

PRODUCTION OF VOLATILE FATTY ACIDS FROM ANAEROBIC DIGESTION USING FOOD WASTE AND SLUDGE

BSc in Chemical Engineering - Applied Biotechnology

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Abstract

Volatile fatty acids (VFAs) have gained a very extensive research interest since they are important building blocks for the chemical industry. These acids can be produced via anaerobic digestion (AD) which has shown to be an environmentally friendly process since it utilizes waste products such as sludge and food waste as substrate. Anaerobic digestion can occur naturally and produces biogas as the main product. VFAs are intermediary products formed during the anaerobic degradation process. The main objective of this study was to investigate how to optimize the degradation process aiming to produce a high amount of VFAs instead of producing biogas. Operation parameters, such as pH and time during anaerobic degradation of sludge and/or food waste in mesophilic conditions were examined. The highest VFA concentrations observed were 15.4 g/L when using sludge as substrate with a yield of 0.77 gVFA/gVS at day 14. For food waste, as substrate, the highest concentrations observed were 9.7 g/L with a yield of 0.49 gVFA/gVS at day 13, while the digestion of a mix of food waste and sludge resulted in 10.92 g/L VFAs production with a yield of 0.55 gVFA/gVS at day 13.

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1 Introduction

Anaerobic digestion (AD) utilizes the unique ability of microorganisms to convert organic waste material into useful products, such as renewable energy. Anaerobic digestion can occur naturally and can be observed in sediments of lakes and swamps as well as the digestive tracts of animals, especially ruminants. (Stephen , et al., 2012).

Figure 1 shows a general scheme of an anaerobic digestion process. In order for AD to take place, organic material needs to be dispensed into the process. The organic material fed into the process can be divided into two categories: first source organic waste, which is waste derived indirectly from humans and second source organic waste, which is produced primarily from humans. A good example for the first one is manure, a waste product produced pre consumption in the aim of producing meat or dairy products. The second category can be described as waste created post consumption

A good example are manures which is a waste product produced pre consumption in the aim of producing meat or dairy products. Second source organic waste is waste produced primarily from humans, this can be described as waste created post consumption, for instance, food waste from food industry and sewage sludge from waste water treatment plants.

Sometimes the organic waste needs to be pre-treated before it could be applied as substrate for anaerobic digestion. Manures sometimes consist of bigger undigested organic material which make it harder for the microbes to break down the substrate. A possible solution to this is to shred it in a mixer into smaller particles, to increase the accessible area for the microbes to be able to break down the substrate.

Mixing of two different substrates can also be applied, for instance food waste that contains more organic materials regarding the total solids (TS) while sludge contains much less organic material but instead contains more moisture. When mixing food waste and sludge a

good balance between content can be put together depending on the inoculum and TS ratio mitigating the water needs at the same time taking advantage of the different characteristics of these waste products.

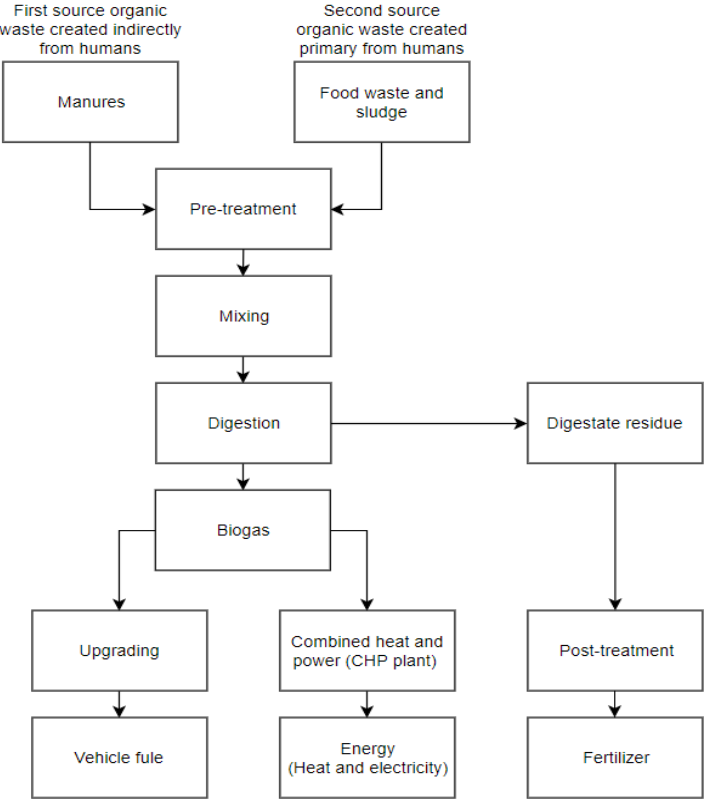


Figure 1 General description of an anaerobic digestion process

The goal of a general AD process is to produce as much biogas as possible in a cost effective time interval. As shown in Figure 1 the biogas can then be further upgraded into vehicle fuel or sent to a combined heat and power plant (CHP) which will produce energy in the form of heat and electricity. The remaining digestate residue is rich in nutrients and can after the anaerobic digestion process be treated and used as fertilizer.

The rapid population growth has led to a growing national economy. This has led to a massive waste generation. Not only has waste management improved but also applications that minimize further environmental degradation and mitigation of environmental pollutions. The

conventional waste management approach have also been improved and mainly focused on meeting the environmental rules and regulations. Resource recovery have a more enlighten approach as it constantly allows minimization of waste and production of value added chemicals from organic waste materials. Among the various applications regarding waste recovery, as mentioned, anaerobic digestion is one possibility, which produces biogas as the end product. As we constantly develop, a new pathway of anaerobic digestion have derived, as shown in Figure 2. This describes other approach that could be taken by modifying the AD process into producing volatile fatty acids (VFAs), which in turn can be utilized for producing other high value chemicals.

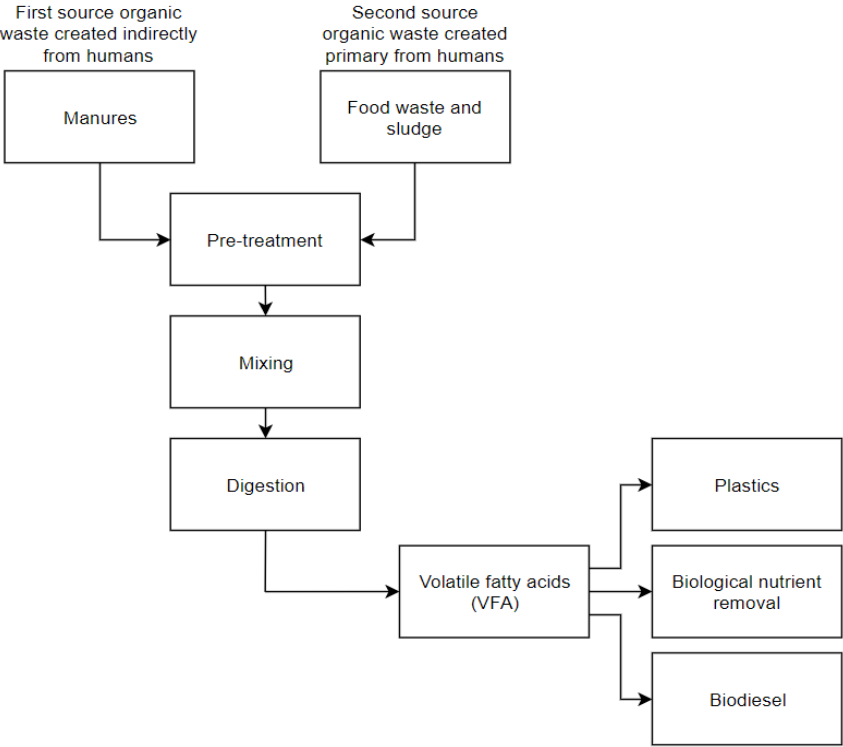


Figure 2 General description of a modified anaerobic digestion with the aim to produce volatile fatty acids (VFAs)

VFAs have been on the market for some time now but often they are produced synthetically since it's easier, but with a high production cost. As it is shown on Figure 2 above, VFAs can

also be formed through a modified anaerobic digestion process, resulting in not only a process utilizing waste, but also a process producing high value chemicals with low production cost.

2 Challenges

Biogas production processes are well established in the industry and its process parameters have been well studied. However the major application of biogas is production of energy as electricity or heat and transport fuel. The economic feasibility of biogas plants can be challenged due to cheap oil prices as well as cheaper renewable sources of energy. Production of VFAs from waste is an alternative way to apply the well-known anaerobic digestion process, since VFAs have a wider spectrum of applications and a higher market value compared to that of biogas. For this study, the impact of pH and the possibility of co-digestion of food waste and sludge is going to be investigated in an attempt to maximize VFA accumulation during the digestion.

