Enhancement of volatile fatty acids production from food waste: Impact of inoculum, pH and retention time

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Abstract

Resource recovery and bio-based production of materials from waste streams is one of the pillars of transitioning into a circular economy and meeting the environmental sustainability agendas set by United Nations. This experimental study aims to investigate the influence of inoculum and other operating parameters on the recovery of volatile fatty acids (VFAs) from batch anaerobic digestion of food waste using different anaerobic digested sludge as inoculum. VFA production was more substantially affected by pH compared to the type of inoculum. The highest VFA contents up to 84% of the soluble COD was achieved under pH 10 for all the different inoculum on day 10, while the highest acidification ratio for pH 5 experiments ranged between 39-42% on day 15. Based on the obtained results, using only digested food waste as inoculum augmented the acidification ratio from 67% to 84% in alkaline conditions, while the presence of food waste in inoculum did not influence the VFA production under acidic conditions.

Key words: food waste; anaerobic digestion; volatile fatty acids; inoculum; pH

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1- Introduction

The escalating global trend of waste generation is a major concern of the 21st century. Food waste is one of the main constituents of the municipal solid waste [1] which can be produced throughout different stages of the food value chain, such as; production, processing, distribution, inventory, retail as well as the consumers stage [2]. It is estimated that one third of the global generated food is discarded as waste which accounts for 25% of the entire water consumption in agriculture sector and is tantamount to 990 billion USD per year [3]. In 2011, the food and agricultural organization reported that 1.3 billion tonnes of food waste was produced globally [4]. Conventional waste treatment techniques such as landfilling and incineration are expensive, energy extensive and impose detrimental environmental effects such as greenhouse gas emissions [1, 5]. Anaerobic digestion (AD) is a cost-effective alternative that is gaining more attention as realization of next generation wastewater treatment plants opens new avenues in proper waste management as well as energy production and recovery of value-added materials from waste streams. Food waste is a suitable feedstock for AD processes due to its facile biodegradability, nutrient-rich nature, high energy and moisture content and rampant availability [5-7].

Anaerobic digestion is comprised of four different stages, namely; hydrolysis, acidogenesis, acetogenesis and methanogenesis involving various types of bacteria and archaea. Volatile fatty acids (VFAs) are the intermediary products of the first three stages of anaerobic digestion that can be exploited as renewable carbon sources in different industries [8]. VFAs are short chain fatty acids containing six or less carbon atoms, with a broad range of applications in bioplastics production [9], biodiesel [10], biological nutrient removal processes [11] as well as electricity generation [12]. To date, VFAs are obtained through chemical processes using petrochemicals as their required raw material. Bio-based production of VFAs through acidogenic fermentation is more appealing as it is cost-effective and environmentally friendly. The global market of bio-based VFAs is estimated to reach 515 billion euros in 2020, which is five times higher compared to 2010 [13] .

Up to now, numerous researches have been conducted on optimizing the operational parameters of anaerobic digestion such as pH [14, 15] , temperature [16, 17], substrate [18], organic loading rate [17, 19] and retention time [20]. It is known that inoculum is also one of the contributing factors to the performance of the AD processes. Nevertheless, the impact of inoculum on the fermentative production of VFAs has not been well studied yet. In the current thesis, three different anaerobic digestion sludge were used to investigate the influence of inoculating media. Furthermore, two different conditions of pH 5 and pH 10 were tested to compare the influence of inoculum and pH on lab-scale VFA production from food waste.

2- Literature review

2.1- Food waste

According to the UN Food and Agriculture Organization (FAO) definition, any wholesome or edible material that is lost, wasted or degraded at any step of the food supply chain is regarded as food waste [3]. The annual mean food waste generation is reported to be 100-170 kg per person in developed countries which is two times higher compared to developing countries [2]. In 2012, the total amount of 90 million tons Mt food waste was generated in Europe, where United Kingdom ranked first with 14 Mt of food waste generation, and this number is estimated to augment to 120 Mt by the year 2020 [3].

2.1.1- Food waste characteristics

Depending on the contents, the physicochemical characteristics of food waste can vary tremendously in different parts of the world. For instance, the food waste only collected from households and restaurants in Europe is comprised of 40% fruits and vegetables, 33% pasta and bread, 17% dairy products and 9% meat and fish residues. Whilst in countries such as Japan, China and south Korea, the food waste composition is 56% fruits and vegetables, 34% rice and noodle and the rest is dairy products, fish and meat residues which is much lower compared to European countries. This difference stems from different eating and dietary habits. Furthermore, the FW composition varies based on which stage of the food supply chain it is collected from. The composition of food waste at production stage and consumer are depicted in figure 1.

In general, when food waste is rich in vegetables, rice and pasta, carbohydrates are the dominant component. However, the FW with higher percentages of meat, fish and eggs are replete with proteins and lipids [3].

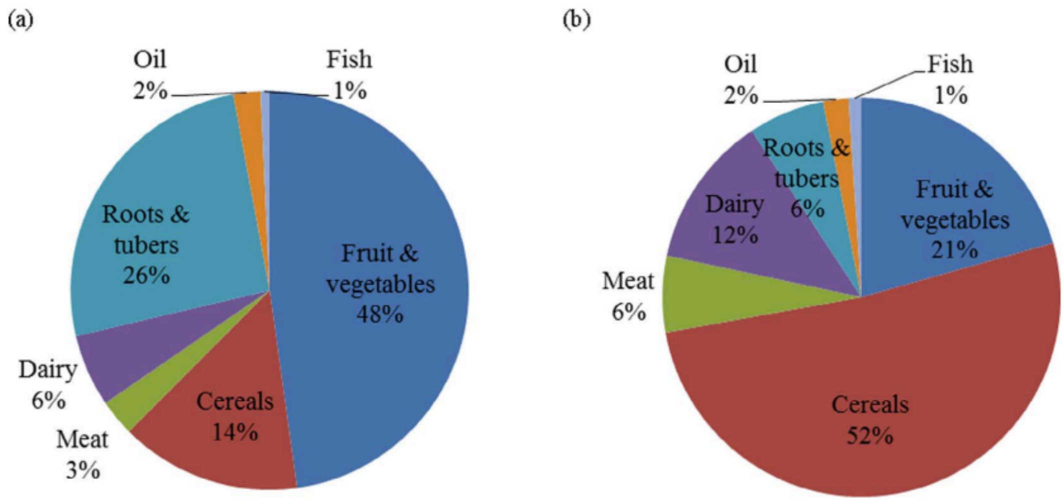

Figure 1- Composition of food waste from production stage (a) and consumer stage (b)

In spite of the variations in the composition, there are common characteristics among the FW throughout the world. In general, the principal components of FW are carbohydrates, proteins and lipids which are present in the ranges 41-62% of carbohydrates, 15-25% proteins and the lipid content is in the range of 13-30% with negligible amounts of heavy metals [3]. Normally, FW streams contain acidic pH with an average of 5.1± 0.7 [21], with comparatively low C/N ratio ranging between 13.2 to 24.5 [22]. The organic content is expressed in terms of total solids (TS) or volatile solids (VS) and the ratio of VS/TS is approximately around 90% which presents a great suitability for biological treatment approaches [2]. The moisture content of the food waste is approximately 70-80% [6]. Table 1 summarizes some of the general characteristics of FW found in literature.

Table 1- General characteristics of food waste

Parameters	Zhang et al. [23]	Zhang et al. [24]	Zhang et al. [25]	Li et al. [26]
TS (% w.b.)	18.1	23.1	30.90	24
VS (% w.b.)	17.1	21	26.35	232
VS/TS (%)	0.94	90.9	85.3	94.1
pH	6.5	4.2	__	__

Carbohydrate (% d.b.)	61.9	__	__	55.2
Protein (% d.b.)	__	__	__	15
Fat (% d.b.)	23.3	__	__	23.9
C (% d.b.)	46.67	56.3	46.78	54
N (% d.b.)	3.54	2.3	3.16	2.4
C/N	13.2	24.5	14.8	22.5

2.2- Anaerobic digestion

Food waste generation has an escalating global trend due to population growth and urbanization and its disposal is becoming troublesome. Disposal methods of food waste, namely incineration and landfilling have adverse environmental impacts. The associated problems are emission of toxic and greenhouse gases that are usually malodourous. The generated leachate pollutes groundwater and furthermore, these processes are energy and cost extensive [1].

