4$ = 10.55 kWh was used. In the first option, the thermal energy yield using a heat boiler assuming an efficiency of 85% (Hakawati et al., 2017) was calculated by:

$$Thermal\ energy\ yield\ with\ heat\ boiler\left[\frac{kWh}{L\ leachate}\right]$$
$$= CH_{4\ yield,COD}\left[\frac{L\ CH_4}{g\ COD}\right]*\frac{10.55\ kWh}{1\ m^3 CH_4}*\frac{1\ m^3}{1000\ L}*85\%\ efficiency*\frac{g\ COD}{L\ leachate}$$

In the second option, a CHP is used to produce heat and electricity. An electrical efficiency of 40% and a thermal efficiency of 45% are assumed (Hakawati et al., 2017). The electricity and heat production is then calculated by:

$$Electricity\ yield\ with\ CHP\left[\frac{kWh}{L\ sludge}\right]$$
$$= CH_{4\ yield,COD}\left[\frac{L\ CH_4}{g\ COD}\right]*\frac{11.5\ kWh}{1\ m^3 CH_4}*41\%\ efficiency*\frac{g\ COD}{L\ leachate}$$

$$Thermal\ energy\ yield\ with\ CHP\ \left[\frac{kWh}{L\ leachate}\right]$$
$$= CH_{4\ yield,COD}\ \left[\frac{L\ CH_4}{g\ COD}\right] * \frac{11.5\ kWh}{1\ m^3 CH_4} * \frac{1\ m^3}{1000\ L} * 45\%\ efficiency * \frac{g\ COD}{L\ leachate}$$

## 2.10. NET PRESENT VALUE AND INTERNAL RATE OF RETURN

To establish whether the investment in either a UASB reactor for the treatment of the leachate coupled with a heat boiler or a CHP unit for energy recovery would be a viable option for Wastech, the Net Present Value is calculated for both options. The Net Present Value is calculated by:

$$NPV = \sum_{t=0}^{T} \frac{CF_t}{(1 + WACC)^t} - Initial\ Investment$$

With $CF_t$ the yearly cash flow, WACC the weighted average cost of capital and t the lifespan of the installation. The $CF_t$ is calculated by:

$$CF_t = Revenue_t - Operating\ Costs_t - Maintenance\ Costs_t$$

The internal rate of return (IRR) is a metric used to estimate the profitability of investment. It is a discount rate that makes the net present value (NPV) of all cash flows equal to zero in a discounted cash flow analysis.

$$0 = NPV = \sum_{t=0}^{T} \frac{CF_t}{(1 + IRR)^t} - Initial\ Investment$$

## 2.11. STATISTICS AND TRIPLICATES

The chemical analyses of COD, TS and VS were performed in technical triplicates with associated averages and standard deviations. The BMP samples and negative control were done in biological triplicates. The analyses of VFA, anions and cations, and biogas composition with GC were performed once per sample.

Data analysis was performed in Excel and R version 4.0.3, with figures generated entirely in R. Relevant libraries used include: ggplot2 (9), ggpubr (10), dplyr (11), tidyverse (12), tidyr (13), cowplot (14), grid (15), and gridExtra (16).

## 2.12. ARTIFICIAL INTELLIGENCE

Linguistics AI tools such as ChatGPT (Open AI) and Copilot (Microsoft Edge) were used occasionally throughout this thesis to paraphrase sentences or passages.

# PART IV. RESULTS

## 1. CHARACTERIZATION LEACHATE AND INOCULUM

At the start of the study, the leachate and the granular sludge (inoculum) were characterized (Table 2). The alkalinity of the leachate was measured and was below the range of the test (0.2-7.2 mmol/L $OH^-$), thus, the alkalinity was less than 0.2 mmol/L $OH^-$. The COD/VS ratio of the leachate was equal to 8.82 ± 0.14 and the VS/TS ratio of the leachate was equal to 0.31 ± 0.03. The concentration of carbohydrates and short-chain alcohols of the leachate was also determined at the end of the study with the HPLC, shown in Table 3. All parameters (except electrical conductivity (EC)) were measured in technical triplicates giving the average value with associated standard deviation.

Table 2. Relevant physicochemical parameters pertaining to the leachate and inoculum

| Parameter | Unit | Leachate | Inoculum |
|---|---|---|---|
| pH | - | 3.67 ± 0.21 | 7.33 ± 0.26 |
| Electrical conductivity (EC) | mS/cm | 8.13 | 4.05 |
| Total COD | g/L | 123 ± 2 | 4.2 ± 0.3 |
| Soluble COD | g/L | 118 ± 3 | 0.25 ± 0.10 |
| Total VFA (COD equivalent) | mg/L | 1662 ± 43 | 13 ± 1 |
| Total phosphate | mg/L | 266 ± 5 | 51 ± 11 |
| Total nitrogen | mg/L | 804 ± 24 | 360 ± 6 |
| Total solids (TS) | g/L | 44.9 ± 2.2 | 11.3 ± 0.7 |
| Volatile solids (VS) | g/L | 14.0 ± 1.5 | 8.7 ± 1.7 |

Table 3. Carbohydrates and short-chain alcohols in the leachate

| Compound | Concentration (g/L) |
|---|---|
| Lactate | 7.5 ± 0.4 |
| Succinate | 12.4 ± 0.3 |
| Acetate | 7.0 ± 1.0 |
| Xylose | 2.1 ± 0.2 |
| Glucose | 5.4 ± 3.3 |
| Ethanol | 43.9 ± 6.9 |
| Galactose | 9.0 ± 0.1 |

The concentrations of anions and cations in the leachate was also measured at the beginning of the study with an IC and are represented in Table 4. The ion concentrations were also measured in technical replicates, providing the average concentration with associated standard deviation.

Table 4. Concentrations of anions and cations in the leachate

| Anions | | Cations | |
|---|---|---|---|
| Compound | Concentration (mg/L) | Compound | Concentration (mg/L) |
| $Cl^-$ | 505 ± 22 | $Na^+$ | 63.0 ± 3.0 |
| $NO_2^-$ | 0.0 ± 0.0 | $NH_4^+$ | 70.0 ± 2.1 |
| $NO_3^-$ | 249 ± 10 | $K^+$ | 3,213 ± 176 |
| $PO_4^{3-}$ | 619 ± 23 | $Ca^{2+}$ | 122 ± 10 |
| $SO_4^{2-}$ | 2.6 ± 0.2 | $Mg^{2+}$ | 115 ± 14 |

## 2. PERFORMANCE OF THE UASB REACTORS

The main goal of this study was to determine the suitability of a UASB reactor for the purposes of treating food processing waste leachate. The main parameters used to determine success were the reactor stability and COD removal efficiency.

### 2.1. REMOVAL EFFICIENCY

The performance of the reactors was evaluated through the capability of the granules to remove COD from the influent, the COD removal efficiency, and convert it into biogas, the normalized biogas yield and biogas composition. In Figure 14, the COD concentration in the influent and effluent of both reactors is shown. The COD concentrations were measured in technical triplicates and in the figure, the average concentrations with associated standard deviations are shown. The raw leachate (influent) is treated in reactor 1 and the filtered leachate (influent) is treated in reactor 2.

In experiment I, the influent was diluted to obtain the desired OLR, explaining the low influent COD concentrations of the raw leachate and the filtered leachate. During this experiment, the target OLR was 14 g COD/L*d. The influent concentrations were lower, because they were diluted. Initially, when the reactors were underfed, the OLR was between 4-5 g COD/L*d and the HRT was set at 8 days, due to pressure build-up in the reactors. The COD removal efficiencies of both reactors reached 88% for reactor 1 and 96% for reactor 2. This removal efficiency dropped to 42% for reactor 1 and 80% for reactor 2 when all the influent was pumped in obtaining the target OLR of 14 g COD/L*d, causing the destabilization of both reactors. Removal efficiencies during this experiment were high (93% for reactor 1, and 96% for reactor 2), followed by acidification after seven days.

In experiment II, the target OLR was decreased to 10 g COD/L*d and the HRT was 12.5 days. The influent concentrations were higher, since they weren't diluted from this experiment onwards. Even though the removal efficiencies during this experiment were high, 93.10% for reactor 1, and 96.07% for reactor 2, the reactors acidified within a period of 7 days.

Experiment III is divided into three stages, because the OLR was simultaneously increased to 10 g COD/L*d (HRT = 12.5 days). During the stages IIIa and IIIb with an OLR of 5 g COD/L*d (HRT = 25 days) and 7 g COD/L*d (HRT = 17 days) respectively, the reactors achieved high removal efficiencies of 99.36% for reactor 1 and 99% for reactor 2 during stage IIIa and, 94% for reactor 1 and 97% for reactor 2 during stage IIIb. When the OLR was further increased to 10 g COD/L*d, in stage IIIc, the removal efficiencies slowly decreased, accompanied by signs of acidification, decrease in biogas etc. In the first week of stage IIIc, the removal efficiency for the reactors were equal to 89% and 91% for reactors 1 and 2 respectively. This further decreased in the second week to 85% for reactor 1 and 71% for reactor 2.
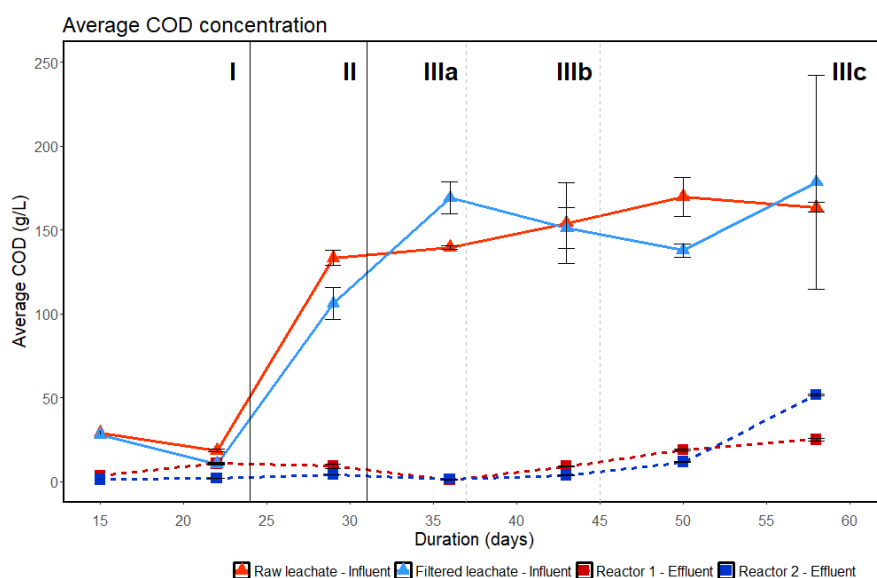


**Figure 14** Average chemical oxygen demand (COD) concentration (g/L) of the effluent of reactor 1 treating raw leachate influent and the effluent of reactor 2 treating filtered leachate influent

The performance of the reactors was also evaluated through the TS and VS concentrations (Table 5) and the corresponding VS/TS ratio (Figure 15) representing the organic fraction of the total solids. The TS and VS concentrations were measured in technical triplicates and in Table 5 and Figure 17, the averages with associated standard deviations are shown.