3 Background

3.1 Food waste

Food is essential for sustaining life on the planet. The bigger the population gets the more food is required to be produced in order to keep our needs. It is approximated that the food production would increase by 60% by 2050. In total approximately 750 trillion dollars' worth of food gets wasted or lost annually worldwide. With the increasing food production comes increasing amounts of food wasted. Consequently, not only does the economy take a cost but also the society and the environment. Food needs to be transported in different transportation systems such as trucks, boats and planes. Since some foods are unique for specific locations in the world it needs to get transported across the world because of the consumers' demand. This

results in a massive usage of fuels that contributes greenhouse gas emissions (Cheryl , 2015). Food waste is basically the food that is intended for consumption but by various reasons gets discarded in the food supply chain (eSchoolToday, 2008). In general approximately one third of the produced food for human consumption gets lost or wasted annually which counts up to roughly 1.3 billion tons of food (FAO, 2019). Since we constantly develop technologies, new food waste management and general improvements on the food chain supply have been implemented. Implementations such as raising awareness and transportation improvements have resulted in some reductions in the produced food waste. Other implementations such as recycle or recovering have also shown to be effective. Since food waste is rich in organic material it automatically qualifies as a great feed stock substrate to be used for anaerobic digestion. (Cheryl , 2015) . In this analysis, food waste is going to be one of the substrates used and it will be also mixed with other organic waste for applications in AD processes.

3.1 Sludge

Sludge, sewage sludge or often called bio solids is the residual solid material accumulated from sewage treatment plants or industrial waste water treatment plants. The treatment methods consist mainly of 3 stages, mechanical, biological and chemical (Figure 3). In the mechanical stage large debris like sand, grit, stones, textiles, plastics and large objects get separated. This is done by using a grit chamber and then sedimentation. The grit chamber separates sand, gravel and stones that get processed for landfill. The sedimentation separates remaining particles by letting heavy particles sink down to the bottom where they get separated. The separated material is called primary sludge (Agency, 2018).

Biological treatment utilizes microorganisms such as bacteria that consume the organic material remaining in the waste water. In this stage almost 90% of the organic materials gets removed and close to 20% of the nitrogen is used by the microbial activity. In activated

sludge processes the microorganism gather in large flocs, these flocs are removed via sedimentation. A part of the sediment sludge is recycled back to the biological treatment, while the rest is removed and called as excess or secondary sludge (Agency, 2018).

Waste water treatment plants can also have a stage called nitrogen removal. This stage is very complicated and only applied in large waste water treatment plants. In this stage nitrifying bacteria oxidizes ammonia to nitrates in the presence of oxygen, then denitrifying bacteria in anoxic conditions convert nitrates into N_2 . This process removes approximately 50 – 75 % of nitrogen (Agency, 2018).

Finally, in the chemical treatment, phosphorus from the wastewater gets removed through chemical precipitation based on aluminum or iron flocculants. These metal ions bind the dissolved phosphorus and which then gets removed through sedimentation processes. This process removes up to 90 % of the phosphorous existing in the wastewater (Trikoilidou, et al., 2016).

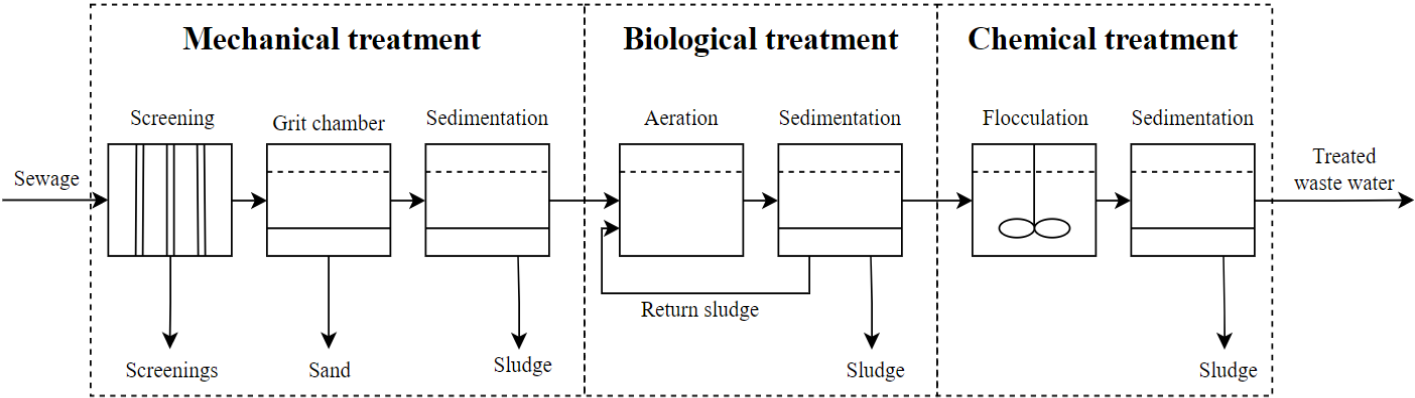


Figure 3 Description of a waste water treatment process

The last stage is filtration. Here filtration is added to increase the purification levels of floating sludge and other particles (Agency, 2018).

Majority of the waste water treatment steps undergo sedimentation that accumulates sludge (Stehouwer, 2010) as shown in Figure 3. The sludge from these stages can be extracted separately or mixed together depending on the application. The biggest application of sludge is to utilize it in anaerobic digestion. Since sludge contains a great amount of organic materials it's a suitable substrate for AD. In this study, sludge obtained from a waste water treatment plant and its mixture with food waste are going to be applied in AD processes.

4 Theoretical background

4.1 Anaerobic digestion

Anaerobic digestion is one of the oldest technologies, however it was the industrialization there the scientific implementation of this process began, for instance the first digestion plant was established in Bombay at 1859, and moreover by the end of 1800 biogas was recovered from sludge from waste water treatment plants and used to torch street lamps in England (Kou & Dow, 2016).

In recent decades the world has witnessed an extensive industrialization and urbanization. Although many people view this as positive, the negative impacts cannot be neglected. As a result of this extensive industrialization and urbanization a great amount of organic waste material is produced. This has triggered a serious global challenge regarding waste management, especially for waste consisting of organic materials. Currently, there is a great variety of diverse organic waste management methods exists, such as composting, home composting as well as a new development, a microbial fuel cell (MCP), which is a bio-electrochemical process that generates electric current by using bacterial activity and mimic

natural bacterial interaction (Wikipedia 1, 2019). However, anaerobic digestion has proven to be the most effective at transforming different organic waste materials into renewable energy and in addition mitigating the environmental impact such as greenhouse gas emissions and subsurface contamination (Mayers, 2016).

AD consist of four different phases, as shown in Figure 4, namely, hydrolysis, acidogenesis, acetogenesis and methanogenesis. Methanogenesis is the last phase where the biogas is produced. For this scientific analysis, AD is going to be manipulated by inhibiting methanogenesis in order to maximize VFAs accumulation. The alkalinity levels will be regulated to better understand what conditions is beneficial for VFA production.

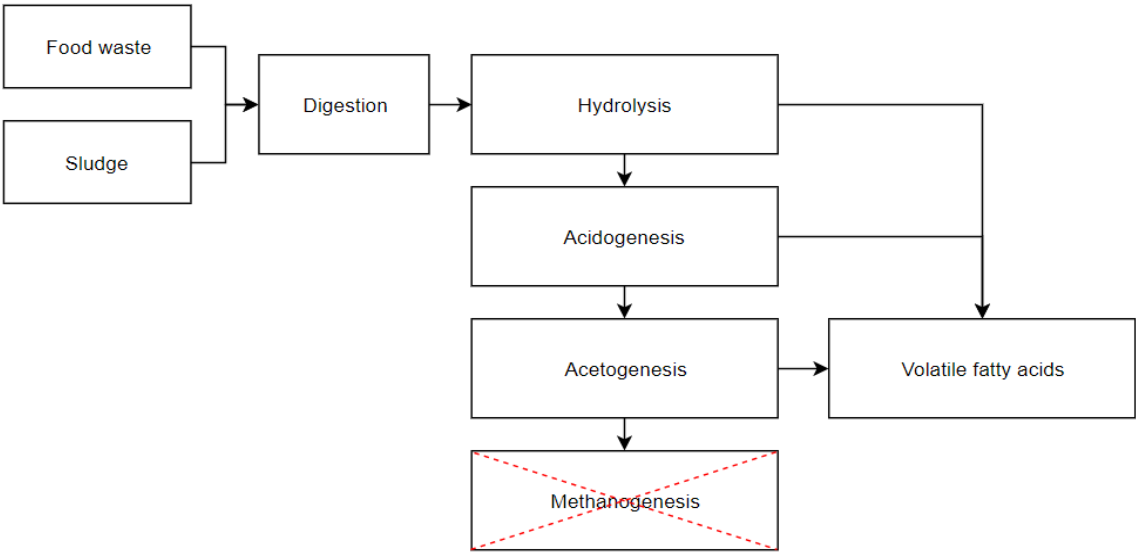


Figure 4 Description of an anaerobic digestion process regarding microbial activity

