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Study of the production of Volatile Fatty Acids in a municipal wastewater treatment plant

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SUMMARY

This study has carried out a literature review to see what are the optimal operating conditions for the production of VFAs for the mono- and co-fermentation of WAS, PS, OFMSW and FW. For WAS mono-fermentation, the optimal operating conditions found are neutral-basic pH (7-8) and thermophilic temperature (55 C). In addition, the best pretreatment of WAS in terms of VFA yield and pretreatment cost is believed by the author to be the pretreatment of 1.54 mg/L FNA + RL. For PS mono-fermentation, the optimal operating conditions found are at basic pH (9) and thermophilic temperature (55 C). Finally, for OFMSW fermentation, the optimal operating conditions found were pH 6-7 and mesophilic temperature (40 C). For co-fermentation, the highest yield from the data collected in this study was (0.867 g CODVFA/ g VS fed) with a substrate with a FW/WAS=5 ratio that was fermented at the following operating conditions: SRT 7 d, OLR 9 g VS/L·d, T 40 °C and uncontrolled pH (5.2-6.4). For the implementation of acidogenic fermentation in a WWTP, the author of this work proposed to work with co-fermentation of mixed sludge and FW at the optimal conditions found in a CSTR reactor using the produced methane to maintain the temperature of the system.

Keywords: Polyhydroxyalkanoates (PHA), Volatile Fatty Acids (VFAs)

RESUM

Aquest estudi ha portat a terme una recensió bibliogràfica per veure quines són les condicions òptimes d'operació per a la producció de AGVs per a la mono- i co-fermentació de fangs de depuradora i residus orgànics. Per a la mono-fermentació del fang secundari, les condicions òptimes trobades d'operació són a pH neutre-bàsic (pH 7-8) i temperatura termofílica (55 °C). A més, el millor pretractament pels fangs secundaris en termes de rendiment d'AGV i de el cost de pretractament, l'autor creu que és el pretractament de 1,54 mg / L FNA + RL. Per a la mono-fermentació de PS, les condicions òptimes d'operació trobades són a pH bàsic (pH 9) i temperatura termofílica (55 °C). Finalment, per a la fermentació de OFMSW, les condicions òptimes d'operació trobades han sigut a pH 6-7 i temperatura mesofílica (40 °C). Per a la co-fermentació, el rendiment més elevat de les dades recopilades en aquest estudi ha estat de (0.867 g CODVFA / g VS fed) amb un substrat amb una relació FW / WAS = 5 que es va fermentar a les següents condicions d'operació: temps de retenció de sòlids de 7 d, taxa de càrrega oràginca de 9 g VS / L d, temperatura de 40 ° C i pH sense controlar (5.2-6.4). Per a la implementació de la fermentació acidogènica en una EDAR, l'autor d'aquest treball proposa de treballar amb la co-fermentació de la barreja del fang primari i secundari amb residus alimentaris en les condicions òptimes trobades en un reactor CSTR i utilitzant el metà produït per mantenir la temperatura de el sistema.

Paraules clau: Polihidroxialcanoats (PHA), Àcids Grassos Volàtils (AGVs)

1. INTRODUCTION

Volatile Fatty Acids (VFAs) are important intermediates products that are increasingly in demand in the market. They are traditionally produced by petroleum-based production methods. However, when these methods are applied to produce them, greenhouse gas (GHG) emissions are remarkably high. For example, GHGs due to the production of acetic acid in the petrochemical industry are 3.3 t CO_2eq./t for cradle-to-grave. Furthermore, the EU committed itself in the Energy Saving 2020 report to reduce its GHG emissions by 80% to 95% by 2050. Ultimately, there is a need to replace this process with one that uses renewable sources and bio-based production methods. Thus, the use of biorefineries has emerged. Biorefineries consist of using organic waste to produce new products with high added value. In other words, the concept of circular economy is applied to the production of bioproducts as the opposite of the linear economy established in the "take, make and dispose" production model.

For example, while potential VFA production capacity of EU countries from dairy industry wastewater is projected as 10 Mt/year (acetic acid eq), an estimated 2 Mt of acetate, 1.28 Mt of butyrate and 1.54 Mt of propionate could be recovered annually. In the global scale, the values are 9.15 Mt acetate, 5.39 Mt butyrate and 6.47 Mt propionate. The calculations were done by Atasoy et al. (2018) according to data from European Commission Statistics (ec.europa.eu/eurostat/data/database).

This work is focused on the production of VFA from waste activated sludge (WAS), the organic fraction of municipal solid waste (OFMSW) and other organic wastes. VFAs are precursors of polyhydroxyalkanoates (PHAs), thermoplastic polyesters with mechanical properties like those of conventional plastics (polyethylene and polypropylene). However, PHAs have the advantage of being biodegradable, biocompatible and bio-based.

Traditionally, what is done with biodegradable organic wastes is to produce methane or compost. The problem with this is that these products have low added value. Therefore, the European Union has launched several research projects to better valorize these wastes: one of them is the *Res Urbis* aimed to optimize and lower the costs of bioplastic production in the form of PHAs from organic city waste (mainly WAS and OFMSW). The PHAs are estimated to be useful for packaging, production of durable plastics (e.g., phone casing) and even for environmental remediation (controlled release materials for remediation of contaminated groundwater or other examples). This project was led by Sapienza University (Rome) and involved numerous universities (included the University of Barcelona), technological centers, industries, and public administration with a total funding of three million euros and had a duration of three years.