In general, TS concentrations tended to be higher in the unfiltered leachate, due to the presence of solids in the liquid. The VS/TS ratio of the raw leachate was lower compared to the filtered leachate. The VS/TS ratios for the raw leachate ranged from 0.24 ± 0.03 to 0.47 ± 0.07, whereas those for the filtered leachate ranged from 0.26 ± 0.03 to 0.40 ± 0.06. Typically, a higher VS/TS ratio indicates a higher content of organic matter.

In the beginning of experiment I, the VS/TS ratio of reactor 2 was higher than the ratio for reactor 1. The TS concentration of both reactors were similar, however the VS concentration of reactor 1 was lower than reactor 2, indicating that in reactor 2 a higher portion of the solids in the effluent are volatile, which may indicate incomplete digestion. At the end of experiment II, both reactors showed similar VS/TS ratios. At this point both reactors had acidified marking the end of the experiment.

In experiment IIIa, the VS/TS ratio was highest for both reactor 1 and reactor 2. The concentration of VS was high in the effluent of both reactors, and because the reactors had just started again, the digestion was still starting up. In stage b, the VS/TS ratio of both decreased, since the reactors were stabilizing and this trend continued until the first week in stage c where the VS/TS ratios of both reactors reached a minimum. At the end of the study, the VS/TS ratios of both reactors increased again corresponding to the destabilization of the reactor.

**Table 5**. Total solids (TS) and Volatile solids (VS) concentration (g/L) of reactor 1 treating raw leachate and reactor 2 treating filtered leachate throughout the three experiments

| Duration (days) | TS concentration [g/L] | | | | VS concentration [g/L] | | | |
|---|---|---|---|---|---|---|---|---|
| | Reactor 1 | Reactor 2 | Raw leachate | Filtered leachate | Reactor 1 | Reactor 2 | Raw leachate | Filtered leachate |
| 15 | 5.85 ± 0.31 | 5.72 ± 1.34 | 14.39 ± 0.34 | 12.85 ± 0.36 | 3.12 ± 0.14 | 4.56 ± 0.71 | 5.21 ± 0.76 | 4.89 ± 0.92 |
| 29 | 2.91 ± 0.13 | 3.23 ± 0.09 | 31.52 ± 0.16 | 28.02 ± 12.50 | 1.60 ± 0.13 | 1.76 ± 0.14 | 7.40 ± 0.76 | 9.18 ± 4.48 |
| 36 | 5.19 ± 1.85 | 4.87 ± 1.54 | 37.10 ± 1.16 | 29.61 ± 10.87 | 3.45 ± 1.18 | 3.07 ± 1.17 | 13.43 ± 2.48 | 7.95 ± 3.53 |
| 43 | 10.52 ± 1.17 | 8.07 ± 1.19 | 35.51 ± 0.89 | 28.23 ± 0.96 | 4.89 ± 0.97 | 4.43 ± 1.02 | 16.13 ± 2.00 | 11.58 ± 1.90 |
| 50 | 7.36 ± 4.88 | 14.68 ± 6.14 | 32.35 ± 3.00 | 25.78 ± 1.94 | 2.70 ± 1.41 | 6.98 ± 3.31 | 9.58 ± 2.74 | 9.63 ± 2.00 |
| 58 | 5.93 ± 0.08 | 8.58 ± 0.10 | 34.38 ± 0.87 | 35.61 ± 11.60 | 3.00 ± 0.16 | 4.72 ± 0.20 | 16.19 ± 2.58 | 11.71 ± 0.69 |



**Figure 15**. VS/TS ratio of reactor 1 treating raw leachate and reactor 2 treating filtered leachate throughout the three experiments

In comparison with the measured COD concentrations (Figure 14) in both influent streams, filtered and raw leachate, the VS and TS concentrations (Table 5) were very low. The leachate contained a lot of volatile solids that evaporated before measurement and thus, the measured VS and TS concentrations in the leachate were underestimations of the actual carbon content. Therefore, the following methane yields were calculated with g COD instead of g VS.

## 2.2. BIOGAS PRODUCTION AND COMPOSITION

The biogas production and composition of the UASB reactors were also monitored. Figure 16 shows the course of methane production per gram of COD for both reactors (normalized methane yield). This figure was compiled by combining various measurements, including the concentration of COD in the influent and effluent and their volumes, the amount of biogas produced, and the concentrations of $CO_2$ and $CH_4$ in the biogas. The calculations and assumptions to arrive at this figure are explained below.

Between day 20 and 34 of the study, the biogas production was not measured due to the inconsistency of the gas counters. Therefore, no methane yield was calculated in this period explaining the gap in the graph. The methane production in experiment I was lower for reactor 1 compared to reactor 2, corresponding with the higher COD removal of reactor 2 compared with reactor 1 (Figure 16). In experiment IIIa, the methane yield reached a maximum after one week of stable operation. This corresponds with the maximum COD removal efficiencies that were reached at this point. As the OLR was increased, throughout experiments IIIb and IIIc, the methane yield decreased very rapidly. The decrease in methane yield of both reactors was quicker than the decrease in removal efficiencies and decrease in pH. Throughout the study, the methane yield of reactor 2 was higher than reactor 1.
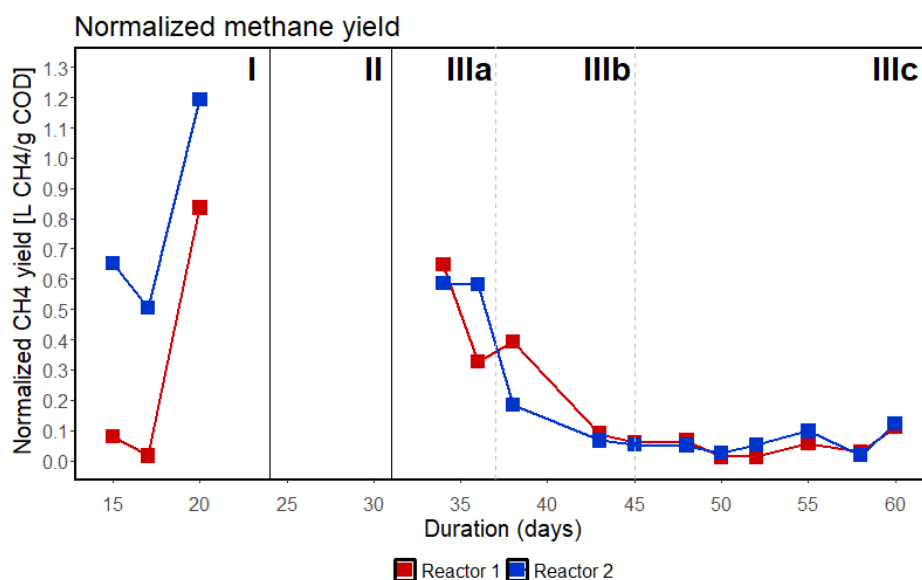


Figure 16. Normalized methane yield (L $CH_4$/g COD) of reactor 1 treating raw leachate and reactor 2 treating filtered leachate throughout the three experiments

The composition of the biogas produced by both reactors throughout the experiment was determined. Reactor 1 exhibited a methane fraction of 52.6% and a $CO_2$ fraction of 47.4%, whereas Reactor 2 had a higher methane content of 64.2% alongside a $CO_2$ fraction of 35.4%. This aligns with the methane yield (Figure 16), where reactor 1 yielded less methane compared to Reactor 2. Notably, in each experiment, Reactor 1 consistently failed prior to Reactor 2, resulting in biogas with a reduced methane content.

## 2.3. REACTOR STABILITY

### 2.3.1. PH

The stability of the reactors was evaluated through the monitoring of the pH. In Figure 17, the evolution of the pH of both reactors is shown for the different experiments. Every time the pH dropped below 6, the reactors were re-inoculated, which marked the end of the experiment and the beginning of the next experiment.

During the experiment I, the reactors were stable for two weeks at an OLR of 4-5 g COD/L*d and an HRT of 8 days. Once the entire volume of leachate was pumped in attaining the target OLR of 14 g COD/L*d, the reactors failed with reactor 1 drop acidifying four days before reactor 2. Since the influent was diluted with tap water during this experiment, the pH was slightly higher, around 4.64 ± 0.17. During experiment II, an OLR of 10 g COD/L*d with an HRT of 12.5 days was chosen and maintained for 7 days at which point the pH of the reactor dropped below 6.

In experiment III, in stage a both reactors remained stable with pH between 7-9. In stage b, the OLR was then increased, which resulted in a slight decrease in pH, but still remained above neutral levels. In stage c, the further increase in OLR resulted in an precipitous drop in pH across both reactors. Reactor 1 acidified first, followed by reactor 2.
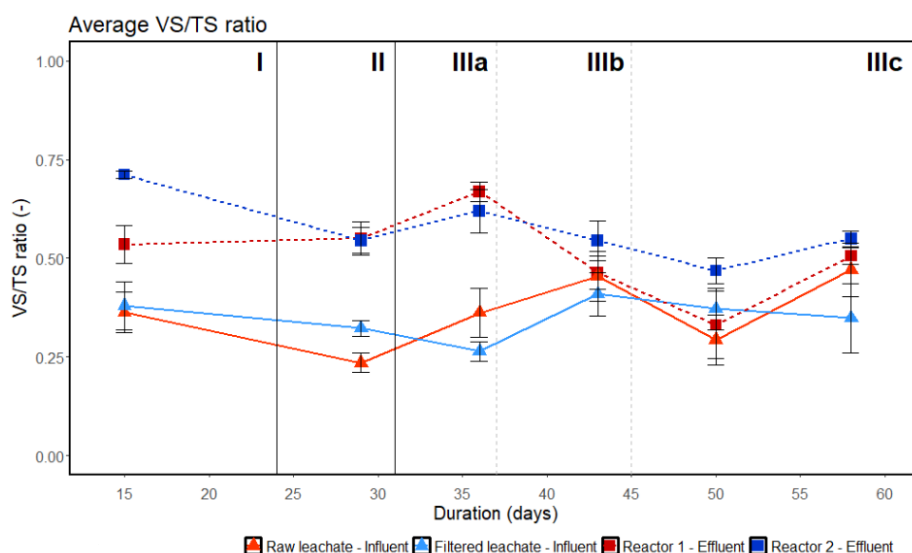


**Figure 17**. pH reactor 1 treating raw leachate and reactor 2 treating filtered leachate throughout the three experiments

The concentration of VFA is another parameter to assess the stability of the reactors. In Figure 18, the evolution of the total concentration of VFA in both the influent and effluent is shown.