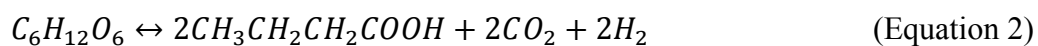
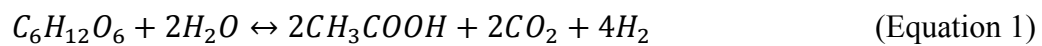
4.1.1 Hydrolysis

Hydrolysis is the first stage of anaerobic digestion. Here the organic material is disintegrated into smaller particles by microbial organisms known as hydrolytic bacteria. Complex biopolymers, such as proteins, carbohydrates and lipids from the organic material, are broken

down into monomers and oligomers, such as sugars, amino acids and peptides. These materials gets then converted into amino acids, long chain fatty acids and monosaccharides. Sometimes the substrate contains high amounts of lignin or cellulose as in cow manure. This will result in hydrolysis being slower which, as it was mentioned before, will require a pre-treatment. (Stephen , et al., 2012)

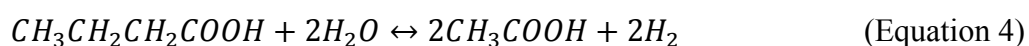
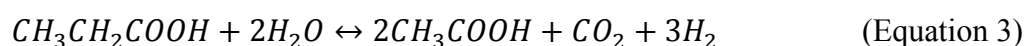
4.1.2 Acidogenesis

In acidogenesis microorganisms continue to break down the products from the hydrolysis step into hydrogen, carbon dioxide, alcohols and volatile fatty acids (VFAs), such as acetate propionate or butyrate for example. A good observation to make is that high amounts of accumulated VFA will result in a pH reduction regarding the batch mixture. Below are two different chemical equations that describe the activity in acidogenesis. Simple sugars, such as glucose, break down into acetic acid and butyric acid with rest products of carbon dioxide and hydrogen (Equation 1, 2). (Stephen , et al., 2012)



4.1.3 Acetogenesis

Acetogenesis is the third phase of anaerobic digestion. Here the longer volatile fatty acids, about C3-C5 or longer are oxidized into acetic acid, carbon hydroxide, hydrogen and water. Below are Equation 3 and 4 that illustrate the chemical activity in this phase. (Stephen , et al., 2012)



4.1.4 Methanogenesis

In the last and final phase of the anaerobic digestion, microorganisms transform acetic acid, or hydrogen and carbondioxide onto methane, water and carbon dioxide. Methanogenesis consists of two extensive microbial reactions, acetoclastic methanogens where acetate is oxidized onto methane and water (Equation 5) and hydrogenotropic methanogens where hydrogen and carbon dioxide are converted into methane and water (Equation 6) (Stephen , et al., 2012).



Since in this study the goal is the production of VFAs, the microbial activity of methanogens needs to be restricted. Therefore, the pH is going to be maintained at certain acidic or alkaline levels to find conditions which prohibit methanogenesis the most (Stephen , et al., 2012).

4.2 Volatile fatty acids (VFA)

Volatile fatty acids have gained a very extensive research interest because of their low production cost when produced via anaerobic digestion using organic waste as feed stock. Not only the low production cost is beneficial, but also the different applications of the produced VFAs in production of bioenergy, nutrient removal process and production of biodegradable plastics. Volatile fatty acids, also known as short chain fatty acids, consisting of carboxylic acids with a carbon length of C2-C6. This gives the advantage that they can be distilled at atmospheric pressure (Nag, et al., 2018). The length of the VFAs is also important, since different applications prefer different carbon lengths. For instance, some applications prefer shorter VFAs, like acetic and propionic VFAs reasoning that microbes can extract energy faster from shorter chained VFAs compared to longer, whereas some other

applications prefer longer chained acids like iso-valeric and valeric acids reasoning the longer carbon lengths for maximized product production.

4.3 General applications of VFAs

4.3.1 Plastics

With a low production cost regarding anaerobic digestion, an extensive interest of research in VFAs aimed for the production of biodegradable plastics has grown (Wee, et al., 2014). An example is polyhydroxyalkanoates (PHAs). PHAs polymers produced by microorganism using VFAs derived from AD as substrates, and they can be used for a wide range of applications as in food packaging and other basic applications that currently are dominated by plastics produced from crude oil and other fossil sources (Wee, et al., 2014). In addition of being biologically synthesized products, PHAs are biodegradable making them a good replacement for other fossil based polymers regarding the current environmental and plastic pollution issues. Not only its environmental friendly aspects but also the low substrate cost that makes this technology a promising option. However, an important aspect is that beside the concentration level of the VFAs the length of the VFAs are also important and plays a significant role in production of PHAs. For instance, a mixture containing acetic and butyric acid benefits the production of 3-hydroxybutyrate (3HB) and a mixture with propionic and valeric acid favors the production of 3-hydroxyvalerate (3HV). This means that even the quality of the VFAs are important, as different mixtures will give rise to the production of different types of PHAs (Nag, et al., 2018).

4.3.2 Biological nutrient removal (BNR)

Volatile fatty acids are essential for biological nutrient removal (BNR). VFAs acts like a carbon substrate to improve the removal of phosphorus and nitrogen from wastewater. As it was discussed earlier, nitrogen can be removed by proceeding an aerobic nitrification and

then an anoxic denitrification step. The removal of phosphorus can also be performed through enhanced biological phosphorus removal (EBPR) (Nag, et al., 2018). Using volatile fatty acids produced via a synthetic pathway can be expensive. A more economical approach is therefore to utilize VFAs produced from anaerobic digestion utilizing renewable waste. To facilitate an effective BNR process, optimum quantities of an organic carbon source needs to be added since the incoming waste water in these last steps of the treatment does not have a sufficient amount of organic carbon anymore (Lystek, 2000). It was found that different molecular weight and different lengths of the VFAs will affect the effectiveness of BNR processes. For instance in the nitrogen removal process where acetate, propionate, butyrate and valerate is presence, the denitrifying bacteria is going to consume the shorter VFAs first, *i.e.* acetate then propionate and so on. This is a result of that the bacteria will choose the simpler metabolic pathway if possible. (Nag, et al., 2018).

4.3.3 Biodiesel

The number one concern about petroleum derived diesel fuel is that it emits powerful pollutants into the air. Biodiesel made out of animal fats or vegetable oils is a replacement. The advantages of using biodiesel are that it lowers greenhouse gas emissions compared to that using normal diesel. In the production of biodiesel impurities of the fat and oil will be first removed and then its viscosity will be changed so that it can burn in a normal diesel engine without causing any troubles in the fuel line. This is done by a chemical reaction, called transesterification. This is simply a reaction of the alcohol and oils mixed together with a catalyst to speed up the reaction. The process is very simple and can be done by adding a certain amount of alcohol to the catalyst and then combining the mixture with the feedstock while it undergoes a heating and stirring process. The result is a layer of biodiesel and glycerol. The biodiesel still needs to undergo a purification process and that is done by for instance using ion exchange beads to wash the produced biodiesel from impurities. (Nag, et

al., 2018). Volatile fatty acids have been drawing attention regarding production of biodiesel. Since the material being used to produce biodiesel are long chain fatty acids, volatile fatty acids are a good alternative of implementation in the biodiesel production process reasoning VFAs low production costs and that it uses waste materials as production source. If implemented this results in generally will lower greenhouse gas emissions compared to that using normal vegetable and animal oil based biodiesel.

5 Operating parameters of anaerobic digestion

5.1 Temperature

The temperature is one of the key factors for an AD process regarding survival of microorganisms. There is three different main temperatures applied to anaerobic digestion. Psychrophilic at 25 °C, mesophilic at 35 °C and thermophilic at 55 °C (Kiros, et al., 2016). Generally higher temperature gives AD a higher production rate of biogas however operating an AD at high temperatures are very energy intensive and harder to control. Not having a stable temperature will result in varying environment for the microbes, which has a negative effect on the microbial growth and will decrease the production of biogas (Vímac, et al., 2015). A mesophilic environment often results in a big variety of different microorganisms and generally in much more stable operation than a thermophilic environment (Lise, et al., 2008) (Wiqvist, 2017).