2. OBJECTIVES

The production of VFA as a precursor of PHA is a very current research topic under development. For example, the Res Urbis Project has been studying it for the last three years. For this reason, it is not yet a systematized field and it is not clear what are the operating factors that enhance VFA production and how they influence VFA composition. A literature review of the available data is needed to see what conclusions can be drawn.

So, the main objective of this study is to review the literature on the current state of VFA production and their conversion to PHA. To do so, the results of research articles on this topic will be analyzed to find the optimal conditions for the VFA production process. Specifically, it is intended to see which operating conditions (including pH, temperature, ORL, HRT and the reactor configuration) produce a higher yield of VFA, since a high concentration of VFA is required to produce PHA from this precursor (Valentino et al., 2017). Another factor to be analyzed is whether pretreatments before acidogenic fermentation are useful to improve VFA production and whether they can be implemented easily and economically. Furthermore, it is intended to evaluate if the co-fermentation of sewage sludge with OFMSW or FW improves the VFA yield with respect to mono-fermentation.

Once this bibliographical review is done, the author of this work will propose some guidelines to apply to a WWTP in such a way that the production of VFA is optimized in a cost-effective way.

3. STATE OF THE ART OF VFA PRODUCTION AND ITS CONVERSION INTO PHA

This chapter will explain what VFA are and their main physicochemical characteristics. Then, the steps in the process of obtaining VFA through acidogenic fermentation will be explained and, finally, the substrates selected in this study by means of which VFA can be produced and their characteristics will be presented.

3.1. VOLATILE FATTY ACIDS (VFAS)

Volatile fatty acids (VFAs) are saturated aliphatic acids containing two to six carbon atoms in their chemical structure, and sometimes even eight (Chwiałkowska and Ole_skowicz-Popiel, 2016; Zielewicz, 2007). They contain an acetyl group that makes them polar compounds. In addition, these compounds are characterized by high vapor pressure, which is exploited for the determination of their concentration in environmental samples (Zielewicz, 2007), and by their strong hydrophilic properties.

VFA are ultimately a valuable intermediate product that can be produced by anaerobic digestion of waste as a feedstock. Among these wastes are primary sludge (PS), secondary sludge or waste activated sludge (WAS), the mixture of both or sewage sludge (SS), food waste (FW) or the organic fraction of the municipal solid waste (OFMSW). However, they can also be produced from petrochemical derivatives. Currently, the large amount of VFA demand in the market is 90% supplied by petroleum-based production methods due to the low performance of anaerobic digestion at low pH (Atasoy et al., 2019).

3.2. ACIDOGENIC FERMENTATION FOR VFA PRODUCTION:

Anaerobic digestion of organic matter occurs in several stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. To produce VFA it is essential to stop the reaction in acetogenesis.

Hydrolisis:

Hydrolysis is the first step of acidogenic fermentation and, during this process, insoluble and polymerized organic compounds are exposed to the activity of the bacteria's extracellular hydrolytic enzymes (amylases, lipases, proteases, etc.), leading to their decomposition and solubilization. Thus, polysaccharides, proteins and fats are hydrolyzed into monosaccharides, amino acids and polyols and fatty acids, respectively. This stage is believed to be the limiting step of acidogenic fermentation (Zielewicz, 2007; Agler et al., 2011; Eastman and Ferguson, 1981). In fact, if the particulate organic matter contained in activated sludge is not adequately solubilized, only 30-50% of the total COD (TCOD) or volatile solids (VS) in WAS are biodegraded within 30 days (Parkin and Owen, 1986).

Therefore, different pretreatments have been used before the anaerobic digestion to hydrolyze the organic matter (see Appendix 1).

The extent of hydrolysis can be defined as stated in equation 1, where $sCOD_{out}$ and $sCOD_{in}$ are soluble COD in the outlet and inlet, respectively, and $tCOD_{out}$ and $tCOD_{in}$ are the total COD in the outlet and the inlet, respectively (Yin et al, 2019).

$$extent\ of\ hydrolisis(\%) = \frac{(tCODin - sCODin) - (tCODout - sCODout)}{tCODin - \ sCODin} * 100\% \qquad (1)$$

Acidogenesis:

The next stage is acidification or acidogenesis, which is also called dark fermentation. The bacteria involved in this process are facultative bacteria or obligate anaerobes and start from the organic matter hydrolyzed in the previous stage. The most common bacteria are Aerobacter, Alcaligenes, Clostridium, Escherichia, Flavobacterium, Pseudomonas, Lactobacterium, Lactobacillus, Micrococcus and Streptococcus.

In addition, these are relatively less susceptible to temperature or pH changes and proliferate much faster than methane-producing bacteria. Through the activity of these bacteria, the hydrolyzed low molecular weight organic compounds are mainly (76%) transformed into VFA (such as acetic acid, butyric acid, propionic acid and valeric acid), alcohols (such as ethanol and methanol) and gaseous products (CO_2 and H_2). The remaining part is broken down into acetates. Also, in this phase proteins are broken down into simple organic acids, thiols, and amines (Zielewicz, 2007; Rosik-Dulewska, 2007; Sadecka, 2010).