In experiment I, the VFA concentration of reactor 1 was significantly higher than reactor 2 corresponding with the fact that reactor 1 destabilized before reactor 2. In experiment II, the VFA concentration was also high in both reactors. This corresponds with the drop in pH at that OLR signifying reactor destabilization.

In experiment III, during stages a and b, the concentration of total VFA were lowest in the effluent of both reactors, which shows that the reactors were stable. However, at the end of experiment IIIb, a slight increase in VFA concentration is detected in reactor 1 showing early signs of VFA accumulation. This trend was quickly followed by reactor 2 in experiment IIIc.
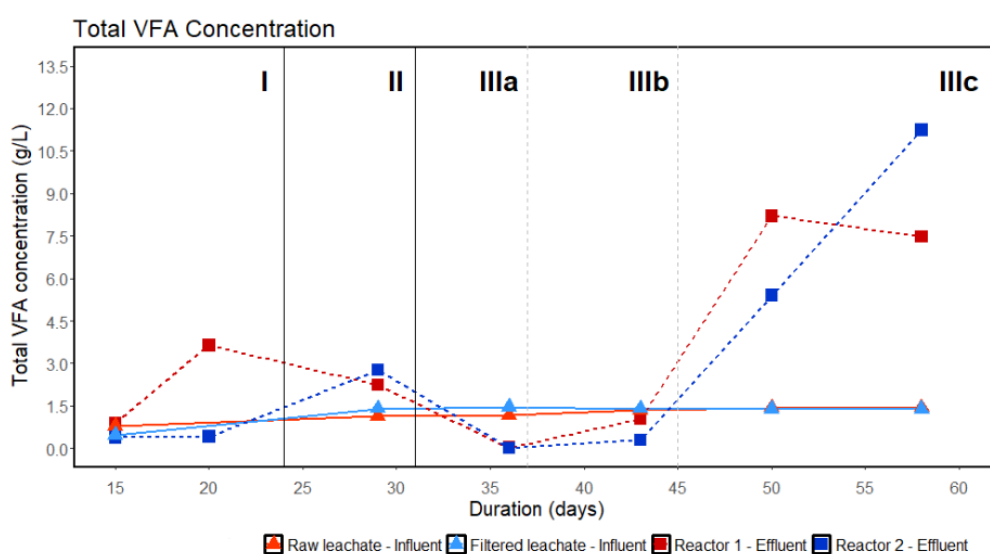


**Figure 18**. Total volatile fatty acids (VFA) concentration reactor 1 treating raw leachate and reactor 2 treating filtered leachate throughout the three experiments

## 2.3.3. ANIONS AND CATIONS

The concentrations of certain anions ($Cl^-$, $NO_3^-$, $PO_4^{3-}$ and $SO_4^{2-}$) and cations ($Na^+$, $NH_4^+$, $K^+$, $Ca^{2+}$ and $Mg^{2+}$) are used to evaluate the stability of the granules and their concentrations throughout the study are depicted in Figure 19 (cations) and Figure 20 (anions).

The concentration of $Na^+$ in the effluent was higher than in the influent throughout the three experiments. The concentrations were also more elevated during the more stable periods of the reactor (experiment IIIa & IIIb). A decrease in sodium levels was detected when the reactors were acidifying. The $NH_4^+$ concentrations were low in the influent. During experiment IIIa and b, the ammonium levels of the effluent were lower than the influent indicating a $NH_4^+$ take-up by the microorganisms. During the periods of reactor acidification, ammonium concentrations of the effluent rose above the concentrations of the influent. The $K^+$ and $Mg^{2+}$ concentrations of the influent were higher than the concentrations of the effluent throughout

the experiment, indicating potassium and magnesium uptake by the granules. Once the reactors started failing and granules were degrading the potassium and magnesium concentrations in the effluent increased. The $Ca^{2+}$ concentrations follow the stability of the reactors well. During experiment III a and b, the $Ca^{2+}$ concentrations of the effluent are lower than the influent indicating an uptake of the cations by the granules. When the reactors failed the concentrations in the effluent approached the concentrations of the influent, meaning the granules didn't take up the $Ca^{2+}$ anymore.



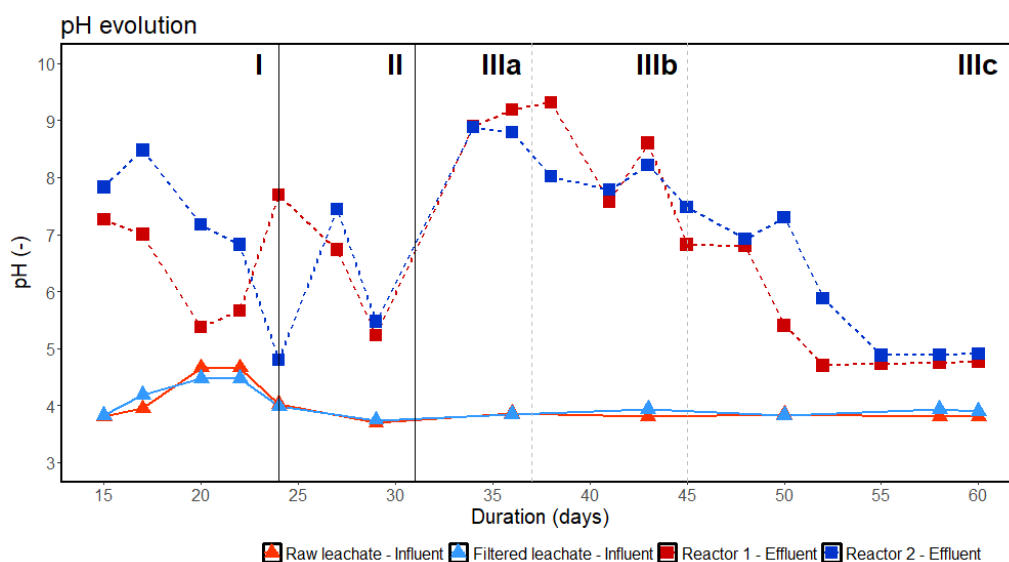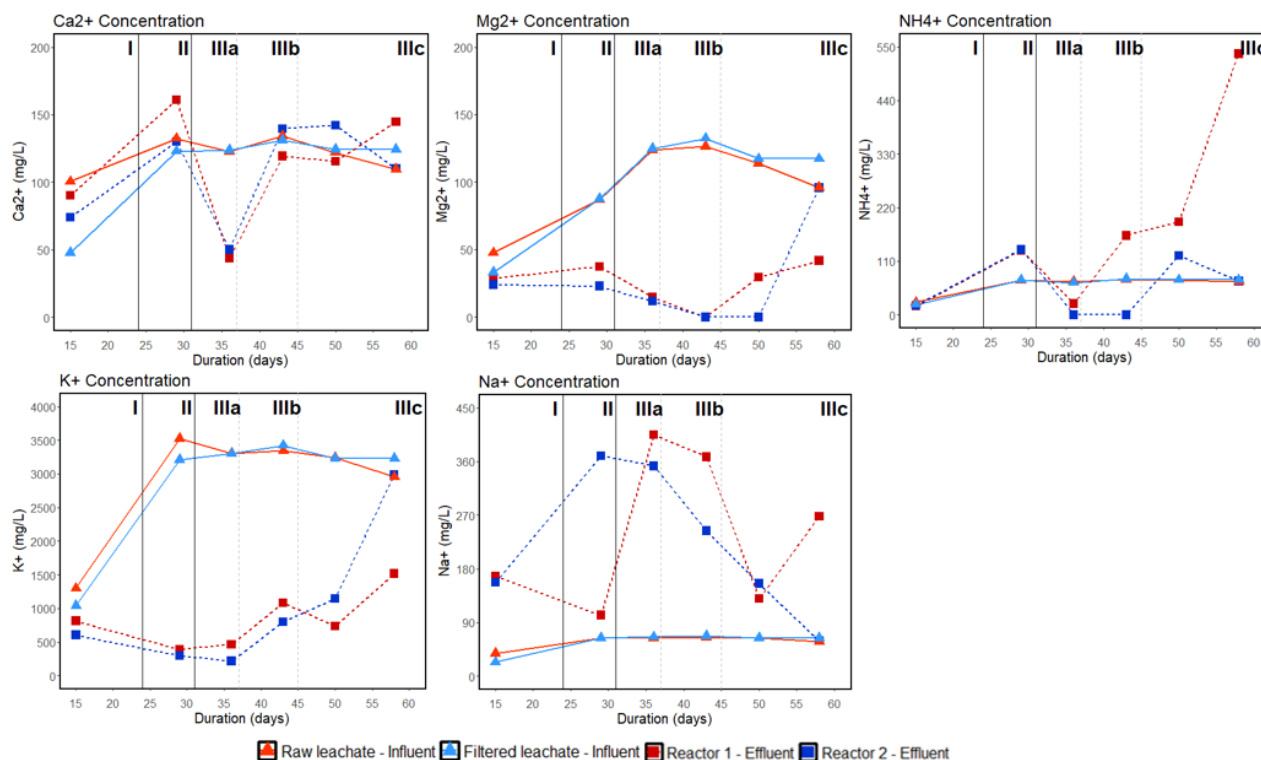**Figure 19**. Cation concentration ($Na^+$, $NH_4^+$, $K^+$, $Ca^{2+}$ and $Mg^{2+}$) of reactor 1 treating raw leachate and reactor 2 treating filtered leachate throughout the three experiments