5.2 pH

Generally microorganism prefer natural pH. Anaerobic digestion processes usually contain multiple variety of microorganisms that require different value of pH in order to maximize the production rate. The optimal pH value to gain best biogas production in anaerobic digestion is approximately 7 +- 0.2 (Miaomiao, et al., 2018). Since AD consists of different chemical and microbial processes the different stages in an AD processes prefer different pH values, for

instance methanogenesis which is the last part of AD works best at pH 7, acidogenesis prefers pH 5-8 since it is a very tolerable process (Miaomiao, et al., 2018). The pH is also very important influencing the balance between ionized and non-ionized molecules. Chemical properties of chemicals will change depending on their ionized or non-ionized forms, for instance, hydrogen sulfate, fatty acids and ammonia are very toxic in their non-ionized forms (Nayono, et al., 2009). Since the pH plays an important role in the production rate of the microorganisms, the pH can be modified in order to aim to improve the production in a specific stage in AD. For instance, in acidogenesis VFAs gets broken down into shorter VFAs, if the certain pH value is applied, the products produced in acidogenesis will be accumulated, since the applied pH will inhibit the next coming step of AD, the methanogenesis. In this last step would VFAs otherwise be broken down into methane. Hence, using an appropriate pH the process can be aborted and the accumulated VFAs can be then extracted. (Nag, et al., 2018).

5.3 Substrate

Substrate characterization is very helpful to understand the different chemical compositions consisting in the substrates. By knowing what is put into the reactor we better can expect what comes out. The TS and VS values are just basic characterizations that can be applied to substrates, like food waste and sewage sludge (Mashhadi, 2018). By knowing these values we can add the same amount of organic material as the feed, since different substrates contain different amount of water and organic material. Substrates does not contain the same organic materials, this results in different amounts of accumulated VFAs depending on which substrate that were applied (Mashhadi, 2018). In this study food waste and sludge were used as substrates. Moreover, these substrates were even mixed together to see if any increase of VFA accumulation took place.

5.4 Agitation

Mixing plays an important role in how efficient the anaerobic process is. Agitation gives a good mixed mixture allowing both the inoculum and substrate to have better contact which gives an increasing digestive process. Not only does it increase microbial activity but also inhibits the formation of scum and formation of chunks of solids by keeping them in suspension (Mashhadi, 2018).

There are a great variety of methods achieving an efficient mixing, for instance using mechanical stirrers, where an electrical stirrer is attached to either a propeller or a stirrer vane. Advantages with mechanical stirring are that the speed and direction of the motion can be modified. In plug flow reactors (PFR) where the accumulated and already existing gas will be pumped back from the bottom into the batch reactor to get the mixing with the help of the gas bubbles. Here the speed of the mixing can be regulated by adjusting the pressure and volume flow of the pump (Nayono, et al., 2009) (Muzaffar, et al., 2016).

5.5 Inoculum

Inoculum is not essential for this process but plays an important role in the performance of the anaerobic digestion. Inoculum consists of a community of microorganisms that acts like a booster for the AD process. This gives a high rate of product production. Adding an inoculum results in adding microbial organisms that instantly start the hydrolytic process and at a much higher rate than when not adding anything (Miaomiao, et al., 2018).

In this study the strategy was to add inoculum to the substrate at specific inoculum substrate VS ratio depending on the substrate characteristics. By using specific pH values for manipulating the AD specific microbial activity can be promoted so that methane production would be inhibited. This will result in high production rate of VFAs which is the product of interest.

6 Objective

Anaerobic digestion is a naturally occurring microbial process. In AD organic waste material will be broken down via different degradation stages to finally produce biogas. AD has a good variety of applications when implemented and often gives environmental benefits regarding greenhouse gas emissions. Thus biogas is very useful biofuel, however its production is not beneficial economically compared to fossil fuel based biogas. The aim of this study was therefore to apply an AD process but instead of aiming to produce biogas, the target was the production of VFAs. To achieve the accumulation of VFAs the AD process needed to be manipulated by applying specific pH in mesophilic conditions. Batch digestion assays were applied using different substrates and substrate mixtures applying the same inoculum and substrate VS ratio. The main goal was to find conditions leading to the most optimal *i.e.* highest VFA production. The specific type of VFAs produced was also determined applying different alkaline conditions and retention times.

7 Materials and methods

7.1 Materials

For the experiments, substrate-inoculum of 1:1 ratio on VS basis were used. Food waste was acquired from Renova Gothenburg with a TS and VS value of 20.37% and 18.27 %, respectively. The Sewage sludge was acquired from Gryaab Gothenburg with a TS and VS value of 5.79% and 4.32%, respectively. The inoculum was obtained from Hammarby Sjöstad Stockholm and had a TS and VS value of 6.48% and 9.55%, respectively.

7.2 Reactors and operation

For the experiment a setup of ten 2L batch reactors were established (Figure 6). The general setup consisted of 2 water baths with a capacity to hold 6 reactors each. The water baths were

set to keep a mesophilic temperature at 37 °C. Since the aimed temperature was set to 37 °C the water baths were set to 38.5 to compensate for eventual heat losses. The water baths were sealed with a plastic overlay with 6 slots to prevent evaporation. Choosing 10 reactors was intentional to facilitate the refilling and observing the water level of the water baths. As Figure 6 shows every batch reactor was given a specific pH value to maintain. pH 5, 8, 10, 12 were set and maintained with an addition of a blank reactor with no pH adjustment. For each sample a duplicate setup was performed to eliminate eventual mistakes and mitigate the margin of error referring to results.

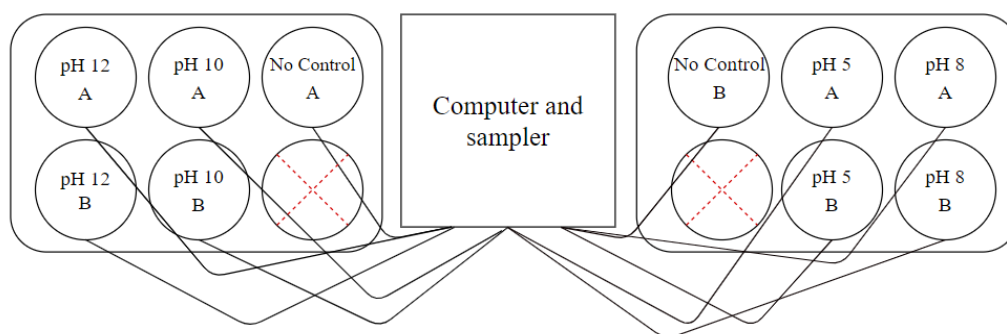


Figure 6 Drawn picture of a 2-dimensional picture of the setup of the batch reactors

Between the water baths with the batch reactors a sampler was established. The roll of the sampler was to measure the volume of the accumulated gas produced in each reactor. Each reactor were connected to the sampler with a rubber tube. The rubber tube had a glass tube that allowed gas sampling for the determination of gas composition.

Figure 7 illustrates the batch reactors used in the experiment. Each reactor had an electrical stirrer which allowed mechanical stirring. The stirring was connected to the computer which allowed different speeds and mechanical technics to be applied regarding agitation. Speed used for mixing was 30 rpm with 50 seconds residence time, for pH adjustment 60 rpm speed was applied with 0 residence time. As mentioned above, the reactors had rubber tubes

connected to the sampler with an attached glass tube that allowed gas sampling. The reactors had 3 lids small lids; 2 of the lids allowed maintenance of the pH by opening them putting a pH meter on one opening and adding acid or base in the other depending on the situation. The third lid was modified with a glass tube that allowed liquid sampling.

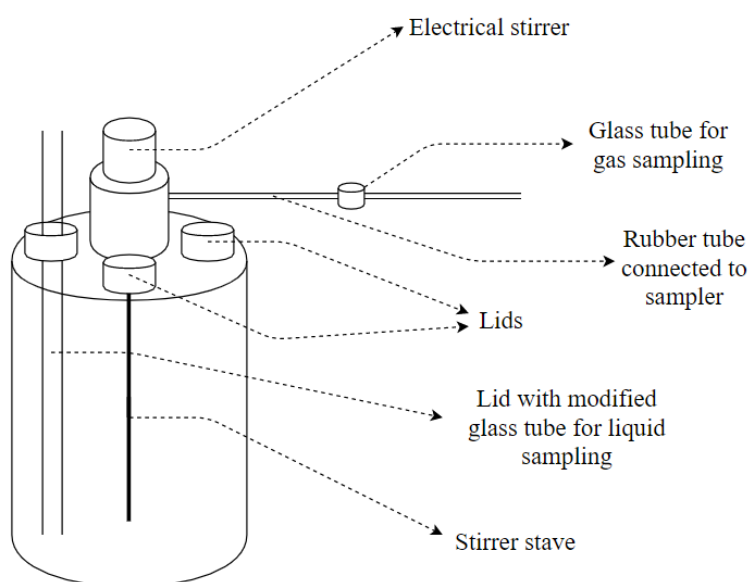


Figure 7 Drawn 3-dimensional picture of one batch reactor

7.3 Analytical methods

Sampling and maintenance were applied every second day with an exception on weekends. First gas sampling was performed using a gas tight syringe. Gas was sampled from each reactor one by one and analyzed by gas chromatography. Peaks of methane (CH_4), Hydrogen (H_2) and carbon dioxide (CO_2) were noted.