Anaerobic digestion is an appealing environmentally-friendly approach which is comparatively cost-effective and presents the opportunity of materials and energy recovery, which is not only economically sensible, but also contributes to the development of a sustainable socio-economy [2, 3].

Anaerobic digestion is a complex biological process were a broad range of bacteria and methanogenic archaea [1] decompose biodegradable and organic substances in the absence of oxygen [27]. Anaerobic fermentation encompasses four successive stages of hydrolysis, acidogenesis, acetogenesis and methanogenesis. In the sections below the first three stages of anaerobic digestion will be discussed in more details.

2.2.1- Hydrolysis

In the first step of AD, complex and high molecular weight organic substances namely; proteins, carbohydrates and lipids are broken down into their constituents; amino acids, glucose and fatty acids, respectively. Hydrolysis takes place with a slow pace and is considered as the rate-limiting step of the overall AD process [27].

The slow nature of the hydrolysis can be attributed to the time-consuming adsorption of enzymes on the surface of the solid substrates [28, 29]. The overall reaction of the hydrolysis step is presented in reaction (1) below:

$$n\ C_6H_{10}O_5 + \mathrm{n}\ H_2O \rightarrow n\ C_6H_{12}O_6 \qquad \text{Rxn. (1)}$$

The hydrolysis is conducted by extracellular enzymes rereferred to as hydrolyses or lyses [27, 30], that are excreted by a group of facultative anaerobe and anaerobic bacteria of genera such as *Streptococcus* and *Enterobacterium [31]*.

2.2.2- Acidogenesis:

In the second stage, the obtained small molecular weight monomers from hydrolysis are further broken down to simpler molecules such as short chain length fatty acids (acetate, propionate, butyrate), aldehydes, alcohols in conjunction with other gaseous side-products namely: carbon dioxide, ammonium, hydrogen and hydrogen sulfide [1, 6, 27, 30] . Drastic pH drops are observed in the second step due to generation of VFAs. The reactions occurring at the acidogenesis step are exhibited in reaction 2 and 3 [27].

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH \qquad \text{Rxn. (2)}$$

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CHOHCOOH \qquad \text{Rxn. (3)}$$

The existence of oxygen consuming bacteria is of paramount importance in this stage as the presence of oxygen and nitrates are regarded toxic to the process [27, 30].

2.2.3- Acetogenesis

In acetogenesis, the products of the acidogenesis step are further digested into acetate, hydrogen and carbon dioxide [6]. During the first three steps of anaerobic digestion that are referred to as acid fermentation, no organic material is eliminated but they are only broken down and primed as substrates for the subsequent methanogenesis step. Reactions 4,5 and 6 summarize the overall process of the acetogenesis step [27].

$$CH_3CH_2\mathrm{COOH} + 2\ H_2O \rightarrow\ CH_3COOH +\ CO_2 + 3\ H_2 \qquad \text{Rxn. (4)}$$

$$CH_3CH_2CH_2COOH + 2\ H \rightarrow\ 2\ CH_3COOH + 2\ H_2 \qquad \text{Rxn. (5)}$$

$$CH_3CH_2OH \rightarrow\ CH_3COOH + 2\ H_2 \qquad \text{Rnx. (6)}$$

Volatile fatty acids are the products of the three aforementioned stages of anaerobic digestion process which then are converted to methane and carbon dioxide in the final step of AD known as methanogenesis. The aim of the current thesis was to enhance the procurement of VFAs through acidogenic fermentation. Therefore, the methanogenesis step will not be addressed.

2.3- Volatile fatty acids

Volatile fatty acids or short chain carboxylic acids [20] contain six or fewer carbon atoms [12] which are building block chemicals that are conventionally produced from petroleum-derived resources. Petrochemical-derived resources are non-renewable and cause adverse environmental effects such as green-house gas emissions [8]. VFAs can also be obtained through bio-based approaches as they are the intermediary products of the second and third steps in anaerobic digestion process. Recently, the generation and recovery of VFA has gained substantial interest due to their broad range of applications including biological nutrient removal (phosphorous and nitrogen)[11], building block in pharmaceuticals, food and chemical industries and furthermore as raw material for production of biogas [32], bio-diesel [33], biohydrogen [34], electricity from microbial fuel cells [35] and polyhydroxyalkanoates (bioplastics) . Recently, a shift towards VFAs through food waste fermentation has gained more attraction as their added value (50–130 $/ton) is higher compared to methane production (0.72 $/m^3) [8].

Acetic acid, propionic acid and butyric acid are the most commonly produced VFAs. Acetic acid is mostly employed in the production of pesticides, paints, plastics, polymer emulsions and paper coatings [36]. Propionic acid is utilized as food and fruits flavors and preservatives [37]. Butyric acid is used in biodiesel production and as an antibiotic in animal feed [38]. Table 2 outlines general properties of the three most common VFAs and their respective production approaches and applications.

Table 2-General properties of VFAs [8]

VFA	Chemical Formula	Market price (€/ton)	Application
Acetic acid	$C_2H_4O_2$	400-800	Food additives, adhesive, solvent, polymers, coatings
Propionic acid	$C_3H_6O_2$	2000-2500	Animal feed, pharmaceuticals, aroma additive in food industry
Butyric acid	$C_4H_8O_2$	1500-1650	Animal and human food additives, solvent, flavoring agent

2.3.1- Applications of waste-derived volatile fatty acids

Volatile fatty acids obtained from waste streams can have numerous applications such as polyhydroxyalkanoates (bioplastic), biological nutrient removal, biodiesel as well as electricity generation. In this part a few of these applications are reviewed.

2.3.1.1- Polyhydroxyalkanoates (bioplastics)

Polyhydroxyalkanoates (PHAs) are microbiologically produced polyesters with tunable mechanical and physical properties, high biodegradability, non-toxicity and low environmental impact [39]. Aforementioned characteristics make PHAs a promising substitute for conventional petrochemical plastics [40]. In spite of the broad range of applications of PHAs, the large-scale production is hindered due to high production expenses. This is ascribed to the high costs of carbon source used as substrate which approximately accounts for 40-48 % of the total production costs [41]. While production of PHAs by pure microbial cultures has higher yield, due to high costs of sterilization, implementation of microbial mixed cultures is preferred. Utilization of microbial mixed cultures can substantially diminish the production costs of PHAs and make them more economically feasible. The PHA production process from microbial mixed culture (MMC) typically encompasses three steps: [40]

1) Supplementation of a proper feedstock enriched with readily biodegradable organic substances. The preferable feedstock is predominantly rich in volatile fatty acids (VFA) which can be obtained through acidogenic fermentation.
2) Production of a microbial mixed culture with high PHA accumulation potential
3) PHA accumulation in the microbial mixed culture

It should be noted that the composition of the VFAs not only stipulates the composition of the PHA monomers [40] but also, a proportional correlation between the length of the fatty acids and the molecular structure of the resulting PHA monomers have been reported [42]. Therefore, controlling the operating parameters of acidogenic fermentation is of great importance [12].

2.3.1.2- Biological nutrient removal

Biological nutrient removal is referred to aerobic nitrification succeeded by anoxic denitrification for biological nitrogen removal purposes or biological phosphorous removal under interchanging anaerobic and aerobic conditions [12]. Normally, a carbon to nitrogen ratio ranging between 5-10 mgCOD/mgN is required for combined nitrification/denitrification process [43]. Commercial chemicals such as methanol and acetate are commonly used as extra carbon sources [8] as the present carbon substrate in wastewater is normally inadequate [12]. Different studies have indicated the positive influence of utilization of waste-derived VFAs as external carbon sources in terms of higher removal efficiencies [44, 45]. The composition of VFAs produced from waste streams impacts the rate and efficiency of the denitrification processes [11].