The concentration of chloride ions in the influent is higher than the concentration in the effluent, suggesting that chlorine is retained in the reactors, potentially within the granular structures. At the end of experiment IIIc, when the reactors had failed due to the accumulation of VFA and granules had started degrading, a slight increase in $Cl^-$ is detected, corroborating the hypothesis. The $NO_3^-$ concentrations were also measured. The concentration of $NO_3^-$ of the influent was high compared to the concentration of the effluent throughout the three experiments, resulting in near complete nitrate removal by the UASB reactors. The $PO_4^{3-}$ concentrations in the influent are also higher than in the effluent throughout the three experiments. However, an increase of $PO_4^{3-}$ is detected in experiment II and IIIc when the reactors were destabilized, due to VFA accumulation. The increase of $PO_4^{3-}$ of reactor 1 during experiment III started before reactor 2, which aligns with the fact that reactor 1 had started failing before reactor 2. The $SO_4^{2-}$ concentrations in the influent are quite low. In experiment III, a small increase in sulphate levels is detected. Especially near the end of the experiment when the granules were degrading.
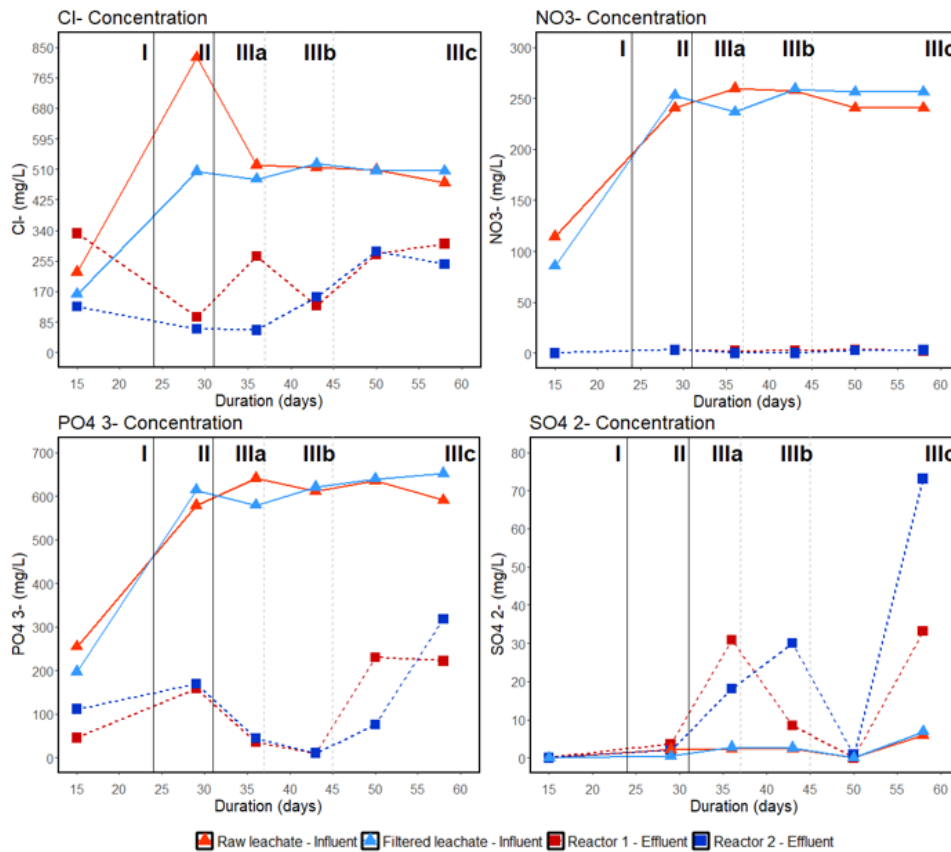
**Figure 20**. Anion concentrations (Cl⁻, NO₃⁻ and SO₄²⁻) of reactor 1 treating raw leachate and reactor 2 treating filtered leachate throughout the three experiments

## 3. BIOGAS AND ENERGY PRODUCTION

### 3.1. BIOMETHANE POTENTIAL TESTS

During the study, a series of biomethane potential tests (BMP) with the anaerobic granules were conducted to determine the maximum methane yield of the unfiltered leachate (TCOD), filtered leachate (sCOD), diluted unfiltered leachate (1/3 TCOD), diluted filtered leachate (1/3 sCOD). The diluted treatments were chosen to see if the pH of the influent would have a positive effect on AD.

In Figure 21, the normalized methane yield across the biological replicates for all treatments is shown. Overall, the methane yield of the undiluted treatments was highest with an average methane yield of 0.15 $\pm$ 0.03 L CH₄/g COD for the raw leachate (TCOD) and 0.15 $\pm$ 0.01 L CH₄/g COD for the filtered leachate (sCOD). There is a notable difference between the methane yield of the replicates of the diluted raw leachate (1/3 TCOD), since in two of the biological replicates VFA started accumulating, resulting in lower methane yield. The average methane yield of this treatment is 0.06 $\pm$ 0.08 L CH₄/g COD. The methane yield for both the diluted TCOD and sCOD treatment are lower than for the undiluted leachates, with an average of 0.06 $\pm$ 0.08 L CH₄/g COD for the diluted raw leachate and 0.14 $\pm$ 0.03 L CH₄/g COD for the diluted filtered leachate.
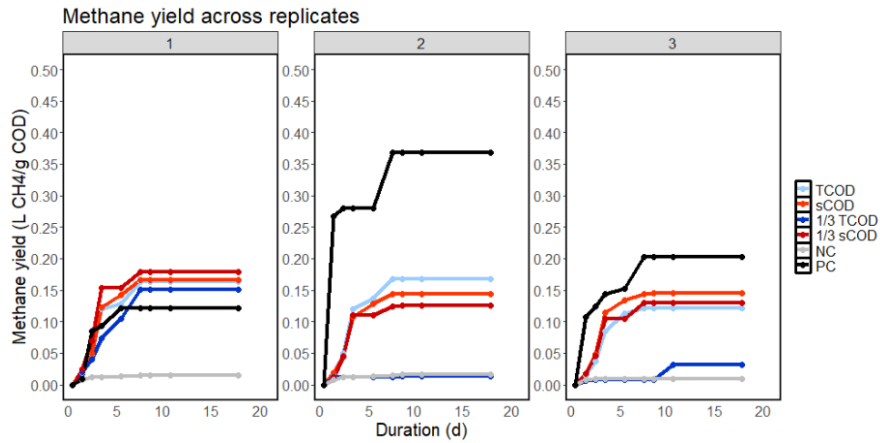
**Figure 21.** Normalized methane yield (NL CH$_4$/ g COD) across the replicates for all treatments during the biomethane potential test

In Figure 22, the average biogas composition (%CH$_4$ and %CO$_2$) for all treatments is shown. The higher the methane fraction, the more energy content the produced biogas contains. Typically, biogas contains between 50-70% CH$_4$ and 20-50% CO$_2$. The methane fraction across all treatments (excluding the negative and positive control) is very similar and around 50%. Both the negative and positive control produced biogas with a higher methane fraction than the different leachate treatments.
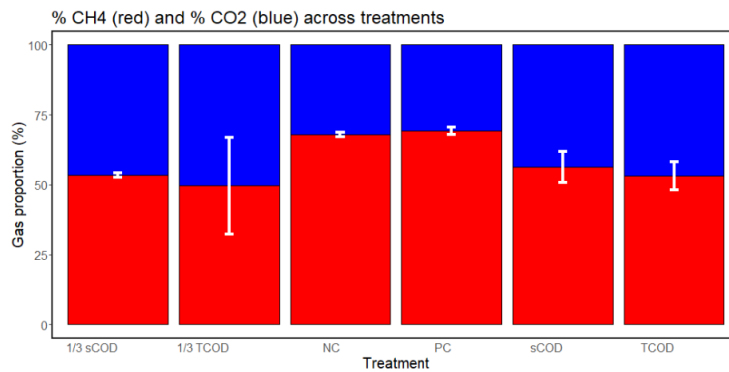


**Figure 22.** Average biogas composition (%CH$_4$ and %CO$_2$) for all treatments from the biomethane potential test

In Figure 23, general parameters determined at the end of the BMP test such as TS/VS ratio, pH, TS concentration, VS concentration, and total VFA concentration for all treatments are shown. The TS/VS ratio was similar for all treatments at the end of the study. The pH at the end of the study was around neutral levels for all treatments, however for treatment 1/3 TCOD, a slightly lower pH is observed, which aligns with the increased total VFA concentration in this treatment. As mentioned above, two of the three biological replicates of this treatment showed lower pH levels and elevated VFA concentrations.
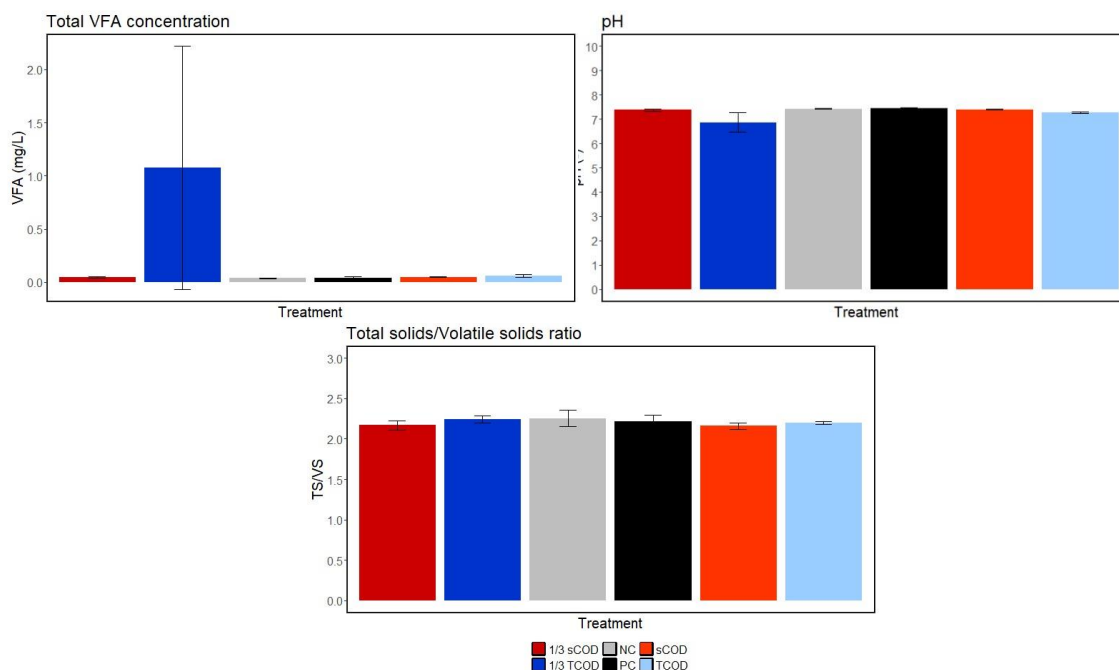
**Figure 23.** General parameters for all treatment determined at the end of the biomethane potential test; Average volatile fatty acids (VFA) concentration, Average pH., Average TS/VS ratio

## 3.2. ENERGY PRODUCTION

Based on the normalized CH$_4$ yield established in the BMP tests, the kWh produced daily per gram COD with a combined heat and power (CHP) unit can be calculated. For the calculations, the case study of Wastech is used (see section 3.3. and 3.5. of the literature study). The energy production is calculated with the COD concentration, the methane yield of all treatments converted to the amount of kWh produced knowing that 1 m$^3$ of methane has an energy content of 10.55 kWh.