The extent of acidification can be defined as shown in equation 2 where COD_{CH4} and COD_{H2} are methane and hydrogen production as COD equivalents, $COD_{VFA,out}$ and $COD_{VFA,in}$ are VFA in the outlet and the inlet as COD equivalents. Finally, $tCOD_{inlet}$ is the total COD in the inlet (Yin et al, 2019).

$$extent\ of\ acidification(\%) = \frac{COD_{VFA,out} - COD_{VFA,in} + COD_{CH4} + COD_{H2}}{tCODin - COD_{VFA,in}} * 100\% \qquad (2)$$

Acetogenesis:

In the third stage, called acetogenesis, ethanol and VFA are converted to CO_2, hydrogen, and acetic acid by bacterial activity.

For example, valeric acid is decomposed into propionic acid and acetic acid by Mbacterium suboxydans, while the decomposition of propionic into acetate, CO2 and CH4 is carried out by Mbacterium propionicum. Finally, for the decomposition of the acetates into methane it is needed the presence of the bacterium Methanococcus mazei (see Figure 1).

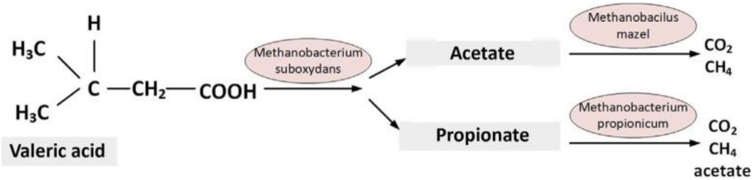

Figure 1 - Activity of bacteria to produce CH_4 from valeric acid. (Worwag et al 2017, based on Sadecka, Z., 2010)

Also, acetogenic bacteria can use other simple compounds (such as carbon dioxide, methanol, or glucose to generate acetate as it is show in equations 3- 6.

$CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$ (3)
$\Delta G° = - 95$ kJ/mol ethyl

$4CO_2 + 2H_2O \rightarrow CH_3COOH + 2CO_2 + H^+$ (4)
$\Delta G° = -175$ kJ/mol ethyl

$4CH_3OH + 2CO_2 \rightarrow 3CH_3COOH + 2H_2O + 3H^+$ (5)
$\Delta G° = -7$kJ/mol ethyl

$C_6H_{12}O_6 \rightarrow 3CH_3COOH + 3H^+$ (6)
$\Delta G° = -104$ kJ/mol ethyl

Bacteria of the acetogenic phase have a long generation time, that is variable depending on the type of substrate available, as represented in Figure 3.XX (Sadecka, 2010). Li et al. (2015) reported that the addition of nitrate might enhance the sludge granulation in acetogenesis because the denitrifying bacteria used the hydrogen produced from acetogenesis to drive the acetogenesis. In fact, when the molecular hydrogen is removed from the reaction environment, the acetogenic and methanogenic bacteria live in symbiosis (Zalewski et al., 2012). That is, both types of bacteria can connect to various aggregates resulting in the decomposition of propionic acid according to equations (7)-(9) (Bitton, 2005; Rosik-Dulewska, 2007; Z˙ ygadło, 1999; Worwag et al., 2017).

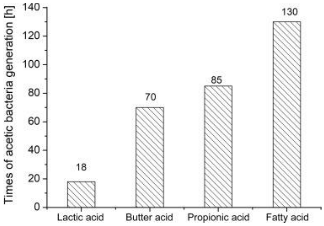

Figure 2. Times of acetic bacteria generation at mesophilic temperatures (35–37°C) depending on the type of substrate. (Worwag et al., 2017; Sadecka, 2010).

Acetogenic bacteria:

$CH_3CH_2COO^- + 3H_2O \rightarrow HCO_3^- + CH_3COO^- + H^+ + 3H_2$ (7)

Methanogenic bacteria:

$HCO_3^- + 4H_2 + H^+ \rightarrow CH_4 + 3H_2O$ (8)

Total reaction:

$4CH_3CH_2COO^- + 3H_2O \rightarrow HCO_3^- + 4CH_3COO^- + 3CH_4 + H^+$ (9)

Disturbance to this symbiotic process is observed when hydrogen is also consumed to reduce sulfates to hydrogen sulfide.

The extent of acetogenesis can be defined using equation 10 where COD_{CH4} and COD_{H2} are methane and hydrogen production as COD equivalents, $COD_{HAc,out}$ and $COD_{HAc,in}$ are the acetic acid concentrations in the outlet and the inlet as COD equivalents. Finally, $tCOD_{inlet}$ is the total COD in the inlet (Yin et al, 2019).

$$extent\ of\ acetogenesis(\%) = \frac{COD_{HAc,out} - COD_{HAc,in} + COD_{CH4} + COD_{H2}}{tCODin - COD_{HAC,in}} * 100\% \qquad (10)$$

Methanogenesis:

The last stage of the fermentation process is methanogenesis, which occurs mainly by methanogenic bacteria belonging to obligate anaerobes of the genera Methanobacterum, Methanococcus and Methanogenium. These bacteria are highly susceptible to temperature and reaction changes. The generation time ranges from 15 to 85 hours depending on the initial substrate as shown in Figure 3 (Sadecka, 2010).