Finishing with the gas sampling the liquid sampling was started. From the modified lids 10 ml of liquid sample were extracted and put into 13 ml plastic tubes. The plastic tubes were then centrifuged 14.0 k-rpm. After centrifugation 1 ml liquid sample was taken out from each plastic tube and then put into 1.5 ml centrifuge tubes adding a duplicate for each reactor resulting in a set of 20 samples. The samples were once again centrifuged and filtered into 1.5

ml tubes with 0.5 ml distilled water, diluting the liquid with a factor of 0.5. The samples were then transferred into HPLC vials and once again filtered to eliminate particles that might result in errors in the measurements. The vials were then placed on an auto-sampling carousel and analyzed by HPLC. The peaks for VFAs were then noted. Excessive liquid samples were preserved in a freezer.

Finally, the pH in every reactor were checked and maintained according to the targeted pH value by adding either sodium hydroxide (NaOH) or hydrochloric acid (HCL) depending on the pH. Finishing the sampling the water baths were filled with water regarding the vaporization to prohibit temperature changes.

7.4 Method description

7.4.1 HPLC

For High performance liquid chromatography (HPLC) analysis, an Acquity Arc HPLC was used with a hydrogen based column (Aminex HPX87-H; BioRAD Laboratories, München, Germany) at 60 °C. The mobile phase was 5 mM H₂SO₄ at 0.6 mL/min. The detection was achieved using ultraviolet absorption detector (UVA) at 210 nm (Waters 2487, Waters Corporation, Milford, CT, USA).

7.4.2 GC

For gas chromatography analysis a gas-tight 0.25 mL syringe was used. The gas was analyzed with a Parkin-Elmer gas chromatograph (Clarus 590) equipped with a packed column (Carboxen TM 1000, 6' × 1.8" OD, 60/80 Mesh, Supelco, Shelton, CT, USA). The detector type was a thermal conductivity detector at temperature of 200 °C. The carrier gas used was N₂ with a flow rate of 30 mL/ min at 75 °C.

7.4.3 Determination of TS and VS

The determination of the total solids (TS) and volatile solids (VS) is described by Figure 5.

The TS and VS value is essential to this analysis because it allows to determine the amount of organic material that needs to be added to the assays at determined substrate to inoculum ratio.

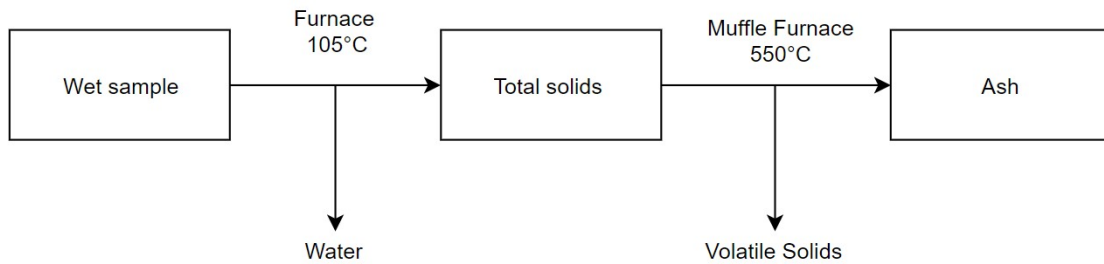


Figure 5 Description of how measurement of the TS and VS value was performed

This measurement method was performed by first determining the empty weight of the aluminum cups used for the determination. Three samples per substrate were measured to reduce the margin of error. A given amount of the substrate was placed into each cup by using a simple electronic scale. The samples were then put into a 105 °C oven. In this stage only the water was vaporized out of the samples, hence the remaining weight represents the TS value. The samples were then placed into a 550 °C muffle furnace. At this temperature a part of the sample will be volatilized leaving the ash behind. The lost weight in this stage represents the volatile solids. Both food waste and sludge were analyzed. The inoculum was pre-analyzed and the TS and VS values were given by the supervisors.

8 Result and Discussion

8.1 pH

Chart 1 shows the pH change in each reactor with a specific set alkaline pH. On the x-axis are the day where the alkalinity was adjusted to match the determined pH level. On the y-axis shows the change in the alkalinity. Negative change means the pH decreased from the last day it was maintained to the pre-determined pH value. For instance on Chart 1, Food waste day 26 we can see that pH 12 had -1 change in the pH meaning it decreased from pH 12 to 11 from day 19 to 26. If the value is positive it means the pH value increased.

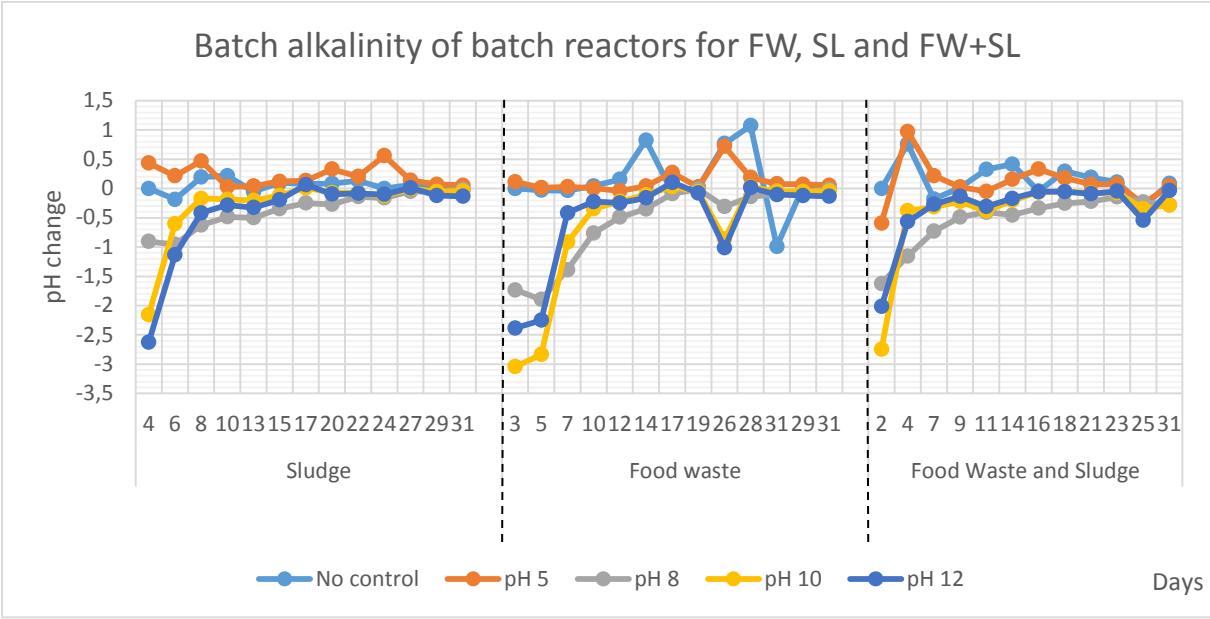


Chart 1: pH-change values for FW, SL and FW+SL

VFA accumulation relates to alkalinity and pH change, as shown in (Chart 1), when initiating the reactors, pH, 8, 10 and 12 we can see that those pH conditions had the largest pH change tending to become more acidic after each day of maintaining the determined pH values. This

indicates the high amount of VFA production as written in the article (Wang, Dou et al., 2017) which shows the rapid decrease of pH as the result of VFA accumulation. Still with the increasing amount of VFA accumulation, after an approximately a 15 day long period the pH changes seem to be stabilized as also shown in Chart 1. This does not necessary mean that the production rate of VFAs decreased but instead shows the ability of the mixture to self-buffering. In the case of co-digestion of food waste and sludge we can see that the pH changes started to be stabilized already after day 7, which is earlier that that observed in case of the single substrates mentioned previously above. This shows that co-digesting food waste with another substrate can increase the self-buffering ability of the mixture as it was shown in the article (Wang, Dou et al., 2017), there it was also found that co-digesting food waste with other substrates can increase the buffering capacity.

8.2 Gas production

Chart 3, 4 and 5 illustrates the percentage of H₂, CH₄, and CO₂ in the total accumulated gas. The days are plotted on the x-axis and on the y-axis the percentage is plotted. In each plotted chart, three different accumulated gases are divided. The lines illustrates the different pH conditions given. Since information regarding the gas volume were unavailable for us, we were only able to analyze the percentage of total accumulated. The big spikes in the chart does not mean the values are bad rather it means the amount accumulated is so low that the percentage shown varies a lot.