2.3.1.3- Biodiesel

Depletion of fossil fuel resources has propelled the production of biodiesel as an environmentally-friendly alternative. Yet, biodiesel production is impeded due to high costs of feedstock that constitute 70-75 % of the total production costs [46]. Lipids are converted to methyl esters of long chain fatty acids also known as biodiesel. Utilization of edible lipids such as rapeseed oil or soybean oil has the conflict of using food resources for fuel generation. VFAs produced from waste streams can be used for microbial lipid synthesis by oleaginous microorganisms; as microbial lipids possess the same composition as jatropha oil or soybean oil [12]. This has been proposed as a plausible solution to decrease the biodiesel production costs [8].

2.3.1.4- Electricity

The chemical energy of organic substances can be exploited as source for electricity generation by using microbial fuel cells (MFCs). A microbial fuel cell is comprised of anodic and cathodic compartments and a proton exchange membrane in the middle [47]. At the anode, electrons, protons and CO_2 are produced from oxidation of VFAs by microorganisms. Electrons move along an external circuit to enter the cathodic compartments, while protons flow through the proton exchange membrane in order to reach the cathode. Water is formed by incorporation of electrons, protons and oxygen in the cathode. Reactions (10) and (11) display the reactions occurring at anodic and cathodic chambers.

$$CH_3COO^- + 2\,H_2O \rightarrow 2\,CO_2 + 7\,H^+ + 8\,e^- \qquad \text{Rxn. (7)}$$

$$O_2 + 4\,e^- + 4\,H^+ \rightarrow 2\,H_2\mathrm{O} \qquad \text{Rxn. (8)}$$

2.4- Operating parameters of an anaerobic digester

Operating conditions in the anaerobic fermentation process play a pivotal role in the process performance and subsequently on the composition and yield of the end-products. In the sections below, operational parameters such as pH, temperature, substrate, inoculum, retention time, agitation, C/N ratio and the respective influence of their alteration is outlined.

2.4.1- pH

The pH value is a crucial operating parameter that influences the digestion process. Different pH intervals are favorable in different stages of an AD process. Also based on the targeted end-product different pH values are of interest. In hydrolysis and acidogenesis steps, a pH range of 5.5-6.5 is preferred. While, in the methanogenesis step, a neutral pH ranging between 6.5 and 7.2 is optimal for the growth of methanogens and biogas production [6, 48]. When the objective of the AD process is biogas production, neutral pH conditions are employed and drastic declination in pH values can cause process failure. Normally, at high organic loading rates, VFA accumulation decreases the pH substantially and can become problematic. According to [49, 50], if the ratio of the concentration of propionic acid to acetic acid exceeds 1.4, the process fails. On the other hand, in the cases with VFA production as the purpose, acidic or alkaline conditions are implemented as they impact both hydrolysis rate and acidogenesis step [8]. It should be noted that acidogens are sensitive to pH conditions and cannot endure extreme acidic (pH 3) or extreme alkaline (pH 12) environments [51].

2.4.2- Temperature

The operating temperature is one of the key influential parameters as it not only affects the enzymatic activities [48], but it also plays a crucial role in the hydrolysis rate, VFA yield [52] and the quality of the digestate [6]. Anaerobic digestion can be operated under four operational temperature ranges; namely, psychrophilic (10-30°C), mesophilic (30-40°C), thermophilic (50-60°C) and extreme thermophilic (60-80°C) [6, 48].

While, elevated temperatures positively influence the metabolic rates of the microorganisms, increase the rate of pathogenic destruction [48] and the total VFA production [12], process control is more complicated at thermophilic conditions and it is more energy-extensive to keep the process at higher temperatures. Maintaining a constant operating temperature is of great importance as temperature variations negatively impact the microbial growth [48] and the improvement in the VFA yield is not that substantial at higher temperatures [12]. While operating under mesophilic conditions, the microbial community can endure and adjust better to environmental changes and the operational costs are lower. The drawback is that longer retention times are required while the biogas production is still lower compared to thermophilic conditions [27].

In the case of food waste, a study by *Komemoto et al.* demonstrated substantially higher overall solubilization rates in mesophilic conditions in comparison to thermophilic conditions [53].

The effect of temperature on the type of obtained VFAs is not substantial compared to pH [12]. However, *Esteban-Gutiérrez et al.* stated that thermophilic conditions lead to higher VFA accumulation. Different compositions are reported in the literature operating under different temperatures of anaerobic digestion of food waste [13]. Operating at 35°C and 45°C, acetate and propionate were the major produced VFAs of the fermentation of FW, while butyrate was the dominant product (81%) when the process was operated at 55 °C [17].

2.4.3- C/N ratio

The ratio of carbon to nitrogen concentration in the digestor plays an important role. Carbon is the energy source and nitrogen contributes to the growth of microorganism [27]. A ratio between 20 to 30 has been reported to be optimal to maintain the appropriate amount of nitrogen required for the process. The biogas production rate declines at high C/N ratios due to expeditious depletion of the nitrogen from the tank. Whereas, low C/N ratios can result in ammonia build-up [12, 27]. *Ghosh et al.* reported an optimal range of 22 to 25 for the anaerobic digestion of fruit and vegetable wastes [54].

2.4.4- Substrate

The characteristics of the substrate used in fermentation process influences both the amount of the produced VFAs as well as their composition. Based on the type of the utilized substrate, different operating parameters might require modifications. For instance, adjustments of C/N ratio, a pretreatment step or different retention times might be necessary to enhance the performance of the AD process based on the type of the used substrate [52]. In a study by *Min et al.* propionate was the dominant acquired VFA (up to 78%) when the substrate was rich in total kjeldahl nitrogen content [55]. While, *Feng et al.* obtained low propionate content using a substrate with high protein content [56]. Co-digestion is a proper solution to increase the yield in the cases where there is a paucity of essential nutrients and trace elements [52].

2.4.5- Agitation (mixing)

Mixing is another important operational parameter as it generates a sufficient contact surface between substrate and inoculum hence, improving the efficiency of the digester. Furthermore, scum formation is avoided, and solids deposition is prevented by keeping them in suspension. Mixing can be achieved by mechanical stirrers or through recirculation of the generated biogas depending on the total solid concentration in the digester tank. It is noteworthy that excessive mixing can be detrimental to the microbial consortium and disturb the syntrophic relationships [27, 57].

2.4.6- Inoculum

Inoculum is one of the integral aspects in the performance of anaerobic digestion, as a functional community of microorganisms disintegrates the organic substances and contributes to the rate of material degradation and products formation. The major microbes involved in anaerobic processes are hydrolytic bacteria, acidogenic bacteria and methanogens [52]. Hampering the activity of methanogens results in higher VFA accumulation which can be carried out in different ways such as pH adjustments and addition of inhibitory factors. Manipulations in the inoculum and microbial communities results in alteration of metabolic pathways, yielding different products for instance, if a specific type of VFA is of interest. To date, the literature on the influence of inoculum on the performance of anaerobic digestion is limited.

2.4.7- Retention time (RT)

Retention time is defined as the average time during which a particular feedstock is remained in the reactor. Retention time of the waste and microbial communities is another influencing parameter in the performance of AD process. Volume of the reactor and consequently capital investments and domination of the main microbial species in the tank are influenced by the retention time [12]. Theoretically, more reaction time is provided between microorganism and substrate at higher retention times, having a beneficial influence on the total VFA production [58].

Retention time can differ depending upon the type of the utilized substrate and other operating parameters such as temperature. Retention time can not only improve the VFA yield but also has an impact on the composition [8]. In a study using paper mill effluent and whey, the production of propionic acid was increased at longer retention times while the butyric acid production was diminished [12].