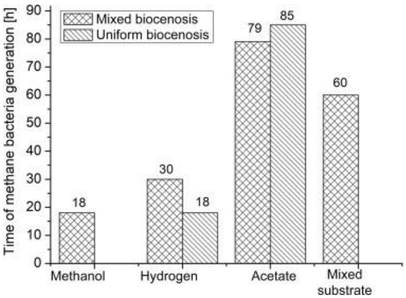

Figure 3- Times of CH_4 bacteria generation at mesophilic temperatures (35–37°C) depending on the type of substrate. (Worwag et al.,2019; Sadecka, 2010)

Two groups of bacteria can be distinguished. The first one generates CH_4 from formic acid, methanol, hydrogen, and CO_2. They have a short generation time and a high tolerance to the reaction changes. The energy yield in these reactions ranges from 106 to 145kJ/mol, and are shown in eq. 11

$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ (11)

$CH_3OH + H_2 \rightarrow CH_4 + 2H_2O$

The second group of bacteria is much poorer, they grow more slowly and are less tolerant to the reaction changes (Sadecka, 2010). Still, it has been proved that about 70% of CH4 is produced from the acetic acid, whereas only the 30% is produced from hydrogen and CO2 (Janosz-Rajczyk, 2008). The reaction is shown in eq. 12.

$CH_3COOH \rightarrow CH_4 + CO_2$ and $\Delta G = -35.9 kJ/mol$ (12)

A key factor in the yield of VFA is the inhibition of the last phase (methanogenesis). This can be achieved by adding a specific inhibitor, such as 2- bromoethane sulphonate (BES) (Eryildiz et al., 2020). Indeed, it has been reported that 10 mM of BES addition could totally inhibit the activity of methanogens in the cow manure and municipal wastewater fermentation (Webster et al., 2016). or working with an operating condition that are unfavorable to methanogens. Indeed, the optimum pH for methanogenic bacteria is pH =6-8. Therefore, operating above or below this pH inhibits this process (Maspolim et al., 2015).

Also, heat shock (70–120 °C, 15–120 min) can inhibit methanogenesis and it has been widely used because of the simplicity of operation and the low cost and no chemical requirement (Wang et al., 2017). This inhibition is due to the inactivation of non-spore-forming microorganisms such as methanogens, while maintaining spore-forming acid producers (Arslan et al., 2016). Moreover, it has been reported that heat shock improves the hydrolysis with a SCOD increase of 2.9–25.6 folds (Carrere et al., 2016).

Several reports have used the heat shock pretreatment to inhibit the methanogens. For example, Jomnonkhaow et al. (2021) and Tampio et al. (2019) worked with cow manure as initial substrate, while Ghimire et al. (2017) worked with the digested sludge fermentation or Mei et al. (2016).

The extent of methanogenesis can be defined as stated in equation 13 where COD_{CH4} and COD_{H2} are methane and hydrogen production as COD equivalents. $tCOD_{inlet}$ is the total COD in the inlet (Yin et al, 2019).

$$extent\ of\ acetogenesis(\%) = \frac{COD_{CH4} + COD_{H2}}{tCODin} * 100\% \tag{13}$$

3.3. BIODEGRADABLE ORGANIC WASTES AS FEEDSTOCK TO PRODUCE VFAS:

As stated previously, several biodegradable organic wastes have been tested to produce volatile fatty acids. In this section, sewage sludge, food waste and/or organic fraction of municipal solid waste and its mixtures as feedstock to produce VFA are discussed.

Primary Sludge (PS)

Primary wastewater treatment consists of gravity sedimentation of wastewater previously screened and degraded in pretreatment to remove settleable solids. Typically, slightly more than half of the suspended solids are removed along with one third of the total BOD.

Although the objective of primary wastewater treatment is to separate suspended solids that are readily removable and BOD, wastewater constituents that exist as settleable solids or are adsorbed on settleable solids may also be removed. Therefore, primary treatment also produces some reduction in the concentration of nutrients and pathogenic organisms in the effluent of nutrients, pathogenic organisms, trace elements and potentially toxic organic compounds.

Table 1 – Main characteristics of the primary sludge used in different research articles.

ARTICLE	Total suspended solid (g/L)	Volatile suspended solid (g/L)	VS/TS (%)	CODsol (g COD/L)	total COD (g COD/L)	sCOD/tCOD (%)	pH	NH_4^+-N (mg/L)	Total carbohydrate (g COD/L)	Total protein (g COD/L)
Wu et al. (2009)	19.30 g/L	11.64 g/L	60	1.76	20.63	8.5	6.0	53	0.312	7.718
Ucisik et al. (2008): Lynetten	33.12 g/L	28.4 g/L	86	3.29	40.06	8.2	5.46	114		
Ucisik al. (2008): Avedøre	34.20 g/L	29.10 g/L	85	0.70	45.45	1.5	6.18	56		
Ucisik et al. (2008): Helsingør	32.80 g/L	27.53 g/L	84	1.73	35.05	4.9	5.78	75		

Thus, primary sludge (PS) is the residue of primary treatment and consists of a concentrated suspension of all the constituents that are removed from the water. Table 1 summarizes the main characteristics of settled primary sludge. As observed in this table, the characteristics of the primary sludge used by different researchers are similar. This is partly due to the fact that there are four different primary sludges used by Usicik et al. (2008) from four different WWTPs:

Lundtofte, Lynetten, Avedøre and Helsingør. Thus, for primary sludge the pH range is 5.5-6.2; the VS/TS range is 60-85%, the sCOD/tCOD range is 0.70-3.5 % and the soluble NH_4^+-N is between the range of 50-120 mg/L.