PERCENTAGE OF TOTAL GAS (SLUDGE)

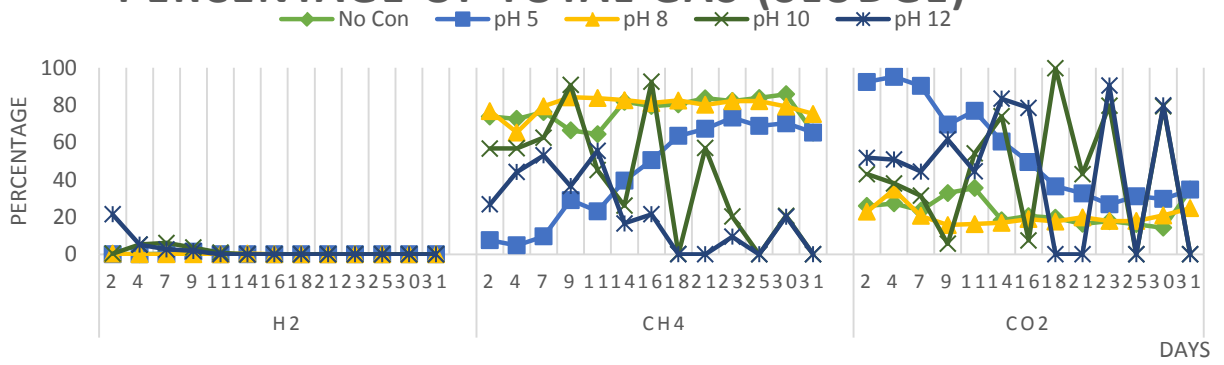


Chart 2: Percentage of total gas (Sludge)

PERCENTAGE OF TOTAL GAS (FOOD WASTE)

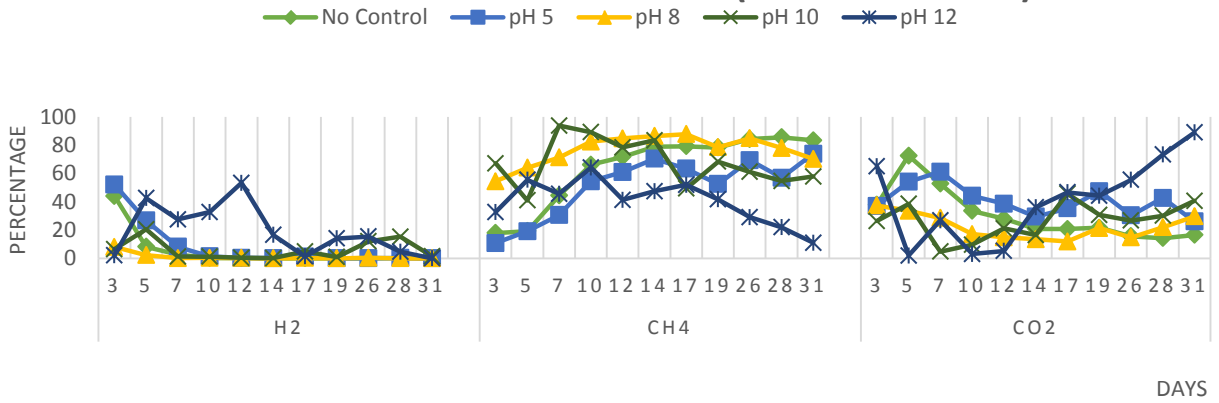


Chart: 3: Percentage of total gas (Food waste)

PERCENTAGE OF TOTAL GAS (FW + SL)

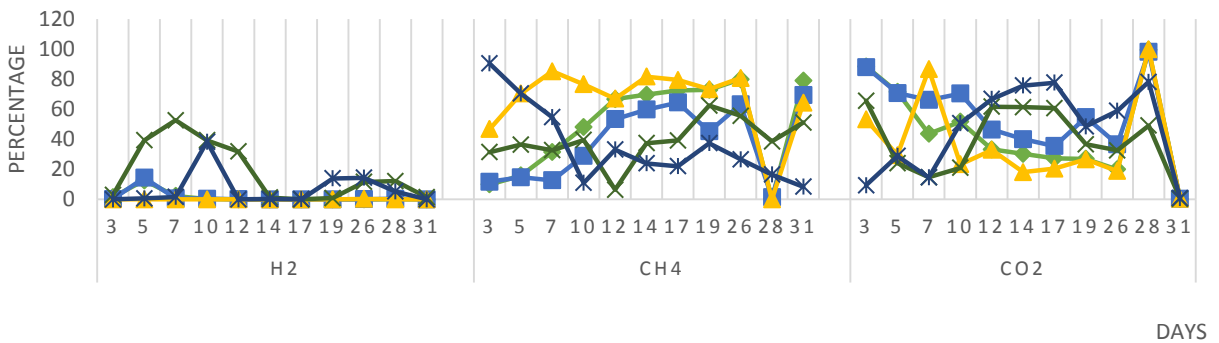


Chart 4: Percentage of total gas (FW + SL)

Chart 2, 3 and 4 show the gas composition there the percentages of different gases, i.e. H₂, CH₄ and CO₂, present in the total accumulated gas mixture were determined. Since the Sampler that collects the volume gas accumulated was out of order, only the percentage of the gases accumulated compared to the total amount accumulated could be analyzed. Still we can see the relations between the different types of gases produced. As shown on Chart 2, 3 and 4, a larger accumulation of H₂ occurred indicating high activity in acidogenesis and acetogenesis as also described in article of (Stephen , et al., 2012). These observations indicate the increasing activity in methanogenesis as also described in (Stephen , et al., 2012) article. Regarding pH 12 and 8, big spikes were observed and the reason is that even though the percentages produced was high only a very small amount was accumulated. This resulted in the GC not being able to read the exact amounts giving the results big deviation.

8.3 VFA accumulation and VFA yield

Chart 5, 6, and 7 shows the amount of accumulated VFA concentrations. For each graph 4 measurement occasions; i.e at day 10, 14, 18 and 26, were chosen for each substrate and substrate mixture. The y- axis illustrates the amount of concentration accumulated were as on the x-axis the different pH conditions are plotted. Each column is divided in different colors representing the distribution of specific VFAs as the entire column represents the total VFA concentration.