3- Materials and methods

3.1- Food waste and Inoculum

In the current thesis, food waste was utilized as the substrate for the anaerobic digestion experiments. Three different mixed culture consortia were assessed as inoculating media for VFA production. The food waste was collected from the Syvab wastewater treatment plant in south of Stockholm, Sweden. The incoming stream to the plant includes household food waste and the faulty batches of breweries which is then heated at 71 °C for 61 minutes according to Swedish hygienization requirements.

The first Inoculum originated from the mesophilic anaerobic digestion tanks of Henriksdal wastewater treatment plant, Stockholm, Sweden, containing a mixture of digested primary sludge (PS), waste activated sludge (WAS) and digested food waste (DFW). The second inoculum was also obtained from mesophilic anaerobic digesters of Henriksdal wastewater treatment plant comprised of PS, WAS and glycerol (G). The third sludge was collected from the facilities of Scandinavian biogas in south of Stockholm which only contained digested food waste. Table 3 summarizes the inoculum which were acclimated to different types of waste.

Table 3- Inoculums and their corresponding acclimated waste stream

Inoculum	Content
Inoculum 1	PS/WAS/DFW
Inoculum 2	PS/WAS/G
Inoculum 3	DFW

PS, primary sludge; WAS, waste activated sludge; FW, food waste; G, glycerol

All the samples of inoculating media and the food waste were stored at 4 °C and no pre-treatment was applied to the samples prior to experiments. The main characteristics of the substrate and inoculum are presented in table 4 .

Table 4- Substrate and inoculum characterization

	FW	Inoculum 1	Inoculum 2	Inoculum 3
TS [mg/L]	146233	23166	23666	41800
VS [mg/L]	132133	15700	15800	30766
VFA [mg/L]	6140	_	_	_
pH	5.53	7.37	7.1	7.58
TCOD [mg/L]	215050	53200	54600	73700

3.2- Reactor and operation: Batch digestion experiments

Serum bottles with a total volume of 150 ml were utilized for all the acidogenic fermentation experiments. The ratio of substrate to microorganism (F/M) influences the VFA cumulation, as well as the pH buffering capacity [59]. The F/M ≈ 2 g COD/ g VS was used in all the reactors [60]. Initially the volume equivalent to 4000 mg/l total COD of food waste and 2000 mg/l VS for each inoculum was calculated to maintain the desired F/M and then the volumes were multiplied to accordingly reach the working volume of 100 ml inside each reactor. The ratio was concluded preferential for higher VFA attainments in literature [60].

In order to suppress the methanogenic activities and augment the VFA production in the reactors, acidic and alkaline conditions were set [16] prior to the process. 2 M solutions of hydrochloric acid (HCl) and magnesium hydroxide ($Mg(OH)_2$) were used to adjust the pH to the initial values of 5 and 10, respectively. Throughout the entire fermentation tests, the pH remained uncontrolled inside the reactors as artificially maintaining the pH values is unrealistic particularly for larger scale applications. All the experiments were conducted in triplicates in order to gain more statistically reliable results. Absence of micronutrients can influence the function and activities of enzymes and alter the oxidative-reductive potential and result in higher production of VFAs [2]. Therefore, no trace element nor media were added to the reactors.

In order to ensure anaerobic conditions and eliminate oxygen, gaseous nitrogen was sparged in all the bottles for 10 minutes. Afterwards, the bottles were capped with rubber stoppers and sealed with paraffin film. All the bottles were situated inside a thermostatic incubator operating at mesophilic temperature conditions (35°C) with orbital shaking of 125 rpm to keep the contents of reactors well-mixed and in suspension.

In total, six different sets of experiments, three different inocula in acidic (pH 5) and alkaline (pH 10) conditions, were conducted. Each experiment prolonged for 30 days to observe the microbial behavior and changes inside the digester in terms of total yield and composition of the VFAs. Samples were taken on day 1, day 5, day 10, day 15, day 20, day 25 and day 30 for analysis of pH, total and soluble chemical oxygen demand (TCOD and sCOD, respectively), total and compositional VFA content as well as microbial samples for genomic DNA extraction. The extracted DNA samples were later sent to high throughput sequencing.

3.3- Analytical methods

Aliquots of the fermentation broth of each reactor was taken and centrifuged at 11000 rpm for three minutes. The obtained supernatant was filtered with 0.45 μm polypropylene filters in order to determine the soluble chemical oxygen demand (sCOD). To measure the total VFA content, the supernatant was passed through a cellulose acetate filter with a pore size of 0.2 μm. Concentrations of total COD (tCOD) and sCOD in each batch reactor were measured using COD cuvette tests (LCK 514 Hach Lange, Germany). Total VFA content of each reactor was evaluated using organic acids cuvette tests (LCK 365 kits, Hach Lange, Germany). An aliquot of 0.4 ml volume from the filtered supernatant was digested at 100°C for 10 minutes. After digestion, all calorimetric results from the HACH kits (both organic acids and COD tests) were measured by a DR 3900 Hach Lange spectrophotometer. The pH was measured by the pH-meter (Mettler Toledo FiveEasyTM pH bench meter, FE20). The acidification ratio was calculated to evaluate the performance of VFA production as the ratio of total VFA concentration expressed in COD equivalent over the sCOD for each retention time.

4- Results

The influence of inoculum and pH on the performance of anaerobic digestion of food waste was studied. Three different anaerobic digested sludge were used as inoculum under acidic and alkaline conditions. Experiments were conducted over a period of 30 days and samples were analyzed every 5 days.

4.1- pH

The value of pH plays a prominent role in the bio-production of VFAs as it regulates the activities of different microbes involved in anaerobic digestion [7]. Figure 3 shows the detailed pH variations over time under both acidic and alkaline conditions. In acidic experiments the pH was initially adjusted to 5. In the inoculum 1 and inoculum 2 experiments, pH values remained within the range of 5 ± 0.4 in the first 15 days due to acids production. After day 15, the pH increased gradually and reached 5.9 ± 0.1 at the end of the experimental period. While the Inoculum 3 experiments exhibited a different trend and had lower pH fluctuations. The pH values were within the range of 5 ± 0.1 throughout the thirty days of experiments.

In the alkaline experiments, pH was initially adjusted to 10 and plummeted to 6.5 after one day in the inoculum 1 and inoculum 2 experiments and continued the declining trend until day 10 where it reached the value of 5.9. Afterwards, pH started to increase until it reached the values of 7.3 ± 0.05 in day 30. In the experiments using inoculum 3 under alkaline conditions, the pH drop was smaller compared to the other two experiments. pH dropped to 7.3 in the first day and continued a declining trend until day 10 where it reached the value of 6.7 and remained constant in the next five days. Afterwards pH started to increase until it reached the value of 7.6 at the end of the experimental period.