Secondary sludge or Waste Activated Sludge (WAS):

Secondary treatment of municipal wastewater consists of a biological treatment process. Suspended microorganisms are used to remove biodegradable organic matter (and nutrients). Thus, part of the organic matter is oxidized by the microorganisms producing carbon dioxide and other end products, while the rest of the organic matter provides the energy and materials necessary to maintain the community of microorganisms. During this process, the microorganisms assimilate nutrients, such as nitrogen and phosphorous. The microorganisms biologically flocculate to form settleable particles and, after biological treatment, this excess biomass is separated in settling tanks as a concentrated suspension called "secondary sludge", "biological sludge" or "waste activated sludge". Table 2 shows a characterization of Waste Activated Sludge, where it is observed that the characteristics of the WAS used by the different authors are similar. Although, the concentration of dissolved NH_4^+-N is very variable. In general, it can be said that for the reported studies, the properties of WAS are: pH 6.5-7.3, VS/TS 60- 95 %, sCOD/tCOD 0-5 %, 1-2 g COD carbohydrate/L, 8-15 g COD protein/L, 0-0.3 g COD lipid/L.

Some wastewater constituents- such as pathogens, trace elements and organic compounds- can be associated with secondary sludge because of microbial assimilation. Quite often both primary and secondary sludges are combined together for further treatment and this mixture is usually called mixed sludge (MS) or Sewage Sludge (SS). As stated in this Table 3, the characteristics of both mixed sludges are similar. Although, the concentration of NH_4^+-N is very variable. For example, the VS/TS is practically the same. The mixed sludge used in the study of Chen et al (2017) is in a relation (1:1) in (w/w) for the mixed PS and WAS.

Table 2- Characteristics of WAS used in different research articles.

ARTICLE	Total solid	Volatile solid	VS/TS (%)	CODsol (g COD/L)	total COD (g COD/L)	sCOD/tCOD (%)	pH	NH_4^+-N (mg/L)	Total carbohydrate (g COD/L)	Total protein (g COD/L)	Lipid and oil (g COD/L)
Moretto et al. (2019)	30 g/kg	21 g/kg	70	0.15			6.6				
Chen et al. (2007)	13.808 g/L	10.815 g/L	78	0.041	13.41	0.3	6.8		1.522	8.180	0.131
Zhang et al. (2009)	15.768 g/L	12.901 g/L	82	0.084	18.66	0.4	6.64		1.067	9.936	0.231
Yu et al. (2008)	7.075 g/L	6.683 g/L	94	0.094	9.105	1.0	6.4				
Wu et al. (2016)	27.63 g/L	15.96 g/L	58	1.19	25.10	4.7	7.03	160.76			
Ucisik et al. (2008)	5.79 g/L	4.68 g/L	81	0.03	6.08	0.5	6.62	2.2			
Jie et al. (2014)	18.10 g/L	10.46 g/L	58	0.298			6.68	56			
Feng et al. (2009)	26.15 g/L	18.31 g/L	70	0.651	26.99	2.4			2.005	15.297	0.297
Chen et al. (2013)	24.4 g/L	16.8 g/L	69	0.6	23.1	2.6	6.9		2.0	13.3	0.26
Vidal-Antich et al. (2021)	48.5 g/L	34.2 g/L	71				7.3				

Table 3 – Characteristics of mixed sludge (PS and WAS) used in different research articles.

ARTICLE	Total suspended solid (g/L)	Volatile suspended solid (g/L)	VS/TS (%)	CODsol (g COD/L)	total COD (g COD/L)	sCOD/tCOD (%)	pH	NH4+-N (mg/L)
Ucisik et al. (2008)	11.08 g/L	8.60 g/L	77	0.65	15.48	4.2	6.47	32
Chen et al. (2017)	16.05 g/L	12.69 g/L	79	2.55	21.43	12	5.7-6.0	163.44

Regarding the potencial VFA production from these sludges, PS have a higher VFA production potential than WAS, because its yields range from 30 to 400 mgCOD g^{-1}VS depending on fermentation conditions (temperature, pH, HRT, ORL, etc.) (Ahn and Speece, 2006; Cokgor et al., 2009; Peces et al., 2016; Ucisik and Henze, 2008). However, the volume and composition of PS is somewhat variable since it is linked to the incoming raw wastewater and retention time in the primary settler. Therefore, the VFA yields and VFA composition of their fermentation are variable (Peces et al., 2016; Ucisik and Henze, 2008, Yasui et al., 2008).