The two options for biogas utilization, namely the direct use of biogas for heat generation with heat boilers and heat and electricity production through a CHP unit, were visualized. The energy production by the first option, namely the heat boiler for the direct use of biogas for heat generation with a thermal efficiency of 80-90%, was calculated. In the calculations to establish how much heat can be produced from the leachate, a 85% conversion efficiency is used. The results are shown in Table 6.

**Table 6.** Heat production from the biogas based on the normalized methane yield with a heat boiler for all treatments

| Treatment | COD concentration (g COD/L leachate) | Normalized CH$_4$ yield (L CH$_4$/g COD) | Heat produced (kWh/L leachate) |
|---|---|---|---|
| TCOD | 123.33 ± 2.05 | 0.15 ± 0.03 | 0.17 ± 0.03 |
| sCOD | 117.83 ± 2.66 | 0.15 ± 0.01 | 0.16 ± 0.01 |
| 1/3 TCOD | 29.07 ± 0.74 | 0.06 ± 0.08 | 0.01 ± 0.02 |
| 1/3 sCOD | 28.03 ± 0.25 | 0.14 ± 0.03 | 0.04 ± 0.01 |

The energy production by the second option, namely the CHP unit for both electricity production and heat production with an electrical efficiency of 41% and a thermal efficiency of 45%, was calculated and the results are shown in Table 7.

The energy production for the undiluted treatments is highest. The diluted treatments yield less energy per liter of leachate and the 1/3 TCOD treatment yields the least energy on average. This is because of the decreased normalized methane yield from the BMP tests were two of the three biological replicates of this treatment were experiencing VFA accumulation.

Table 7. Heat and electricity production from the biogas based on the normalized methane yield with a Combined Heat and Power unit (CHP) for all treatments

| Treatment | COD concentration (g COD/L leachate) | Normalized CH$_4$ yield (L CH$_4$/g COD) | Electricity produced (kWh/L leachate) | Heat produced (kWh/L leachate) |
|---|---|---|---|---|
| TCOD | 123.33 ± 2.05 | 0.15 ± 0.03 | 0.080 ± 0.014 | 0.088 ± 0.012 |
| sCOD | 117.83 ± 2.66 | 0.15 ± 0.01 | 0.078 ± 0.006 | 0.085 ± 0.006 |
| 1/3 TCOD | 29.07 ± 0.74 | 0.06 ± 0.08 | 0.007 ± 0.010 | 0.008 ± 0.008 |
| 1/3 sCOD | 28.03 ± 0.25 | 0.14 ± 0.03 | 0.018 ± 0.012 | 0.019 ± 0.003 |

Wastech is currently making plans for a new "plug & play" installation aiming to treat 1,500 tons of food waste annually producing 750 m$^3$ of leachate. Considering the annual requirements for this new installation at Wastech for 40 MWh electrical and 15 MWh thermal energy, they would need to treat up to 90 m$^3$ of leachate with a UASB reactor every year to meet their thermal energy requirements with a heat boiler or up to 500 m$^3$ of leachate to meet their electrical requirements with a CHP unit. Both the heat boiler and the CHP unit would allow Wastech to meet their energy requirements reducing their operational costs.

### 3.3. ECONOMIC FEASABILITY OF THE UASB REACTOR AND GAS UTILIZATION INSTALLATION

To establish the economic feasibility of the UASB reactor and a gas utilization technology (heat boiler or CHP unit), three scenarios are compared; In the first scenario, Wastech decides not to invest in the UASB reactor coupled with a heat boiler or CHP unit. The treatment of the leachate will be done externally and the thermal and electrical energy will be bought. In the second and third scenario, Wastech decides to invest in the UASB reactor, where in the second scenario it will be coupled with a heat boiler, and in the third scenario, it will be coupled with a CHP unit. In both scenarios 2 and 3, the leachate will be treated on site. In scenario 2, the thermal energy is provided by the heat boiler and electrical energy is bought. In scenario 3, both thermal and electrical energy is provided by the CHP unit. An estimation of the NPV of the two investments (UASB + heat boiler and UASB + CHP) is calculated and compared.

In the first scenario, Wastech decides not to invest in an UASB reactor coupled with either a heat boiler or a CHP unit, they would pay up to €10,000 per year for their thermal and electrical energy requirements (see literature section 3.3.).

In the second scenario, Wastech invests in a UASB reactor coupled with a heat boiler. First, the Total Capital Investment of the UASB reactor is calculated (Table 8). The size of the UASB reactor is estimated based on the leachate flow rate, the COD content of the leachate, and the OLR. Wastech will treat 750 m$^3$/year of leachate which gives a flow rate of 2.06 m$^3$/d, the COD concentration is equal to 123.33 g COD/L and an OLR of 5 g COD/L*d is assumed. In this study, the OLR of 5 g COD/L*d gave the highest methane yield and most stable reactor. This gives a reactor volume of 51 m$^3$ with a height of 8 m and a diameter of 2.84 m (Alaerts et al., 1990.; Lettinga & Hulshoff Pol, 1991).

Two pumps are needed, one for feeding the leachate to the UASB reactor and one for the recirculation line. A heat exchanger is necessary for heating the leachate to obtain a temperature of 34°C. The biogas produced by the reactor is collected from the top of the reactor and is compressed towards either the heat boiler or the CHP unit (Spachos & Stamatis, 2011; *U.S. Inflation Calculator: 1635→2024, Department of Labor Data*, n.d.).

Table 8. Total Capital Investment (TCI) of the Upflow Anaerobic Sludge Bed (UASB) reactor

| Compound | Cost (€) | Source |
|---|---|---|
| Pump #1 | 3,000 | Spachos & Stamatis, 2011 |
| Pump #2 | 3,000 | Spachos & Stamatis, 2011 |
| UASB reactor | 20,400 | Alibaba.com |
| Heat exchanger | 6,667 | Spachos & Stamatis, 2011 |
| Biogas compressor | 5,000 | Airhorse8888.com |
| Rest (Piping, valves and their installation) | 5,000 | Spachos & Stamatis, 2011 |
| Cost of equipment (PEC) | 43,067 | |
| Measuring and sampling devices (6% of PEC) | 2,584 | Spachos & Stamatis, 2011 |
| Electric works, boards (19% of PEC) | 8,183 | Spachos & Stamatis, 2011 |
| TOTAL TCI UASB reactor | 53,834 | |

The operational costs of the UASB reactor include energy and repair & maintenance costs. Electricity is also needed for the two pumps, measuring and sampling devices and the compressor and heating is necessary to obtain mesophilic temperatures. The average electricity demand of a UASB reactor is 86 MJ$_e$ per day or 8.76 MWh annually and the average heating demand is equal to 195.7 MJ$_t$ per day or 20 MWh annually depending on the temperature of the influent and the usage of the heat exchangers. The UASB reactors comprise parts that are sensitive to wear and tear (gas collection pipes, inlet pipes, sludge bleed valves etc.) Maintenance and repair costs are 4-6% of the investment in equipment (Alaerts et al., 1990).

Biogas boilers are typically rated based on their output (e.g., kW or BTU/hr). The capacity required depends on the energy content of the biogas. Based on the results from the BMP tests we know that annually 127,500 kWh of thermal energy is produced from 750,000 L of leachate. The required capacity of the boiler is;

$$Boiler\ capacity = \frac{127,500\ kWh/year}{8,760\ hours/year} = 15\ kW$$

Next, the costs of the heat boiler are estimated. The total cost of investment is shown in Table 9. The compressed biogas coming from the UASB reactor goes to the burner where it is burned with ambient air. The gases are directed to a steam boiler and are disposed at a mean temperature of 200°C. The steam produced in the steam boiler is directed to the plant to cover part of its thermal needs. A small part of the steam produced is use to heat up the hot water needed in the second heat exchanger, and to heat up the condensate in the deaerator to the temperature of 102°C. Because of the losses taking place in the plant, the condensates returning to the deaerator are not equal to the steam delivered to the plant. The rest of the quantity needed is covered with the addition of fresh water (Spachos & Stamatis, 2011; *U.S. Inflation Calculator: 1635→2024, Department of Labor Data*, n.d.).

Wastech can apply for subsidies from the Flemish Government for this investment, called de 'Ecologiepremie'. The net subsidy for this technology is 49.5% of the total investment cost (*Subsidies & Financiering | VLAIO*, n.d.).

**Table 9**. Total Capital Investment heat boiler and Upflow Anaerobic Sludge Bed (UASB) reactor

| Compound | Cost (€) | Source |
|---|---|---|
| Steam boiler | 10,000 | Spachos & Stamatis, 2011 |
| Steam separator | 2,000 | Spachos & Stamatis, 2011 |
| Heat exchanger #2 and pump #3 | 2,222 | Spachos & Stamatis, 2011 |
| Burner | 2,750 | Spachos & Stamatis, 2011 |
| Air compressor | 6,000 | Airhorse8888.com |
| Deaerator | 4,000 | Spachos & Stamatis, 2011 |
| Others (piping, valves, racks) | 3,750 | Spachos & Stamatis, 2011 |
| **Cost of equipment boiler (PEC)** | 30,722 | |
| **Total cost of equipment UASB + boiler** | 84,556 | |
| Installation cost (20% of PEC) | 16,911 | Spachos & Stamatis, 2011 |
| Contingency (10% of PEC) | 8,456 | Spachos & Stamatis, 2011 |
| Engineering costs (10% of PEC) | 8,456 | Spachos & Stamatis, 2011 |
| **Total cost of investment UASB + heat boiler** | 118,379 | |
| **Total cost of investment with subsidies** | 58,598 | *VLAIO.be* |

The maintenance cost for the pumps and the compressors is assumed to be equal to 4% of the purchase cost and the cost for the maintenance cost for the rest of the equipment, such as the reactors, the steam boiler, the deaerator, the heat exchangers, the piping and the valves is equal to 4% of the purchase cost. The electricity consumption for the operations of the pumps and compressors is equal to 21.55 kWh monthly.

If Wastech treats 750 m$^3$ of leachate per year (at 0.17 kWh$_t$/L leachate), 127.5 MWh of thermal energy is produced by the heat boiler (see Table 6). In Belgium, the gas price in 2024 is equal to €0.10/kWh$_t$ (*Energieprijzen Anderlecht (Brussel) Mei 2024 | Mijnenergie.Be*, n.d.). Wastech thermal energy requirement is only 15 MWh, the rest of the gas can be sold.