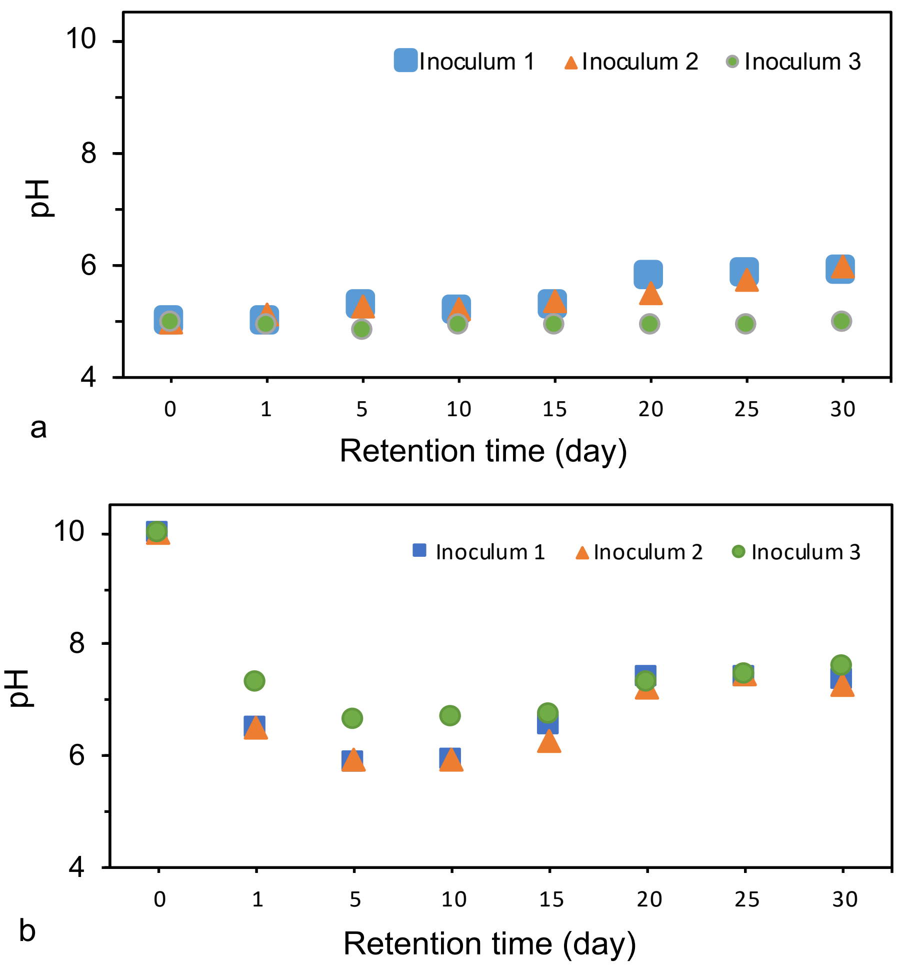

Figure 2- Variation of pH with respect to retention time under pH 5 (a) and pH 10 (b) experiments

4.2- Chemical oxygen demand

sCOD is an indication of the extent of the hydrolysis in anaerobic digestion processes. The changes in sCOD with respect to retention time for acidic and alkaline experiments are given in figure 4. In the inoculum 1 and inoculum 2 experiments under acidic conditions, sCOD initially decreased to 12500 ± 150 mg/L from day 1 to day 5. Afterwards, the values increased and reached 13500 ± 300 mg/L on day 10 and slightly decreased in the next five days. After day 15, the values declined more sharply and reached 8700 ± 300 mg/L at the end of the fermentation process. In the inoculum 3 experiments under pH 5, the sCOD had an increasing trend until day 20 when it reached 14800 mg/L. The hydrolysis occurred with a slower pace for Inoculum 3 experiments, but prolonged for a longer period and reached higher values compared to the Inoculum 1 and Inoculum 2 experiments.

In the Inoculum 1 and Inoculum 2 experiments under alkaline conditions, sCOD did not decrease in the beginning unlike the acidic experiments. sCOD values increased to their highest value of 12700 ± 150 mg/L on day 10. In the next five days the values slightly decreased and then sharply dropped from day 15 to 20 and reached 3400 ± 200 mg/L at the end of experimental period. While in the Inoculum 3 experiments, sCOD values had an increasing trend until day 15 when it reached 19500 mg/L and slightly decreased in the five subsequent days.

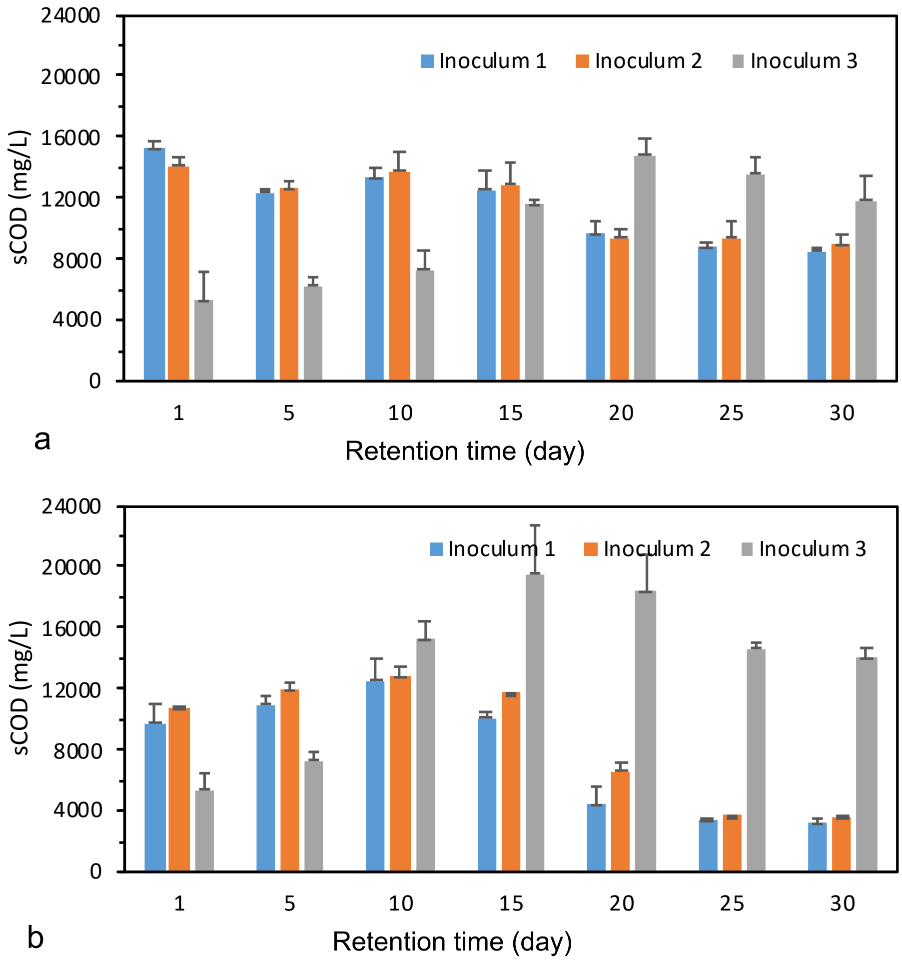

Figure 3- Variation of sCOD with respect to retention time under pH 5 (a) and pH 10 (b) experiments

4.3- Acidification ratio

Acidification ratio is defined as the ratio of total VFA content (COD basis) over the total sCOD. Acidification ratio is a manifestation of the readily fermentable substances that are present in the soluble fraction of the fermentation broth [16]. Figure 5 shows the acidification ratio under acidic and alkaline conditions. In pH 5 experiments, acidification prolonged till day 15 until it reached its highest value of 40 ± 2% for all three different inoculums. After day 15, the acidification ratio had a decreasing trend. The highest acidification ratio under alkaline conditions was 84% for inoculum 3, two times higher than the obtained ratio using the same inoculum under acidic pH which was 41%. In the inoculum 1 and inoculum 2 experiments under alkaline conditions, highest acidification ratios were achieved in day 5 which was faster than the inoculum 3 experiments that was obtained in day 10. The highest acidification ratios were 73% and 69% for inoculum 1 and inoculum 2, respectively.