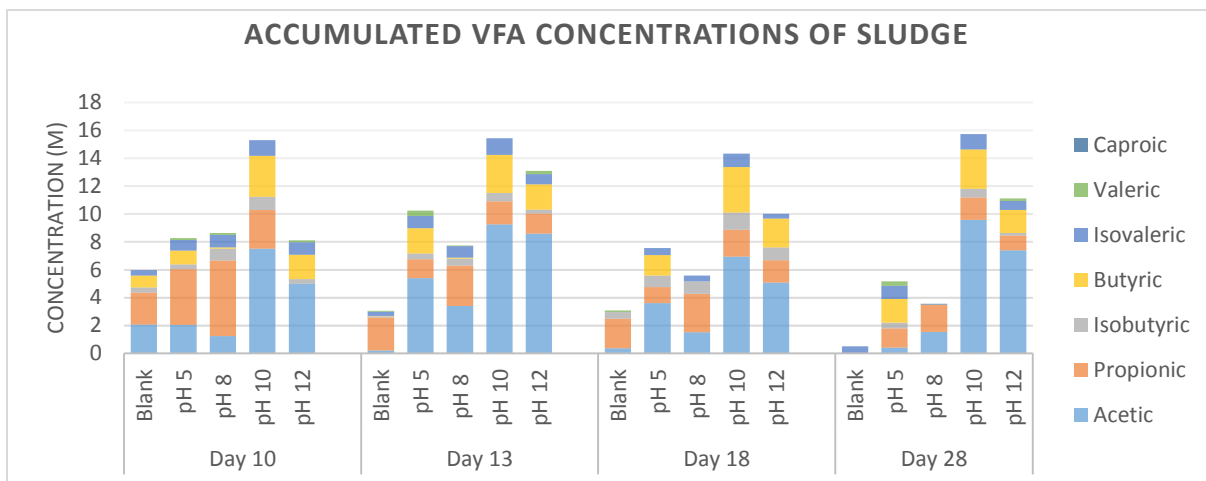


Chart 5: Accumulated VFA concentrations of sludge

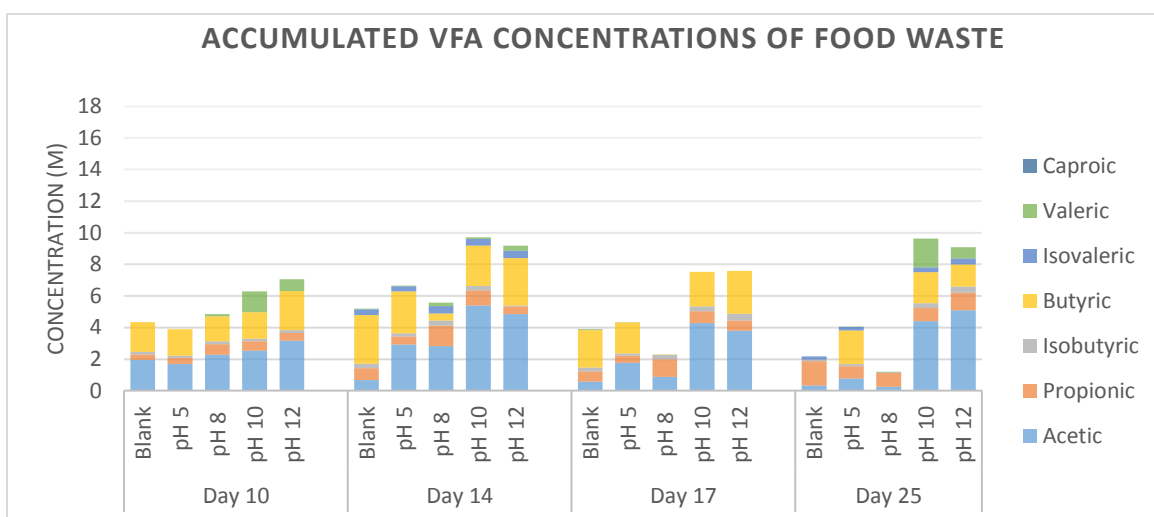


Chart 6: Accumulated VFA concentrations of Food waste

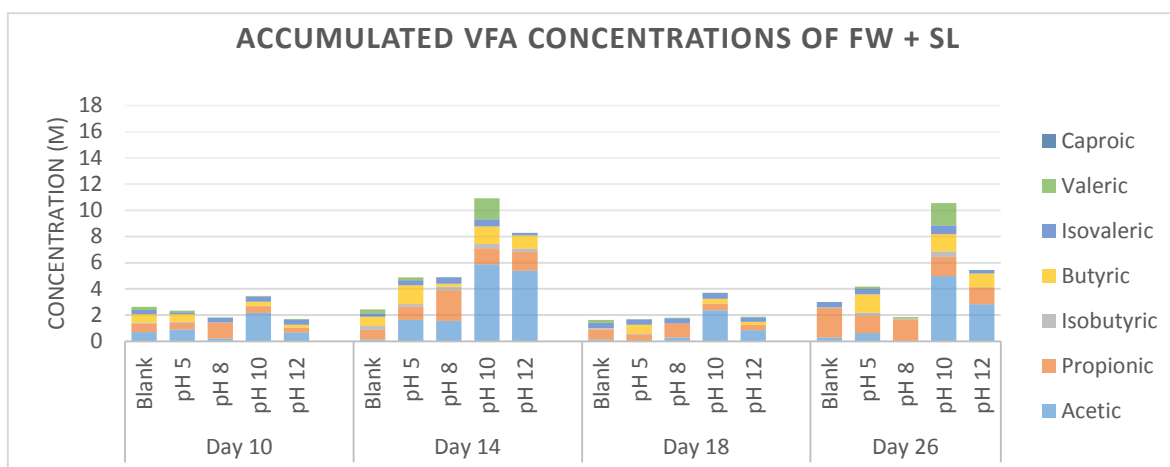


Chart 7: Accumulated VFA concentrations of Food waste and Sludge mixed together

Chart 8, 9 and 10 illustrates the yield of VFAs obtained at the 3 different pH conditions. The yield was calculated by measuring the amount (g) of accumulated VFAs and then dividing that with the amount (g) of VS added in each reactor. The yields were determined at 4 different measurement occasions, i.e. at day 10, 14, 18 and 26 as above.