**Table 10**. Total cash flow of the Upflow Anaerobic Sludge Bed (UASB) reactor coupled with a heat boiler

| Type of cost | Cost (€/year) | Source |
|---|---|---|
| **Cash outflow** | | |
| Electricity costs | 6,057 | Spachos & Stamatis, 2011 |
| Maintenance costs UASB reactor | 2,153 | Allaerts et al., 1990 |
| Maintenance costs heat boiler | 1,229 | Spachos & Stamatis, 2011 |
| **Cash inflow** | | |
| Revenue from heat | 12,750 | *mijnenergie.be* |
| **Total cash flow** | 3,311 | |

Based on the total capital investment and the total cash flow of the UASB reactor coupled with a heat boiler, the net present value of the installation is calculated. A lifespan of the installation of 15 years and a weighted average cost of capital (WACC) of 5% is assumed. The NPV of the UASB coupled with the heat boiler is – €25,000. To make this investment economically viable (NPV > 0), Wastech should treat at least 900 m$^3$ of leachate per year, which is 150 m$^3$ more than their current plans for the plug-and-play installation.

In scenario 3, Wastech invests in the UASB reactor coupled with a CHP unit. The dimensions of the UASB reactor and its associated investment and operational costs are the same as in scenario 2 (Table 8). The initial investment cost of the CHP unit is shown in Table 11. The installation consists of a CHP unit, a heat exchanger to recover heat from the engine exhaust and jacket water, an electrical system including a generator, inverters and transformers and other components such as piping and valves. Also for this investment, Wastech could receive an 'Ecologiepremie' from the Flemish Government of 49.5% of the total investment cost.

The CHP units are also rated based on their output (e.g., kW or BTU/hr). Based on the results from the BMP tests we know that annually 60,000 kWh of electrical energy is produced from 750,000 L of leachate. The required capacity of the CHP is;

$$CHP\ capacity = \frac{60,000\ kWh/year}{8,760\ hours/year} = 7\ kW$$

**Table 11**. Total cost of investment of the Upflow Anaerobic Sludge Bed (UASB) reactor coupled with a Combined Heat and Power (CHP) unit

| Item | Cost (€) | Source |
|---|---|---|
| CHP unit | 32,500 | Epa & CHP, n.d. |
| Heat exchanger | 6,667 | Spachos & Stamatis, 2011 |
| Piping and valves | 3,750 | Spachos & Stamatis, 2011 |
| Control system | 2,000 | |
| Cost of equipment CHP (PEC) | 44,917 | |
| Total cost of equipment UASB + CHP | 98,805 | |
| Installation cost (20% of PEC) | 19,761 | Spachos & Stamatis, 2011 |
| Contingency (10% of PEC) | 9,881 | Spachos & Stamatis, 2011 |
| Engineering costs (10% of PEC) | 9,881 | Spachos & Stamatis, 2011 |
| Total cost of investment UASB + CHP | 138,328 | |
| Total cost of investment with subsidies | 68,472 | |

The maintenance costs of the UASB reactor are the same as in scenario 2, as the dimensions of the reactor have not changed and the maintenance costs of the CHP unit are 4% of the cost of equipment of the CHP unit.

Wastech treats 750 m$^3$ of leachate annually at 0.088 kWh$_t$/L leachate and 0.08 kWh$_e$/L leachate. The energy prices in Belgium are currently (May 2024) equal to electricity for 0.3778€/kWh and gas for 0.1€/kWh (*Energieprijzen Anderlecht (Brussel) Mei 2024 | Mijnenergie.Be*, n.d.).

**Table 12**. Total cash flow of the Upflow Anaerobic Sludge Bed (UASB) reactor coupled with a Combined Heat and Power (CHP) unit

| Type of cost | Cost (€/year) | Source |
|---|---|---|
| Cash outflow | | |
| Maintenance costs UASB reactor | 2,153 | Allaerts et al., 1990 |
| Maintenance costs CHP | 1,797 | Spachos & Stamatis, 2011 |
| Cash inflow | | |
| Revenue from heat | 6,600 | *mijnenergie.be* |
| Revenue from electricity | 22,668 | *mijnenergie.be* |
| Total cash flow | 25,318 | |

Based on the total capital investment and the total cash flow of the UASB reactor coupled with the CHP unit, the net present value of the installation is estimated. The same assumptions in terms of lifespan and WACC as in scenario 2 are made. The NPV of the UASB coupled with the CHP unit is €195,000 with an internal rate of return (IRR) of 37%.

# PART V. DISCUSSION

## 1. SUITABILITY OF THE LEACHATE FOR UASB REACTORS

The first objective of the study was to determine whether the leachate produced by the start-up Wastech was suitable for anaerobic digestion treatment with a UASB reactor. In the characterization of the leachate, several important aspects of the leachate were discovered that should be considered.

### 1.1. ALKALINITY AND PH

The leachate, a byproduct of processing fruit and vegetable waste, has typically low pH and alkalinity. This low alkalinity presents a challenge when treating it using UASB reactors. These reactors require a certain level of alkalinity to handle pH fluctuations and maintain an optimal environment for microbial granules.

When the influent stream has a low pH, it's crucial to have adequate buffering capacity to stabilize pH levels within the reactor. This buffering capacity, primarily provided by alkalinity, helps counteract fluctuations in volatile fatty acid (VFA) concentration. The recommended alkalinity range in the influent is between 250 and 950 mg/L (Singh et al., 1999; M. K. Tiwari et al., 2006). However, the alkalinity of the leachate was zero, indicating a low buffering capacity in the influent. Consequently, even a small increase in VFA concentration led to a drop in pH in all three experiments (Figure 19). Moreover, adequate alkalinity is essential for the formation and maintenance of microbial granules. The composition and strength of the substrate, as well as intra-granular diffusion, strongly influence the microstructure of these granules (Rizvi et al., 2018). The lack of alkalinity in the leachate can explain why the reactors destabilized rapidly.

The concentration of $PO_4^{3-}$ in the leachate was $61.1 \pm 2.7$ mg/L, which should provide a certain buffer capacity (0.6 mM). However, as can be seen in Figure 21, the concentration of $PO_4^{3-}$ in the effluent was always smaller than the concentration in the influent for both reactors, indicating $PO_4^{3-}$ was taken up by the microorganisms in the granular sludge and didn't contribute to the buffer capacity.

During digestion, certain buffers are also created, namely $CO_2$ is produced by the methanogens (see II. Literature section 1.3.2). The solubility of $CO_2$ depends on the pH in the reactors (Figure 24). At neutral pH levels (between pH 6-9), $HCO_3^-$ is the main species and contributes to the buffer capacity of the reactors. As the pH decreases, the solubility of $CO_2$ decreases and won't contribute to the buffer capacity anymore. This creates a positive feedback loop in the reactors; The buffer capacity was already low making the reactors sensitive to pH fluctuations when VFA started accumulating, decreasing the pH, which decreased the solubility of $CO_2$ and decreased the buffer capacity further (König et al., 2019).
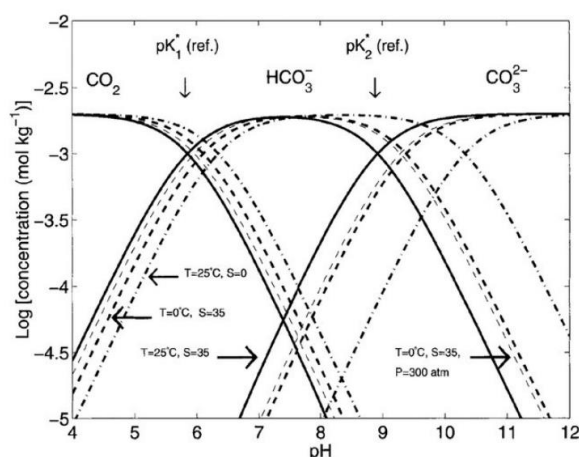
**Figure 24.** Solubility of CO$_2$ in water as a function of the pH value (König et al., 2019)

To ensure stable operation of their UASB reactors, Wastech must prioritize increasing the alkalinity of the leachate. Two main solutions are proposed to increase the alkalinity of the influent and make the UASB reactors more robust against pH fluctuations, namely co-digestion or the addition of buffers.

Co-digestion of different feedstocks has as main advantages the balance of nutrients, C/N ratio equilibrium and the minerals and metals required for equilibrium, and an increased buffering capacity of the system, enhancing the overall process stability and performance of organic matter biodegradation, optimizing the biogas and methane yield (Huang et al., 2016; Khalid et al., 2011; C. Zhang et al., 2014). In a study conducted by Pavi et al. (2017), fruit and vegetable waste was digested with the organic fraction of municipal solid waste (OFMSW), which has a higher alkalinity than fruit and vegetable waste. This helped to increase the overall alkalinity and to control the possible acidification during the fruit and vegetable waste (FVW) digestion. The most significant biogas yield occurred with an OFMSW/FVW ratio of 1:3. Therefore, if Wastech can access another waste stream with higher alkalinity, co-digesting it with the leachate could be beneficial. Examples of such waste streams are brewery, dairy or paper mill wastewater. However, if obtaining such a waste stream requires collaboration with an external partner, transportation logistics may reduce its appeal. Additionally, co-digestion would necessitate a pretreatment step to ensure proper mixing of the two waste streams at the desired ratio.

Adding an additional buffer to the influent will also increase the buffering capacity and make the UASB reactors less sensitive to pH fluctuations. Several buffers are often used in UASB reactors such as a phosphate buffer ($KH_2PO_4$/$K_2HPO_4$) or a bicarbonate buffer ($HCO_3^-$/$CO_3^{2-}$) (Gutiérrez et al., 1991; Singh et al., 1999). As discussed in the literature review (Literature section 1.3.2.), the addition of buffers also has disadvantages such as costs and the interference of the added chemicals with the microbial processes.

## 1.2. CARBON CONTENT OF THE LEACHATE

The carbon content of the leachate was measured as chemical oxygen demand, total solids and volatile solids. Total solids measures the amount of organic and inorganic solids in the feedstock and volatile solids analysis determines the total amount of organic matter in a feedstock. Chemical oxygen demand (COD) is

the amount of oxygen needed to oxidize the organic matter and used to estimate the energy content of the organic matter. As a rule of thumb, the total COD is equal to 1.6x the VS. However, in the leachate the COD/VS ratio was much higher, namely 8.82 ± 0.14. In general, feedstocks with a high COD/VS ratio contain organic matter that is high in energy. This high COD/VS ratio (> 2) also indicates that the leachate contained a lot of volatile organics that were already removed during the TS phase, therefore the TS and VS measurements were an underestimation of the actual solids in the leachate (Hamilton, n.d.).