On the other hand, starting from the same WWTP, WAS production and composition are more constant, making it a more reliable substrate for VFA production despite lower VFA yields. Without pretreatment, WAS fermentation yields range from 18 to 240 mgCOD g^{-1}VS depending on fermentation conditions (Chen et al., 2007; Ucisik and Henze, 2008; Xiong et al., 2012; Yuan et al., 2009)

Both the VFA yields of WAS and PS are low since no more than 20% of the chemical oxygen demand (COD) contained in the sludge is converted to VFA. Therefore, it is advisable to co-ferment the WAS, the PS, or the SS with other cosustrates, such as OFMSW or FW.

Food Waste (FW):

Food waste (FW) is an organic substrate with diverse origins: household canteens, restaurants, catering, vegetable and fruit waste from markets, supermarkets, and food factories. FW, like any other organic substrate, are essentially composed of three groups of macromolecules from which VFAs will be produced: carbohydrates, proteins, and lipids.

Although FWs have diverse origins, they exhibit characteristics that, despite being variable and sometimes seasonally dependent, are somewhat similar (Traverso et al., 2000; Stenmarck et al., 2016). Indeed, FWs contain high levels of organic matter (approximately 15-20% TVS) and high concentrations of nitrogen and phosphorus (2-15 g/kg and 0.5-1.0 g/kg, respectively), which are indispensable nutrients for the metabolic pathway of microorganisms. These chemical characteristics make FWs optimal substrates to produce VFA through acidogenesis or dark fermentation (Strazzera et al., 2018).

Moreover, FWs is an abundant substrate and the Food and Agriculture Organization of the United Nations (FAO) reported that one-third of global food production is lost or wasted along the food supply chain, including the final stages (households, restaurants, and canteens). Specifically, the EU-28 Member States produced approximately 89 million tons of food waste in 2012 (Braguglia et al., 2018; Lucifero, 2016). The sectors contributing to the production of FWs are households (47 million tons ± 4 million tons) and the processing industry (17 million tons ± 13 million tons) (Stenmarck et al., 2016). Traditionally, these FWs are disposed in landfills or sent to incineration with no or very little resource recovery which also originates high emission of GHGs and toxic compounds into the atmosphere and soil (Ren et al., 2018). Therefore, the production of VFAs from FW is a sustainable and efficient alternative to mitigate their impact to the environment.

Organic Fraction of Municipal Solid Waste (OFMSW):

The definition of the organic fraction of municipal solid waste (OFMSW) is not uniform but varies both regionally and nationally. Thus, in the EEUU, OFMSW is considered a mixture of food, garden waste and paper (Palmisano and Barlaz, 1996). In contrast, in the European Union, it is considered a mixture of park, garden and kitchen waste (Al Seadi et al., 2013). Moreover, the production and composition of MSW depends on the geographical region, the number of inhabitants and their social status, the predominant economic activities, regional food habits, the season of the year and the collection system (VALORGAS, 2010, Hansen et al., 2007b, Palmisano and Barlaz, 1996).

Global municipal solid waste production is approximately 1300 million tons per year (Hoornweg and Bhada-Tata, 2012, Al Seadi et al., 2013) and it is estimated that, by 2025, production will increase to 2200 million tons per year with approximately 46% organic content (Al Seadi et al., 2013).

Campuzano et al. (2016) made an analysis of the OFMSW characteristics of cities from different countries and compared them with their corresponding methane production. She concluded that the characteristics with lower variability are carbon composition (40 -50 %), hydrogen composition (5,5-7%), humidity (50-80%), starch (14-20%) and VS/TS ratio (60-96 %). Moreover, the lowest variability resulted from OFMSW with higher food waste and easily degradable compounds. In the other hand, the highest variability was observed for total phosphorus, sulphur, hemicellulose, Total Kjeldahl nitrogen, free sugars, lignin and raw fibers.

Traditionally, municipal waste was disposed of in landfills but, due to its environmental impact, regulations are now strict and allow landfilling only under special considerations (Browne and Murphy, 2013, Fdez.-Güelfo et al., 2011).

Anaerobic digestion of OFMSW and other organic wastes is a way to produce VFA and recover energy in the form of biogas (methane). Moreover, anaerobic digestion does not represent a potentially polluting process when the biogas produced is properly utilized (Wang et al., 2014, Dong et al., 2010, Guendouz et al., 2010) and the costs are relatively lower than those of aerobic treatment (Mata-Alvarez et al., 2000).

Therefore, many researchers, companies and government agencies are actively working to improve the processes (Wang et al., 2014, Guendouz et al., 2010, Mata-Alvarez et al., 2000), such as the Res Urbis project mentioned above.

Mixture of substrates:

Co-fermentation of complementary substrates could lead to an enhanced production of VFAs when compared with their momo-fermentation, such as in the study of Vidal-Antich et al. (2021) where WAS and FW were cotreated. The main advantages of this co-fermentation are the following (i) increase of organic content; (ii) dilution of inhibitory and/or toxic compounds; (iii) C/N ratio balance; (iv) reduction of reactor volume; (v) improvement of buffer capacity; and (vi) optimization of rheological qualities (Banerjee et al., 1998; Gomez et al., 2006; Li et al., 2018; Mata-Alvarez et al., 2000).

3.4. COMPARISON OF PRETREATMENTS APPLIED TO WAS, FW AND OFMSW.

As hydrolysis is the limiting step of anaerobic fermentation, pretreatments that induce further hydrolysis of WAS and thus also further production of VFA have been extensively studied. The most used pretreatments are explained in appendix 1 and they are compared in Table 4 to see which is the best in terms of production of VFA and cost.