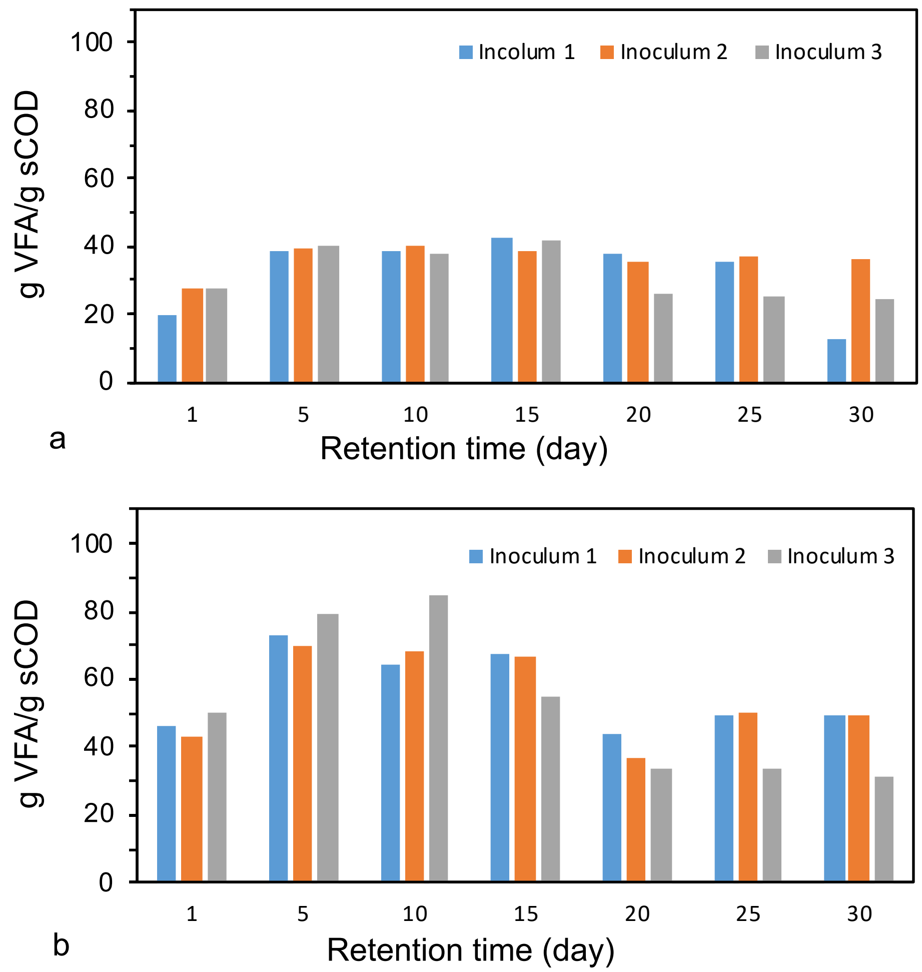

Figure 4- Acidification ratio of different retention times under pH 5 (a) and pH 10 (b) experiments

4.4- Total VFA content

The total VFAs concentrations at pH 5 and pH 10 are shown in Figure 6. The VFA concentrations at pH 10 were considerably higher compared to those at pH 5 conditions, irrespective of the used inoculum.

The highest VFA accumulation under acidic pH was between day 10 and 15 of the experiments. In the subsequent 5 days the VFA concentrations sharply decreased. The highest VFA concentrations in pH 5 experiments were 5600 mg/L, 5300 mg/L and 4900 mg/L for inoculum 2, inoculum 1 and inoculum 3, respectively.

The VFA concentrations obtained under alkaline pH was substantially higher, i.e. 8100 mg/L, 8700 mg/L and 12900 mg/L for inoculum 1, inoculum 2 and inoculum 3, respectively. In the alkaline experiments, the peak VFA concentration was obtained on day 10 which was faster compared to acidic experiments.

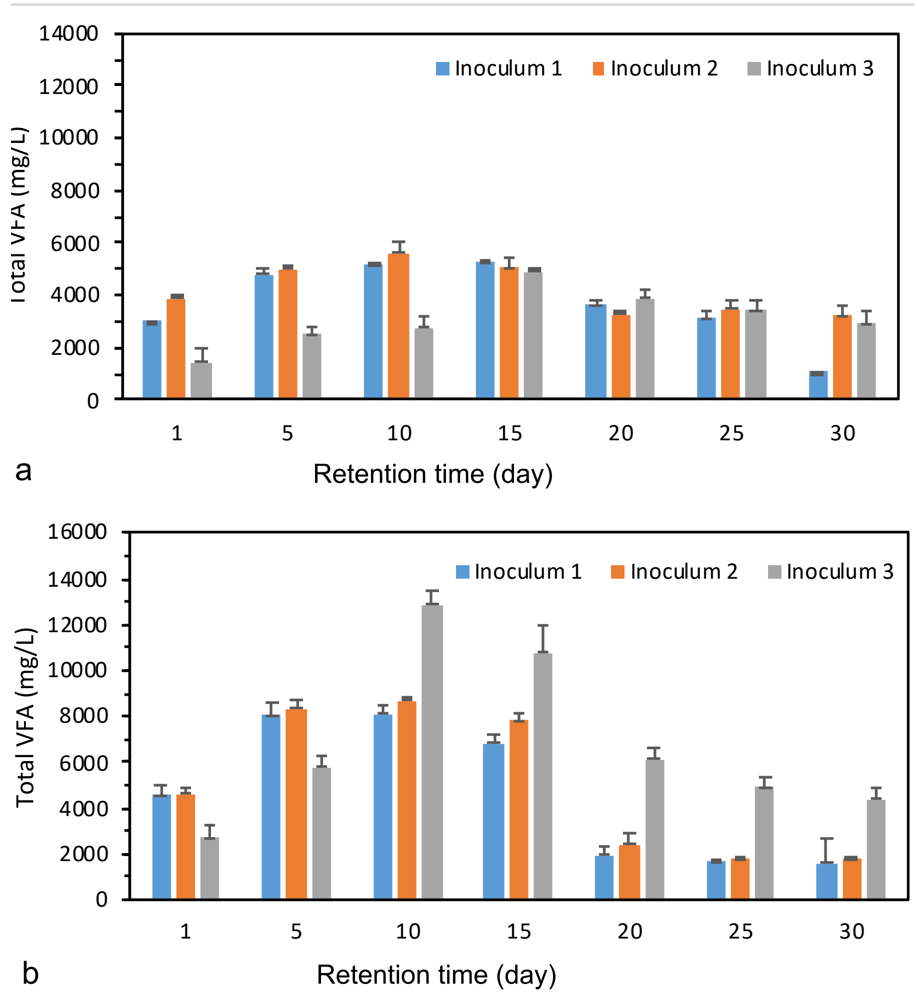

Figure 5- Total VFA content of different retention times under pH 5 (a) and pH 10 (b) experiment

5-Discussion

Based on the acquired results, pH plays a more important role compared to inoculum in the fermentation of volatile fatty acids. It was observed that initial alkaline pH was more beneficial to the overall performance of the process resulting in higher solubilization of the materials and subsequently higher VFA production. The initial alkaline condition can be considered as a pre-treatment step augmenting the rate of hydrolysis [16]. The extracellular polymeric substances of sludge contain charged functional groups such as carboxylic groups. Under alkaline conditions these charged groups get ionized which improves the hydrolysis [61]. These findings are consistent with previous works in literature where higher concentrations of VFAs were obtained under alkaline conditions [62-64]. *Bermúdez-Penabad et al*. conducted batch anaerobic digestion experiments under different pH conditions ranging from 5 to 10 using tuna waste and anaerobic sludge as feedstock and inoculum, respectively. Similar to our results, they obtained the highest acidification ratio of 81% under alkaline conditions of pH 8 and 9 [65].

In both acidic and alkaline experiments, the VFA concentration and pH had an increasing trend up to a certain point. As the pH values stabilized around neutral conditions the VFA concentrations started decrease which is an indication of the initiation of the methanogenesis step [1]. Higher VFA concentrations were obtained under alkaline conditions compared to acidic conditions. The peak VFA concentration was in day 10 for pH 10 whereas the highest VFAs concentration were achieved after 15 days in pH 5 experiments. The obtained results demonstrate a lower pace of VFA accumulation process compared to previous studies. *Lim et al.* studied the influence of retention on VFA production from food waste operating at pH 5.5 in semi-continuous mode. The highest VFA in their study was obtained in 8 days and afterwards no significant change was observed in the total VFA amount [66]. One of the reason for the different required retention time can be attributed to different characteristics of the used substrates in different studies [52].

Regardless of the initial pH, the experiments with inoculum 3 had a delay and overall slower hydrolysis. This can be attributed to the higher lipid content of the inoculum which only contained digested food waste. Whereas, primary and waste activated sludge are rich in proteins and carbohydrates [67].