The VS/TS ratio of a substrate also provides information on the suitability of the substrate for AD treatment. A high VS/TS ratio, at least 0.6-0.7, indicates the presence of high levels of organic matter in the feedstock, which is suitable for biogas production (Ahmadi-Pirlou & Mesri Gundoshmian, 2021; Hamilton, n.d.). The VS/TS ratio of the leachate was equal to 0.31 ± 0.03, which means that only 31% of the solid components were organics (Khatri et al., 2015). This value was most likely an underestimation of the actual VS/TS ratio, but it is difficult to say since the TS and VS measurements were not reliable.
The VS/TS ratio of the leachate can be increased by removing the solids from the leachate, like in the filtered leachate influent in the study. However, the centrifugation of the leachate to obtain the filtered leachate only slightly increased the VS/TS ratio. By removing the solids, the hydrolysis step of AD could be faster producing VFA intermediates at a faster rate and if the methanogens are able to grow along this VFA accumulation, biogas production will be increased.

The COD/N ratio of the leachate is very high, namely 153.75 meaning that the leachate is very carbon-rich compared to nitrogen. As discussed in the literature study (section 1.3.4.), a high C/N ratio induces slow degradation rates and leads to low total ammonia nitrogen and low free ammonia concentrations in the digester. The optimal C/N ratio for AD is between 20-35, with the ratio of 25 the most commonly used. If the C/N ratio is too high, there is insufficient nitrogen to maintain biomass, reducing biogas production (Mao et al., 2015). Co-digestion offers a potential solution to this challenge. Mixing the leachate with a waste stream rich in nitrogen, such as dairy wastewater, can effectively lower the overall C/N ratio, achieving the desired balance for optimal anaerobic digestion.

The composition of the carbon in the leachate was determined at the end of the study. The concentrations of carbohydrates and short-chain alcohols in the leachate stream varied widely. While some components, like lactate, succinate, acetate, and xylose have relatively low concentrations, others, such as glucose, ethanol and galactose were much higher. Carbohydrates and short-chain alcohols are generally biodegradable compounds that can be converted into biogas in an UASB reactor. However, very high concentrations of certain compounds, notably ethanol, were discovered which means that the ethanol concentration evolved during storage, since no ethanol was present in the leachate when it was collected in January. An excessive concentration of ethanol can inhibit methanogenic activity and decrease the biogas yield. It is advised that Wastech treats their leachate with an UASB reactor rapidly after it is produced to avoid ethanol accumulation during storage. An optimization analysis conducted by Y. Wang et al. (2009) showed that ethanol at a concentration of 1.6 g/L led to the highest accumulative methane yield and a maximum methanogenic bacteria concentration. The concentration of ethanol (43.9 ± 6.9 g/L) in the leachate at the end of the study exceeded this value extensively.

It is interesting to look at the cation concentrations in the leachate, as certain cations have an influence on the granulation process, microbial activity and process stability.

According to the multi-valence positive ion-bonding model for the granulation process proposed by Mahoney et al. (1987), cations such as calcium ($Ca^{2+}$) and magnesium ($Mg^{2+}$) have a positive impact on the formation of granules. Bacteria have negatively charged surfaces under neutral pH conditions, and multi-valence cations, such as calcium or magnesium reduce the electrostatic repulsion between negatively charged bacteria. Concentrations of 80-200 mg/L of $Ca^{2+}$ and 12-120 mg/L of $Mg^{2+}$ were found to increase the rate of anaerobic granulation in UASB reactors (Liu et al., 2003; Mahoney et al., 1987; Schmidt & Ahring, 1993). Compared to these values, the leachate contains the right range of concentration of $Mg^{2+}$ (115 ± 14 mg/L) and $Ca^{2+}$ (122 ± 10 mg/L). The calcium ions also stimulate methanogen growth and conversion of contaminants into methane (Vanček et al., 2006). Wastech could dose calcium in the UASB reactors to enhance anaerobic granulation, however, it is important to note that high calcium concentrations (< 500 mg/L) also cause serious operation problems, such as precipitation and accumulation of calcium in anaerobic granules and reduced microbial activity of granules (Liu et al., 2003).

Sodium is also essential for methanogens for its role in the formation of adenosine triphosphate or in the oxidation of NADH. Optimal $Na^+$ were reported in the range of 100-200 mg/L to be beneficial for the growth of mesophilic anaerobes, namely mesophilic acetolactic methanogens and mesophilic hydrogenotrophic methanogens. The concentration of $Na^+$ in the leachate (63.0 ± 3.3 mg/L) was below this optimal range, which can explain why the reactors were sensitive to the VFA accumulation; The metabolism of methanogens was disrupted decreasing their growth rate (Dimroth & Thomer, 1989; McCarty, 1964). Wastech could dose sodium in the UASB reactors in the form of $Na_2HCO_3$ which will increase the $Na^+$ concentration and act as a buffer against pH fluctuations.

Potassium influences the performance of the UASB reactor as well. Karnchanawong & Kabtum (2014) reported that slight inhibitions on COD removal were detected at $K^+$ concentrations of 3,920 mg/L, and from concentrations above 7,320 mg/L, strong inhibitions were observed in both COD removal and biogas production. $K^+$ is a monovalent cation and has a very high water solubility for most forms of its salts. It remains unchanged throughout AD, causing its apparent toxicity to anaerobes. The moderate and severe toxic levels of $K^+$ to anaerobes are reported to be 2,500-4,500 and > 12,000 mg/L, respectively. The $K^+$ concentration of the leachate is 3,213 ± 176 mg/L, which lies within the moderate levels of toxicity for anaerobes and could explain the unstable reactor performance. Due to the high potassium levels, industrial biogas units for distillery wastewater typically operate with a low COD loading rate (around 3 kg/m³·d). This would result in larger reactor sizes and higher investment costs compared to other industrial WWTP. One basic and straightforward approach to decrease the concentration of $K^+$ is dilution. However, while dilution can improve process efficiency, it also increases the volume of treated wastewater, leading to higher disposal costs (Femandez & Eforster, 1993; Karnchanawong & Kabtum, 2014; Srisuwanno et al., 2023).

## 2. SUITABILITY OF RAW VERSUS FILTERED LEACHATE FOR UASB REACTORS

In the second part of the study, a comparison between the treatment of the raw leachate and the treatment of filtered leachate was established. The reactor performance and stability of both reactors are compared.

### 2.1. PERFORMANCE OF THE REACTORS

As the primary objective of Wastech is to treat the leachate with UASB reactors, the COD removal of both reactors throughout the study was compared.  The COD removal efficiencies of both reactors were high and very similar in the three experiments, so removing the solids from the leachate doesn't seem to have a big influence on the overall COD removal (Figure 14). Also, the average VS/TS ratio of the effluent from both reactors was within the same range throughout the study (Figure 15).

A notable difference in performance between the two reactors was observed in terms of the methane yield (Figure 16). In experiments I and III, the reactor treating the filtered leachate consistently exhibited a higher methane yield compared to the reactor treating the raw leachate. This can be attributed to the filtration process removing suspended solids, leaving behind soluble COD that is generally more biodegradable and more easily degraded by microorganisms. These compounds are often rich in readily available carbon sources, such as simple sugars and fatty acids. Consequently, they are metabolized more efficiently by the microbial community leading to a higher methane yield.

It's imperative to assess whether the UASB reactors adequately remove carbon, nitrogen, and phosphorus from the leachate to ensure that the effluent concentrations of these compounds meet the established discharge standards in Flanders for the discharge of wastewater in surface water. In this region, wastewater discharge standards for industrial sectors are outlined in VLAREM II as sectoral conditions. Chapter 5 (Appendix 5.3.2 17°) of VLAREM II specifies discharge standards for fruit and vegetable processing companies, including limits of 125 mg COD/L for Chemical Oxygen Demand (COD), 10 mg total N/L for nitrogen, and 5 mg P/L for phosphorus. The effluent of the UASB reactors do not meet any of these discharge standards. The effluent consistently demonstrated COD concentrations above 1000 mg COD/L. The ammonium concentration of the effluent for both reactors exceeded 10 mg N/L, so the total N in the effluent will also exceed the discharge limit. Phosphate concentrations in the effluent of both reactor also surpassed 5 mg P/L, consequently the total phosphorus concentration also exceeded the discharge limit. With such a performance of the UASB reactors, Wastech will not be allowed to discharge these effluents in the surface water in Flanders and will have to pay for an additional after-treatment of the effluent, which is typically an aerobic wastewater treatment.

### 2.2. STABILITY OF THE REACTORS

In the study, the stability of both UASB reactors, treating the raw leachate and treating the filtered leachate, was studied and compared in terms of pH and VFA concentration (Figure 17 & 18). In general, the reactor treating the raw leachate was more sensitive to VFA accumulation and the pH dropped more rapidly at the same OLR values, suggesting a lower buffering capacity in this reactor.

This can be attributed to the higher solids content present in the raw leachate, which tends to accumulate in the reactor sludge, thereby causing recurrent clogging of the recirculation line. The recirculation line recirculates the biomass and keep the upflow velocity sufficiently high (in this case 1 m/h). López-López et al. (2015) reported that the recirculation line could reduce the impact of VFA and organic matter concentration present in the influent and increases the buffer capacity of the reactor, inducing the stability of the reactor. However, the recirculation line of the reactor treating the raw leachate (reactor 1) clogged frequently because of the accumulation of solids, which could explain why this reactor was more sensitive to VFA accumulation and acidified more rapidly at the same OLR.

The stability of the UASB reactors in terms of anion and cation concentrations was also studied (Figure 19 & 20). As mentioned before, divalent cations have a positive effect on anaerobic granulation. These cations help bind negatively charged cells together to form microbial nuclei that promote further granulation. The use of divalent cations to assist granulation lies in their ability to condense the diffusive double-layers resulting in relatively stronger effect of van der Waals forces (Mahoney et al., 1987; Schmidt & Ahring, 1993). The $Ca^{2+}$ and $Mg^{2+}$ are, thus, incorporated in the granular structures, explaining why their concentrations in the effluent follow the stability of the reactor very well. As the stability of the reactors decrease due to overloading and consequently VFA accumulation, the granules start disintegrating and the concentrations of $Ca^{2+}$ and $Mg^{2+}$ in the effluent increase.