Ultimately, pretreatments target both solubilization and hydrolysis of WAS organic matter to increase the availability of hydrolyzed substrate for the acidogenic stage. Specifically, the pretreatments target extracellular polymers (EPS), which are macromolecular organic polymers secreted by the microorganisms in the WAS and attached to the cell walls of the microorganisms (see Figure 4). It is known that proteins and polysaccharides are the main components of EPSs (Kavitha et al., 2016, Sharmila et al., 2015). For this reason, their breakdown is fundamental because polysaccharides and proteins are the basic substrates for microbial VFA production (Cui and Shen, 2012; Yuan et al., 2006). In addition, EPS play an important role in maintaining floc structure and microbial growth (Ni et al., 2009). So, EPS disintegration can increase the rate of sludge biodegradation during the fermentation process (Zhao et al., 2017b, Li et al., 2014).

On the other hand, in addition to increasing VFA production, pretreatments are secondarily aimed at decreasing fermentation times. And is that, shortening the fermentation time for higher VFA production gives many benefits, such as reducing the device volume (i.e., space required), from an engineering point of view.

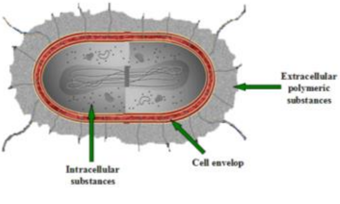

Figure 4 - Schematic illustration of EPS and cell envelope protecting cells against hostile environments. (Zhao et al. (2015))

In Table 4, we can see that pretreatments combined with operation pH 10 give the best yields. The best yield is 445 mg COD/g VSS corresponding to the Ultrasonic pretreatment (Yan et al., 2010) operating at pH 10. Next comes the yield of 378 mg COD/g VSS corresponding to the pretreatment with RL operating at an initial pH of 10 (He et al., 2016) and finally comes 370.1 mg COD/g VSS corresponding to the FNA pretreatment (Zhao et al., 2015) also operating at pH 10. Indeed, their times are 3 days for Ultrasonic and RL, while they are only 2 days for FNA. With a lower yield, the pretreatment with 500 mg/L sulfite combined with an operating pH 9.5 gave a yield of 324.8 mg COD/g VSS with a fermentation time of 4 days (Liu et al., 2020).

Another high yield that can be observed in Table 4 is 368 mg COD/g VSS which corresponds to a thermophilic operating temperature (55 C) and an alkaline operating pH (pH 8). Despite having such a high yield, its fermentation time is long (8 days) as it has no pretreatment. Fermented WAS at mesophilic temperature and alkaline pH (pH 9) gives a much lower yield (298 mg COD/g VSS) but is achieved with a shorter fermentation time (5 days) (Zhang et al., 2009). Finally, in WAS fermented at room temperature (20 C) and alkaline pH (pH 10) yield decreases even more (256.2 mg COD/g VSS) and fermentation time increases (8 days) (Yuan et al., 2006).

In all these cases, the increased cost of chemical addition of NaOH to control the pH would make it difficult to apply on an industrial scale (Wu et al., 2017).

The next highest yields are those of FNA+RL (352.62 mg COD/g VSS; 3 days of fermentation; Wu et al., 2017); FNA+APG (354.6 mg COD/g VSS; 5 days of fermentation; Liu et al., 2018) and FNA+SDBS (334.5 mg COD/g VSS; 4 days of fermentation, Zhao et al., 2016). Within these, the best pretreatment is FNA+RL which gives a yield very similar to that of FNA+APG with the shortest fermentation time. Furthermore, although compared to the combined pretreatments with alkali operation pH the production of Wu et al 2017 was a little lower, the low-cost and reproducible treatment materials allowed this method to be more cost-effective and environmentally friendly (Wu et al., 2017).

There are two other high yields in the table: 70 °C (during 1 h) + SDBS of 0.01 g/g TS (320 mg COD/ g VS; fermentation time of 4 days; Wan et al., 2020) and sole RL (0.2 g RL/g TSS) (311 mg COD/g VSS; fermentation time of 5 days, Huang et al., 2015).

Then, a set of pretreatments with low yield (between 250 and170 mg COD/g VSS) can be observed: SDS+ME (240.8 mg COD/g VSS; Luo et al., 2011), 0.02 g SDBS/ g DS (240.5 mg COD/g VSS; Jiang et al., 2007), Ultrasonic (28 kHz and 60 minutes) combined with pH 12 (230 mg COD/g VSS; Liu et al., 2009), ME (211.7 mg COD/g VSS; Luo et al., 2011), Ultrasonic (energy density of 1.0 kW/L, 10min) (196.7 mg COD/g VSS, Yan et al., 2010), 1.8 mg FNA/L (185.7 mg COD/g VSS, Li et al., 2016).

The pretreatment with the lowest yield is nano zero-valent iron (5.0 g/L) (154.8 mg COD/g VSS; Luo et al., 2014) which is the double of the production of VFA with WAS fermented at 20 °C and uncontrolled pH (76.7 COD/g VSS).