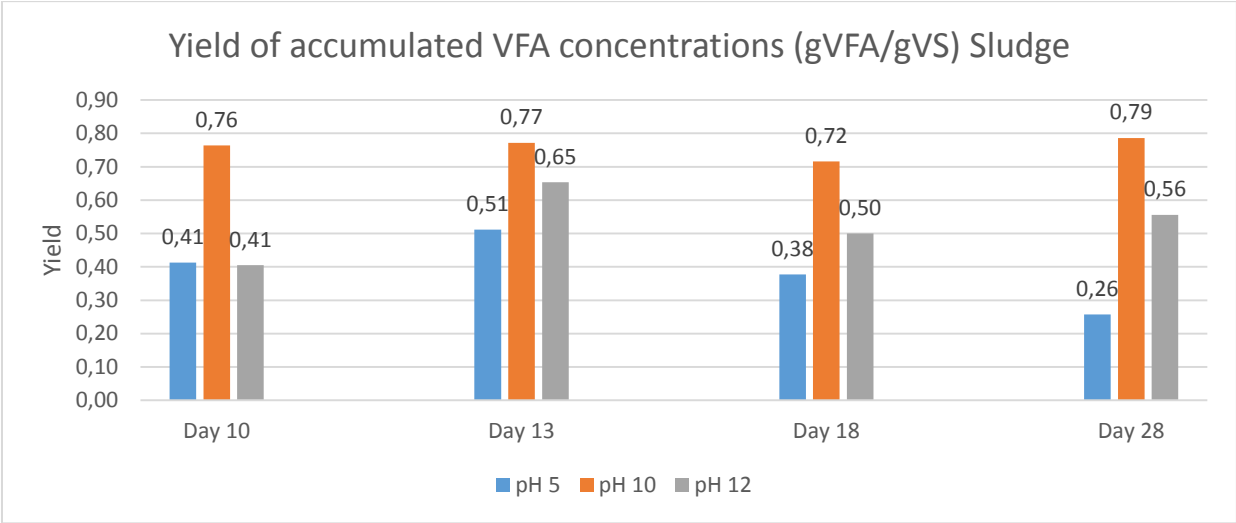


Chart 8: Yield of accumulated VFA concentrations (gVFA/gVS) Sludge

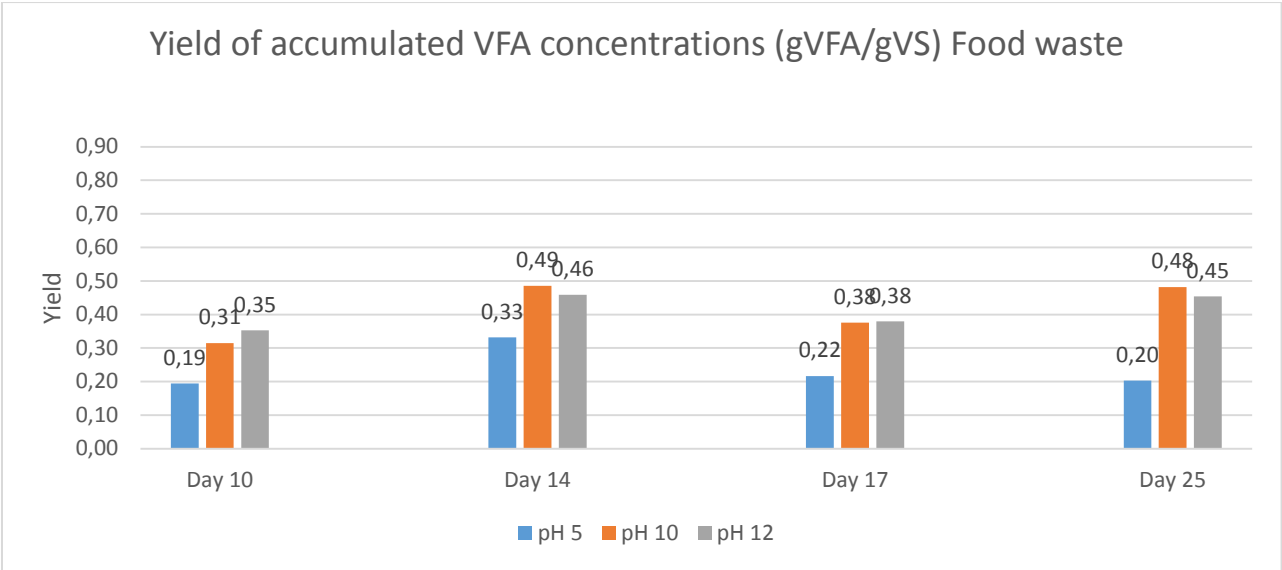


Chart 9: Yield of accumulated VFA concentrations (gVFA/gVS) Food waste

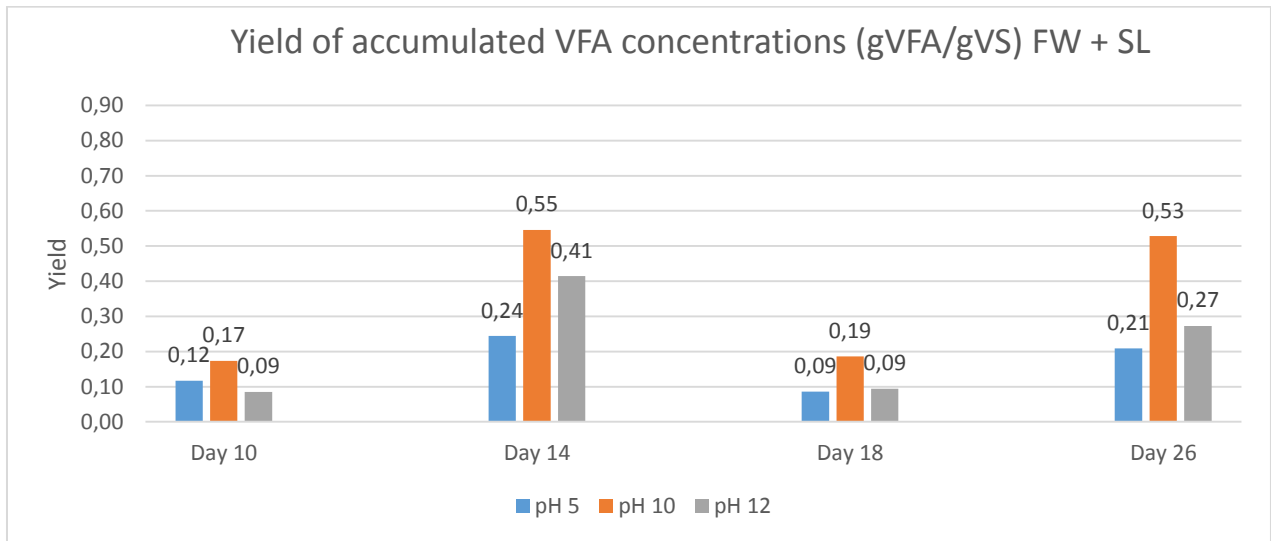


Chart 10: Yield of accumulated VFA concentrations (gVFA/gVS) FW + SL

As illustrated on (Chart 5, 6 and 7) on each chosen day regardless what substrate and substrate mixture used, pH 10 showed to be the most effective pH condition in accumulating highest concentrations of VFAs. The day showing highest VFA concentrations is around day 10-14, which is very similar to what (Wainaina, et al., 2018) previously showed in their article. Comparing the different substrates and the substrate mixture, sludge showed to be the best substrate to accumulate the highest VFA concentration, i.e. 15.43 g/L at pH 10 (Chart 5) and with a yield of 0.77 gVFA/gVS at day 13 (Chart 8). Both food waste and food waste + sludge showed overall a lower concentration of VFAs accumulated, however there were higher concentrations of longer VFAs accumulated in the total amount of VFAs produced. The highest concentrations observed for both food waste and food waste + sludge was calculated to be at 9.7 and 10.92 g/L, respectively (Chart 6 and 7), together with yields of 0.55 and 0.44 gVFA/gVS, respectively (Chart 9 and 10). However even though the accumulated amounts were lower, according to (Wainaina, et al., 2018) a product yield of 0.5 gVFA/gVS is a high

product yield value. Furthermore, degradations at pH 12 also showed high VFAs accumulations and yields. As observed in the case of sludge as substrate, at pH 12 the concentration of VFAs was 13.08 g/L corresponding to a yield of 0.65 at day 13, these values are similar to those observed at pH 10 with the same substrate.

9 Conclusion

The aim of this analysis was to study the impact of pH and the possibility if co-digestion of food waste and sludge is possible. As well as to find conditions leading to the most optimal *i.e.* highest VFA production. The specific type of VFAs produced was also investigated by applying different alkaline conditions and retention times. As a conclusion we can say that during the 10 first days of AD, the process required more pH maintenances when using food waste or sludge as substrates, based on the results showing high changes in pH during degradation. On the other hand, co-digesting food waste with another substrate showed to be effective for self-buffering since the alkalinity was more stable when using a substrate mixture. For gas production pH 10 and 12 showed a lower concentrations of gas accumulated compared to those at pH 5 in general. For producing highest concentration of VFAs under mesophilic conditions, pH 10 and 12 showed to be the best at day 10-14, regardless of the substrate used. The substrate achieving the highest concentrations of VFAs was sludge, with concentrations of 15.43 and 13.08 g/L corresponding to yields of 0.77 and 0.65, at pH 10 and 12, respectively at day 14. When looking for a better variety of VFAs regarding the total amount accumulated in the aim of applying it to a specific application that prefers longer VFAs, FW at pH 10 at day 13 seemed to be most optimal.

From the results of this study we can conclude that if volatile fatty acids is the main goal of an anaerobic digestion, applying pH 10 regardless of the substrate used is the most effective way to accumulate highest concentrations of VFAs. For the most cost-effective day to extract the

accumulated VFAs showed to be day 10 to day 14 regardless of the substrate. Co-digestion with food waste and sludge is also an interesting way of producing VFAs since here two different organic waste sources are being utilized there for different bacterial cultures are forced to work together in order to accomplish an anaerobic digestion. For future studies it is recommended that for the first 15 days of the AD, analyze and maintain pH of the batch reactors more often since it witnessed high changes. After approximately 15 days the mixture became more self-buffering meaning less maintenance was required regarding pH values.

References

- Agency, S. E. P., 2018. *Wastewater treatment in Sweden*, Stockholm: Naturvårdsverket.
- Cheryl , J. B., 2015. *The 10 Principles of Food Industry Sustainability*. 1 ed. Massachusetts: Wiley blockwell.
- eSchoolToday, 2008. *What is food loss and food waste?*. [Online]
Available at: <http://eschooltoday.com/global-food-waste-and-food-loss/what-is-food-waste.html>
[Accessed 20 05 2019].
- FAO, 2019. *SAVE FOOD: Global Initiative on Food Loss and Waste Reduction*. [Online]
Available at: <http://www.fao.org/save-food/resources/keyfindings/en/>
[Accessed 20 05 2019].
- Kiros, H. et al., 2016. Anaerobic co-digestion process for biogas production: Progress, challenges and perspectives. *Renewable and Sustainable Energy Reviews*, 76(4), pp. 1485-1496.
- Kou, J. & Dow, J., 2016. Biogas production from anaerobic digestion of food waste and relevant air quality implications. *Journal of the Air & Waste Management Association* , 67(9).
- Lise, A., Baeyens, J., Degreve, J. & Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in Energy and Combustion Science*, 1(34), pp. 755-781.
- Lystek, 2000. *Leaders in Biosolids & Organics Management*. [Online]
Available at: <https://lystek.com/solutions/bnr-optimization/>
[Accessed 15 07 2019].
- Mashhadi, K. K., 2018. *Enhancement of volatile fatty acids production from food waste: impact of inoculum, pH and retention time* , Linköping: KTH.
- Mayers, J. J., 2016. Integrating Microalgal Production with Industrial Outputs. *Industrial Biotechnology*.
- Miaomiao, Z., Binghua, Y., Wong, J. W. & Zhang, Y., 2018. Enhanced volatile fatty acids production from anaerobic fermentation of food waste: A mini-review focusing on acidogenic metabolic pathways. *Bioresource Technology*, 248(1), pp. 68-78.
- Muzaffar, A. M., Athar, H. & Chanchal, V., 2016. *Design considerations and operational performance*, Greater Noida: Cogent engineering.
- Nag, K. J., Seong, L. J. & Ho, C. N., 2018. *Volatile Fatty Acid Platform: Concept and Application*. 1 ed. Beijing: Wiley Online Library.

- Nayono, S. E., Winter, J. & Herman, H. H., 2009. *Anaerobic Digestion Of Organic Solid Waste For Energy Production*, Karlsruhe: s.n.
- Stehouwer, R., 2010. PennStateExtension. *What is sewage sludge and what can be done with it?*, 15 09.
- Stephen , M. J., Cigolotti, V. & Moreno, A., 2012. *Fuel Cells in the Waste-to-Energy Chain*. 1 ed. London: Springer-Verlag London.
- Trikoilidou, E., Samiotis, G., Bellos, D. & Amanatidou, E., 2016. *Sustainable operation of a biological wastewater*, Kozani: IOP Conference Series: Materials Science and Engineering.
- Vímac, N. A., Marc , S. & Tegetmeyer, H. E., 2015. *Anaerobic digestion of the microalga Spirulina at extreme alkaline conditions: biogas production, metagenome, and metatranscriptome*, Leuven: Front Microbiolci.
- Wainaina, S., Mahboubi, A. S., Parchami, M. & Sárvári Horváth, I., 2019. Food waste-derived volatile fatty acids platform using an immersed. *Bioresource Technolog*, 274, pp. 329-334
- Wang, Dou et al., 2017. Improving anaerobic digestion of easy-acidification substrates by promoting buffering capacity using biochar derived from vermicompost. *Bioresource Technology*. [Online] 227286–296..
- Wee, S. L., Adeline, S. M. C., Hak, K. Y. & Gek, C. N., 2014. A review of the production and applications of waste-derived volatile fatty acids. *Chemical Engineering Journal*, 235(2), pp. 83-99.
- Wikipedia 1, 2019. *Waste managment*. [Online]
Available at: https://en.wikipedia.org/wiki/Waste_management
[Accessed 19 05 2019].
- Wiqvist, W., 2017. *Swedish Waste Management*, Malmö: Avfall Sverige.



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