Under acidic conditions lower VFA concentrations were produced in Inoculum 3 experiments compared to the other two inoculums. The lipid content of the third inoculum is expected to be higher as it was only comprised of digested food waste. *Dahiya et al.* [64] and *Noike et al.* [63] observed lower solubility of lipids under acidic conditions as well, which can be the reason of lower VFA yield. Meanwhile, highest VA concentrations under alkaline conditions were obtained from the inoculum 3 which was substantially higher compared to the other inoculums. This can be due to hinderance of methanogenic activity because of the higher lipid content in the fermentation tanks [68, 69] that results in formation of long chain fatty acids from lipid disintegration [70].

6- Conclusions

Food waste is a suitable raw material for VFA production through anaerobic digestion. Inoculum, pH and retention time are three of the parameters influencing the VFA production process. Based on the obtained results, pH played a more significant role in batch fermentation experiments. Initial alkaline conditions resulted in a more rapid hydrolysis as well as higher VFA concentrations (up to 12900 mg/L). while under acidic conditions, significantly lower concentrations of VFAs were obtained (ranging 4900-5600 mg/L).

7- Future suggestions

Anaerobic digestion of food waste has drawn attention as a proper waste management technique which presents the opportunity of recovery of high value materials. Different aspects of the process and the influence of individual parameters have been studied. Yet, there are numerous grey areas that needs to be addressed. Below some suggestion for future research are presented:

1- Co-digestion of food waste with other waste streams is an alternative for future research endeavors to manipulate the content of the substrate and have different TS and VS in the reactors. In this thesis the exact constituents of the food waste in terms of protein, lipid and carbohydrate content were not characterized. It would be beneficiary to determine the exact contents of the substrate and monitor their changes with respect to time as the process proceeds and observe their influence on the overall performance.

2- The experiments of this thesis were conducted at 35 °C. One suggestion is to conduct the experiments at higher temperatures and observe the potential impacts on the composition as well as higher or more rapid hydrolysis and product yield.

3- The influence of a pretreatment step such as enzyme addition or ultrasonic on the extent of acidification and the required retention time can also be investigated.

8- References

1. Ren, Y., et al., *A comprehensive review on food waste anaerobic digestion: Research updates and tendencies.* Bioresource Technology, 2018. **247**: p. 1069-1076.
2. Xu, F., et al., *Anaerobic digestion of food waste–challenges and opportunities.* Bioresource technology, 2018. **247**: p. 1047-1058.
3. Braguglia, C.M., et al., *Anaerobic bioconversion of food waste into energy: a critical review.* Bioresour Technol, 2017. **248**(Pt A): p. 37-56.
4. Ong, K.L., et al., *Trends in food waste valorization for the production of chemicals, materials and fuels: Case study South and Southeast Asia.* Bioresource technology, 2017.
5. Hobbs, S.R., et al., *Enhancing anaerobic digestion of food waste through biochemical methane potential assays at different substrate: inoculum ratios.* Waste management, 2018. **71**: p. 612-617.
6. Zhang, C., et al., *Reviewing the anaerobic digestion of food waste for biogas production.* Renewable and Sustainable Energy Reviews, 2014. **38**: p. 383-392.
7. Ye, M., et al., *Improving the stability and efficiency of anaerobic digestion of food waste using additives: A critical review.* Journal of Cleaner Production, 2018. **192**: p. 316-326.
8. Atasoy, M., et al., *Bio-based volatile fatty acid production and recovery from waste streams: Current status and future challenges.* Bioresource Technology, 2018.
9. Shen, L., et al., *Production of poly (hydroxybutyrate–hydroxyvalerate) from waste organics by the two-stage process: focus on the intermediate volatile fatty acids.* Bioresource technology, 2014. **166**: p. 194-200.
10. Chi, Z., et al., *Oleaginous yeast Cryptococcus curvatus culture with dark fermentation hydrogen production effluent as feedstock for microbial lipid production.* International Journal of Hydrogen Energy, 2011. **36**(16): p. 9542-9550.
11. Li, C., et al., *Comparison on kinetics and microbial community among denitrification process fed by different kinds of volatile fatty acids.* Process Biochemistry, 2015. **50**(3): p. 447-455.
12. Lee, W.S., et al., *A review of the production and applications of waste-derived volatile fatty acids.* Chemical Engineering Journal, 2014. **235**: p. 83-99.
13. Esteban-Gutiérrez, M., et al., *From sewage sludge and agri-food waste to VFA: Individual acid production potential and up-scaling.* Waste Management, 2018. **77**: p. 203-212.
14. Wang, K., et al., *Anaerobic digestion of food waste for volatile fatty acids (VFAs) production with different types of inoculum: effect of pH.* Bioresource technology, 2014. **161**: p. 395-401.
15. Ma, H., et al., *Improved volatile fatty acids anaerobic production from waste activated sludge by pH regulation: alkaline or neutral pH?* Waste management, 2016. **48**: p. 397-403.
16. Garcia-Aguirre, J., et al., *Selective VFA production potential from organic waste streams: Assessing temperature and pH influence.* Bioresource technology, 2017. **244**: p. 1081-1088.

17. Jiang, J., et al., *Volatile fatty acids production from food waste: effects of pH, temperature, and organic loading rate.* Bioresource technology, 2013. **143**: p. 525-530.
18. Jankowska, E., et al., *Volatile fatty acids production during mixed culture fermentation–The impact of substrate complexity and pH.* Chemical Engineering Journal, 2017. **326**: p. 901-910.
19. Agyeman, F.O. and W. Tao, *Anaerobic co-digestion of food waste and dairy manure: effects of food waste particle size and organic loading rate.* Journal of environmental management, 2014. **133**: p. 268-274.
20. Jankowska, E., et al., *Conversion of organic waste into volatile fatty acids–The influence of process operating parameters.* Chemical Engineering Journal, 2018. **345**: p. 395-403.
21. Fisgativa, H., A. Tremier, and P. Dabert, *Characterizing the variability of food waste quality: A need for efficient valorisation through anaerobic digestion.* Waste Management, 2016. **50**: p. 264-274.
22. Capson-Tojo, G., et al., *Food waste valorization via anaerobic processes: a review.* Reviews in Environmental Science and Bio/Technology, 2016. **15**(3): p. 499-547.
23. Zhang, L., Y.-W. Lee, and D. Jahng, *Anaerobic co-digestion of food waste and piggery wastewater: Focusing on the role of trace elements.* Bioresource Technology, 2011. **102**(8): p. 5048-5059.
24. Zhang, C., H. Su, and T. Tan, *Batch and semi-continuous anaerobic digestion of food waste in a dual solid–liquid system.* Bioresource Technology, 2013. **145**: p. 10-16.
25. Zhang, R., et al., *Characterization of food waste as feedstock for anaerobic digestion.* Bioresource Technology, 2007. **98**(4): p. 929-935.
26. Li, R.P., et al., *Characteristics and anaerobic digestion performances of kitchen wastes.* Renewable Energy Resources, 2010. **28**(1): p. 76-80.
27. Mir, M.A., A. Hussain, and C. Verma, *Design considerations and operational performance of anaerobic digester: A review.* Cogent Engineering, 2016. **3**(1): p. 1181696.
28. Veeken, A. and B. Hamelers, *Effect of temperature on hydrolysis rates of selected biowaste components.* Bioresource Technology, 1999. **69**(3): p. 249-254.
29. Coelho, N.M.G., R.L. Droste, and K.J. Kennedy, *Evaluation of continuous mesophilic, thermophilic and temperature phased anaerobic digestion of microwaved activated sludge.* Water Research, 2011. **45**(9): p. 2822-2834.
30. Kondusamy, D. and A.S. Kalamdhad, *Pre-treatment and anaerobic digestion of food waste for high rate methane production–A review.* Journal of Environmental Chemical Engineering, 2014. **2**(3): p. 1821-1830.
31. Ali Shah, F., et al., *Microbial ecology of anaerobic digesters: the key players of anaerobiosis.* The Scientific World Journal, 2014. **2014**.
32. Begum, S., et al., *Evaluation of single and two stage anaerobic digestion of landfill leachate: Effect of pH and initial organic loading rate on volatile fatty acid (VFA) and biogas production.* Bioresource Technology, 2018. **251**: p. 364-373.
33. Christophe, G., et al., *Production of oils from acetic acid by the oleaginous yeast Cryptococcus curvatus.* Applied biochemistry and biotechnology, 2012. **167**(5): p. 1270-1279.