The $K^+$ ions play a crucial role in the membrane potential of the bacteria and archaea. These ions contribute to the electrochemical gradients across the cell membrane, which are essential for various cellular processes. The $K^+$ ions typically have a high intracellular concentration, while $Na^+$ ions have a higher extracellular concentration, creating a gradient used for nutrient uptake and waste expulsion. The movement of these ions through specific channels and transporters regulates the membrane potential, enabling processes such as ATP synthesis, motility and the maintenance of cell homeostasis. When bacteria and archaea are inhibited by living in an environment that is too acidic, the concentrations of $K^+$ can be affected, disrupting their normal physiological functions. In an acidic environment, the concentrations of protons ($H^+$) outside the cells is much higher. This leads to an increased influx of $H^+$ ions into the cell, which disrupts the intracellular pH balance and affect the ion gradients across the membrane. This causes an efflux of $K^+$ ions as the cell tries to counterbalance the increased positive charge from the influx of $H^+$, which disrupts the cell's membrane potential and impair cellular functions (Alberts et al., 2002). This can be seen in Figure 19 in Experiment IIIc, as the reactors acidified, the $K^+$ concentrations in the effluent of both reactors increased.

## 2.3. BIOMETHANE POTENTIAL

Biomethane potential tests were conducted to determine the maximum possible methane yield of both the raw and the filtered leachate, and to see whether the removal of solids has a positive effect on the methane yield, like in the UASB reactors (Figure 21). Moreover, the influence of dilution to increase the pH of the leachate on the methane yield is evaluated.

When comparing the results of the BMP tests of the raw leachate and the filtered leachate (both undiluted and diluted), it is noted that the methane yield of the undiluted filtered leachate ($0.15 \pm 0.03$ L $CH_4$/g COD) and the methane yield of the undiluted raw leachate ($0.15 \pm 0.01$ L $CH_4$/g COD) are similar, but the methane yield of the diluted raw leachate ($0.06 \pm 0.08$ L $CH_4$/g COD) is lower than the methane yield of the diluted filtered leachate ($0.14 \pm 0.03$ L $CH_4$/g COD). The removal of solids don't seem to have a positive effect on the methane yield of the batches, which does not correspond to the results of the UASB reactors, where the methane yield from reactor 2 (treating the filtered leachate) was always higher than the methane yield of reactor 1 (Figure 16). In continuous operation, the removal of solids has a positive effect on the reactor performance compared to the batch experiments. The accumulation of solids in the UASB reactor treating raw leachate caused frequent clogging in the recirculation line, decreasing the process performance and stability.

From the comparison between the diluted and the undiluted treatments, it can be noted that the normalized methane yield from the undiluted filtered leachate is the highest. The increased pH of the leachate when it is diluted with water does not influence the methane yield nor has an effect on the overall stability. On the contrary, for the diluted raw leachate, as two of the three biological replicates showed VFA accumulation, indicating that dilution destabilized the batches compared to the undiluted batches. So, the diluted leachate both didn't improve the performance of the biogas production and didn't improve the stability of the batches.

## 3. ENERGY PRODUCTION FOR WASTECH

Finally, the potential biogas yield and its energy content were evaluated to assess the economic benefits for Wastech. Two options for utilizing the biogas were explored: direct use for heat generation with heat boilers, and combined heat and power (CHP) production for both heat and electricity.

### 3.1. HEAT BOILER

The heat boiler utilizes biogas produced by the UASB reactors and produces thermal energy in the form of steam, which can be used in the Wastech process. The biogas boilers are very similar to natural gas boiler, which makes them easy to install and maintain. The thermal efficiency of a biogas boiler is also high, namely between 80-90% (Hakawati et al., 2017). A heat boiler system is generally a simple system with a relatively low investment cost. The operational complexity is low and is easy to manage.

In Table 8, the energy production of the heat boiler was calculated based on the normalized methane yield obtained in the BMP tests. The highest energy production was obtained with the raw leachate ($0.17 \pm 0.03$ kWh/L leachate), and is very similar to the energy production of the filtered leachate ($0.16 \pm 0.01$ kWh/L leachate). With this thermal energy production, Wastech would have to treat 95 $m^3$ leachate annually to meet their annual thermal energy requirements of 15 MWh. In the new 'plug' and 'play'-installation of Wastech 750 $m^3$ of leachate will be produced which is largely sufficient for the thermal energy requirements and the additional gas can be sold providing an additional revenue. However, this excess heat may be wasted if not managed properly or if there is a lack of heat storage solutions.

## 3.2. COMBINED HEAT POWER UNIT

The combined heat and power (CHP) unit utilizes the biogas produced by the UASB reactors to generate heat and electricity. Compared to the heat boilers, CHP requires a larger investment cost and since it is a more complex process, it requires more technological knowledge. This can also lead to an increased operational cost and the need for skilled personnel. The efficiencies of the CHP unit are typically lower with an electrical efficiency of 41% and a thermal efficiency of 45% (Hakawati et al., 2017).

In Table 9, the thermal and electrical energy production based on the normalized methane yield obtained in the BMP tests is shown. The highest energy production was obtained with the raw leachate like for the heat boilers. With this thermal energy production, Wastech would have to treat 500 m$^3$ leachate annually to meet their annual thermal energy requirements of 15 MWh and electricity requirements of 40 MWh. In the new 'plug' and 'play'-installation of Wastech 750 m$^3$ of leachate will be produced which is largely sufficient for the thermal and electricity energy requirements and the additional electricity and gas can be sold providing an additional revenue.

## 3.3. ECONOMIC FEASIBILITY

In the economic analysis, the three possible scenarios, one without investment in a UASB reactor, one with a UASB reactor coupled with a biogas boiler and one with a UASB reactor coupled with a CHP unit, were described and compared. The appeal of different scenarios depends on the amount of leachate produced during the process operation. If Wastech successfully minimizes leachate production and keeps it well below the discharge limits, Scenario 1 is the most attractive option. In this case, no investment in a UASB reactor, heat boiler, or CHP unit is necessary, and the leachate can be discharged into local sewage systems. Annual electrical and thermal energy costs would be around €10,000. This scenario is also operationally simpler, avoiding the complexities of running and stabilizing a UASB reactor. However, Wastech would be vulnerable to fluctuating energy prices in times of crisis. In 2022, during the energy crisis, caused by the Russian invasion of Ukraine, the gas and electricity prices were at a record height. Between December 2020 and 2022, the average electricity price had risen by 110.5%. A reoccurrence of these prices, could be detrimental to a start-up like Wastech and given the current geo-political scene in Europe, it is not excluded that this could happen again. Therefore producing heat and electricity on site and being independent of external gas and electricity prices offers a lot of financial stability.

If Wastech prioritizes producing its own gas and electricity and cannot minimize leachate production or keep its content below discharge limits, necessitating treatment, Scenario 3 is preferable. Investing in a UASB reactor coupled with a CHP unit is economically viable, with a NPV of €195,000 and an IRR of 37%. This investment would make Wastech energy independent from the grid and less vulnerable to extreme energy prices during crises. On-site treatment would eliminate the need for external treatment expenses. Scenario 2, involving investment in a UASB reactor with a heat boiler, is the least favorable. It only becomes economically viable when Wastech treats more than 900 m³ of leachate per year. Additionally, the heat boiler only produces heat, requiring Wastech to purchase electricity externally, making them vulnerable to rising electricity prices in times of crisis.

# PART VI. CONCLUSION

Wastech, a Belgian start-up, effectively addresses food processing waste by re-integrating it into the food system through the cultivation of black soldier fly larvae for animal feed production. This innovative process generates a waste stream called leachate, which is currently untreated and disposed of in local sewage systems. This leachate, rich in organic matter, presents an opportunity for further circularity. By treating it with anaerobic digesters, Wastech could produce biogas, a renewable energy source, thus enhancing the sustainability of their operation.

This study evaluated the suitability of the leachate produced by Wastech for anaerobic digestion treatment using a UASB reactor in the first part, compared the treatment of raw leachate filtered leachate with UASB reactors secondly and assessed the economic feasibility of the UASB reactors coupled with a gas utilization technology.

The leachate exhibited low pH and zero alkalinity, posing a challenge for UASB reactor stability. Adequate buffering capacity is essential to counteract pH fluctuations, caused by volatile fatty acid (VFA) accumulation. The lack of alkalinity led to rapid reactor destabilization, emphasizing the need for Wastech to increase the leachate alkalinity. Potential solutions include co-digestion with waste streams of higher alkalinity or adding chemical buffers, though the latter comes with cost and potential microbial process interference. The leachate showed a very high COD/VS ratio, indicating the presence of a lot of volatile solids. The high COD/N ratio suggested an imbalance in nutrients, which can be addressed by co-digestion with nitrogen-rich waste streams. Additionally, high concentrations of ethanol in stored leachate could inhibit methanogenesis, necessitating rapid treatment post-production to avoid ethanol buildup. The concentrations of essential cations, like $Na^+$, in the leachate were below optimal levels for anaerobic granulation and methanogenic growth. Increasing these cation levels could enhance granulation and reactor stability, though care must be taken to avoid excessive concentrations that could lead to operational issues.

Secondly, comparing raw and filtered leachate treatments revealed that filtration and removing solids improved methane yield and reactor stability. The removal of suspended solids in the filtered leachate left more readily biodegradable organics, enhancing microbial activity and methane production. However, both treatments failed to meet regional discharge standards for COD, nitrogen, and phosphorus, indicating the need for additional effluent treatment.

Lastly, evaluating biogas utilization options, the study found that both raw and filtered leachate could produce sufficient biogas to meet Wastech's energy requirements. A combined heat and power (CHP) unit was deemed an economically viable investment, making Wastech energy-independent with a NPV of €195,000 and IRR of 37% over a lifespan of 15 years. In contrast, a heat boiler-only option was less favorable unless treating over 900 m³ of leachate per year.

Overall, while challenges exist, strategic enhancements and thorough evaluations could significantly improve the efficiency and reliability of UASB reactors for treating Wastech's leachate, paving the way for sustainable waste management and energy recovery.

# FUTURE PROSPECTS

For future research, it would be valuable to explore various strategies to enhance the suitability of leachate for UASB reactors, aiming for extended periods of stable reactor operation. One area of interest is investigating the impact of mixing leachate with other waste streams to improve key properties such as alkalinity, C/N ratio, and cation concentrations. This approach could be compared to conventional methods like adding buffers or diluting the leachate to determine which method provides better long-term stability and reactor performance.

Additionally, a comparative study between raw leachate and filtered leachate could be undertaken to evaluate which option offers more long-term stability in UASB reactors. This would involve analyzing the performance of the reactors over an extended period to uncover any potential advantages or disadvantages associated with using raw versus filtered leachate. This could provide deeper insights into optimizing leachate treatment processes and improving the overall efficiency and reliability of UASB reactors.

Lastly, conducting a more elaborate technological and economic comparative study between a UASB reactor coupled with a biogas boiler or a CHP unit, and a scenario without a UASB reactor for biogas recovery would be valuable. This analysis would help determine the viability of these investments for the start-up, providing insights into the potential benefits and drawbacks of each option.

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