34. Sydney, E.B., et al., *Screening and bioprospecting of anaerobic consortia for biohydrogen and volatile fatty acid production in a vinasse based medium through dark fermentation.* Process Biochemistry, 2018. **67**: p. 1-7.
35. Zheng, X., et al., *Using Mixed Sludge-derived Short-chain Fatty Acids Enhances Power Generation of Microbial Fuel Cells.* Energy Procedia, 2017. **105**: p. 1282-1288.
36. Bhatia, S.K. and Y.-H. Yang, *Microbial production of volatile fatty acids: current status and future perspectives.* Reviews in Environmental Science and Bio/Technology, 2017. **16**(2): p. 327-345.
37. Moresi, M. and E. Parente, *FERMENTATION (INDUSTRIAL) | Production of Some Organic Acids (Citric, Gluconic, Lactic, and Propionic)*, in *Encyclopedia of Food Microbiology (Second Edition)*, C.A. Batt and M.L. Tortorello, Editors. 2014, Academic Press: Oxford. p. 804-815.
38. Zacharof, M. and R. Lovitt, *Recovery of volatile fatty acids (VFA) from complex waste effluents using membranes.* Water Sci. Technol, 2014. **69**(3): p. 495-503.
39. Dietrich, K., et al., *Producing PHAs in the bioeconomy—Towards a sustainable bioplastic.* Sustainable Production and Consumption, 2017. **9**: p. 58-70.
40. Valentino, F., et al., *Carbon recovery from wastewater through bioconversion into biodegradable polymers.* New biotechnology, 2017. **37**: p. 9-23.
41. Schmidt, M., et al., *Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) production in a system with external cell recycle and limited nitrogen feeding during the production phase.* Biochemical engineering journal, 2016. **112**: p. 130-135.
42. Rodriguez-Perez, S., et al., *Challenges of scaling-up PHA production from waste streams. A review.* Journal of environmental management, 2018. **205**: p. 215-230.
43. Henze, M., *Capabilities of biological nitrogen removal processes from wastewater.* Water Science and Technology, 1991. **23**(4-6): p. 669-679.
44. Kim, H., et al., *Continuous fermentation of food waste leachate for the production of volatile fatty acids and potential as a denitrification carbon source.* Bioresource Technology, 2016. **207**: p. 440-445.
45. Zhang, Y., et al., *Effect of fermentation liquid from food waste as a carbon source for enhancing denitrification in wastewater treatment.* Chemosphere, 2016. **144**: p. 689-696.
46. Xue, F., et al., *Studies on lipid production by Rhodotorula glutinis fermentation using monosodium glutamate wastewater as culture medium.* Bioresource Technology, 2008. **99**(13): p. 5923-5927.
47. Du, Z., H. Li, and T. Gu, *A state of the art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy.* Biotechnology Advances, 2007. **25**(5): p. 464-482.
48. Hagos, K., et al., *Anaerobic co-digestion process for biogas production: Progress, challenges and perspectives.* Renewable and Sustainable Energy Reviews, 2017. **76**: p. 1485-1496.
49. Pullammanappallil, P.C., et al., *Stable performance of anaerobic digestion in the presence of a high concentration of propionic acid.* Bioresource technology, 2001. **78**(2): p. 165-169.
50. Hill, D., S. Cobb, and J. Bolte, *Using volatile fatty acid relationships to predict anaerobic digester failure.* Transactions of the ASAE, 1987. **30**(2): p. 496-0501.
51. Liu, H., et al., *Acidogenic fermentation of proteinaceous sewage sludge: Effect of pH.* Water Research, 2012. **46**(3): p. 799-807.

52. Zhou, M., et al., *Enhanced volatile fatty acids production from anaerobic fermentation of food waste: a mini-review focusing on acidogenic metabolic pathways.* Bioresource technology, 2017.
53. Komemoto, K., et al., *Effect of temperature on VFA's and biogas production in anaerobic solubilization of food waste.* Waste Management, 2009. **29**(12): p. 2950-2955.
54. Ghosh, S. and F.G. Pohland, *Kinetics of substrate assimilation and product formation in anaerobic digestion.* Journal (Water Pollution Control Federation), 1974: p. 748-759.
55. Min, K., et al., *Acidogenic fermentation of blended food-waste in combination with primary sludge for the production of volatile fatty acids.* Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology, 2005. **80**(8): p. 909-915.
56. Feng, L., Y. Chen, and X. Zheng, *Enhancement of waste activated sludge protein conversion and volatile fatty acids accumulation during waste activated sludge anaerobic fermentation by carbohydrate substrate addition: the effect of pH.* Environmental Science & Technology, 2009. **43**(12): p. 4373-4380.
57. Nayono, S.E., *Anaerobic digestion of organic solid waste for energy production*. Vol. 46. 2010: KIT scientific Publishing.
58. Bengtsson, S., et al., *Acidogenic fermentation of industrial wastewaters: effects of chemostat retention time and pH on volatile fatty acids production.* Biochemical Engineering Journal, 2008. **40**(3): p. 492-499.
59. Vavilin, V.A., et al., *Hydrolysis kinetics in anaerobic degradation of particulate organic material: An overview.* Waste Management, 2008. **28**(6): p. 939-951.
60. Silva, F., et al., *Acidogenic fermentation towards valorisation of organic waste streams into volatile fatty acids.* Chemical and biochemical engineering quarterly, 2013. **27**(4): p. 467-476.
61. Nielsen, J., et al., *Colony-level and season-specific variation in cuticular hydrocarbon profiles of individual workers in the ant Formica truncorum.* Insectes Sociaux, 1999. **46**(1): p. 58-65.
62. Wu, H., et al., *The effect of pH on anaerobic fermentation of primary sludge at room temperature.* Journal of Hazardous Materials, 2009. **172**(1): p. 196-201.
63. Noike, T., et al., *Characteristics of carbohydrate degradation and the rate-limiting step in anaerobic digestion.* Biotechnology and bioengineering, 1985. **27**(10): p. 1482-1489.
64. Dahiya, S., et al., *Acidogenic fermentation of food waste for volatile fatty acid production with co-generation of biohydrogen.* Bioresource Technology, 2015. **182**: p. 103-113.
65. Bermúdez-Penabad, N., C. Kennes, and M.C. Veiga, *Anaerobic digestion of tuna waste for the production of volatile fatty acids.* Waste Management, 2017. **68**: p. 96-102.
66. Lim, S.-J., et al., *Anaerobic organic acid production of food waste in once-a-day feeding and drawing-off bioreactor.* Bioresource Technology, 2008. **99**(16): p. 7866-7874.
67. Zhang, P., Y. Chen, and Q. Zhou, *Waste activated sludge hydrolysis and short-chain fatty acids accumulation under mesophilic and thermophilic conditions: Effect of pH.* Water Research, 2009. **43**(15): p. 3735-3742.
68. Yuan, H. and N. Zhu, *Progress in inhibition mechanisms and process control of intermediates and by-products in sewage sludge anaerobic digestion.* Renewable and Sustainable Energy Reviews, 2016. **58**: p. 429-438.

69. Alves, M.M., et al., *Waste lipids to energy: how to optimize methane production from long-chain fatty acids (LCFA).* Microbial biotechnology, 2009. **2**(5): p. 538-550.
70. Wan, C., et al., *Semi-continuous anaerobic co-digestion of thickened waste activated sludge and fat, oil and grease.* Waste Management, 2011. **31**(8): p. 1752-1758.