In the case of fermentation of PS (Ji et al., 2010), the VFA production is greater than WAS (Zhao et al., 2015). But, the production of VFA of the mixed sludge (PS+WAS) with the pretreatment of SDBS (0.02 g/gTSS; Ji et al., 2010) is lower than WAS with the same pretreatment (SDBS 0,02 g/g DS; 240.5 mg COD/g VSS; Jiang et al., 2007). This might be since the optimal concentration of SDBS in the mixed sludge is not the same of only WAS.

Table 4. VFA production for different pretreated WAS.

Sludge	pH	T (°C)	Pretreatment method	VFA production	Fermentation time (days)	References
WAS	uncontrolled	20	-	76.7 mg COD/g VSS	12	Zhao et al., 2015
PS	uncontrolled	21	-	85 mg COD/g VSS	6	Ji et al., 2010
WAS	10	20	-	256.2 mg COD/g VSS	8	Yuan et al., 2006
WAS	9	35	-	298 mg COD/g VSS	5	Zhang et al., 2009
WAS	8	55	-	368 mg COD/g VSS	9	Zhang et al., 2009
WAS	6	35	Heat-alcali (90 C and pH 12, 2h)	220 mg COD/g VSS	-	Liu et al., 2009

WAS	9,5	20	500 mg/L sulfite	324.8 mg COD/g VSS	4	Liu et al., 2020
WAS	uncontrolled (inicial pH 7)	20	1.8 mg FNA/L	185.7 mg COD/g VSS	6	Li et al., 2016
WAS	10	20	1.54 mg FNA/L	370.1 mg COD/g VSS	2	Zhao et al., 2015
WAS	uncontrolled (inicial pH 7)	20	1.54 mg FNA/L + SDBS (0.02 g/g dry sludge)	334.5 mg COD/g VSS	4	Zhao et al., 2016
WAS	uncontrolled (inicial pH 7)	20	1.54 mg FNA/L + APG	354.6 mg COD/g VSS	5	Liu et al., 2018
WAS	uncontrolled	21	SDBS [d] (0.02 g/g dry sludge)	240.5 mg COD/g VSS	6	Jiang et al., 2007
PS + WAS	uncontrolled	21	SDBS: 0.02 g/g TSS.	173,9 mg COD/g VSS	6	Ji et al., 2010
WAS	uncontrolled	37	70 °C (during 1 h) + SDBS of 0.01 g/g TS	320 mg COD/ g VS	4	Wan et al 2020
WAS	uncontrolled	30	Rhamnolipid dosage 0.02g/g dry sludge	311 mg COD/g VSS	5	Huang et al., 2015
WAS	initial pH 10	35	RL (0.2 g RL/g TSS)	378 mg COD/g VSS	3	He et al., 2016
WAS	uncontrolled (inicial pH 6)	25	0.67 mg FNA/L + RL (0.04 g/g TSS)	352.62 mg COD/g VSS	3	Wu et al., 2017
WAS	10	20	Ultrasonic(frequency of 20 kHz, energy, density of 1.0 kW/L, 10min)	445 mg COD/g VSS	3	Yan et al., 2010
WAS	uncontrolled	20	Ultrasonic(energy density of 1.0 kW/L, 10min)	196.7 mg COD/g VSS	0.5	Yan et al., 2010

WAS	pH 6	35	Ultrasonic(28 kHz and 60 minutes) – pH 12	230 mg COD/g VSS	-	Liu et al., 2009
WAS	uncontrolled	20	nano zero-valent iron (5.0 g/L) addition	154.8 mg COD/g VSS	4	Luo et al., 2014
WAS	uncontrolled	50	SDS+ME [b]	240.8 mg COD/g VSS	7	Luo et al., 2011
WAS	uncontrolled	50	ME [c]	211.7 mg COD/g VSS	6	Luo et al., 2011

FW and the mixed substrate for fermentation can also be pretreated although is less studied. In Figure 5 it is shown the results of Farouk et al. (2019) and Moretto et al. (2019). Farouk et al. (2019) did several pretreatments in the WAS: heat-alkali pretreatment (HA), heat pretreatment (H) and chemical pretreatment (C). In addition, they did these three pretreatments at various pHs: 5, 6 and 7. They found that the one that gave the highest VFA productivity of all was pH 6 with C pretreatment (55.44 g/L). On the other hand, at pH 7 the pretreatment that gave the best result was H pretreatment (50.25 g/L). In this graph, we can also see that the productivity of pH 5 (were 21.07 g/L, 21.78 g/L and 23.63 g/L for HAT, HT, and CT pretreatments, respectively) is very low compared to that of pH 6 or 7.

At pH 5, there is great more acid acetic than the other fermentation at different pH, and the order of the pretreatments with more acetic is the following: C>HA>A. At contrary, at pH 6 were a low composition of acetic acid and the composition of butyric increased. The order of the pretreatments with more butyric acid is the same (C>HA>A). At pH 7, the distribution of VFA were not affected by the pretreatment.

Moretto et al. (2019) WAS + 30% OFMSW with thermal pretreatment. As we can see in Fig. 3XX, when the substrate was pretreated, the production and yield of VFA was increased at fermentation with initial pH 7 and 10. When the initial pH was 5, the pretreatment did not increase significantly neither the yield nor the production of VFA.

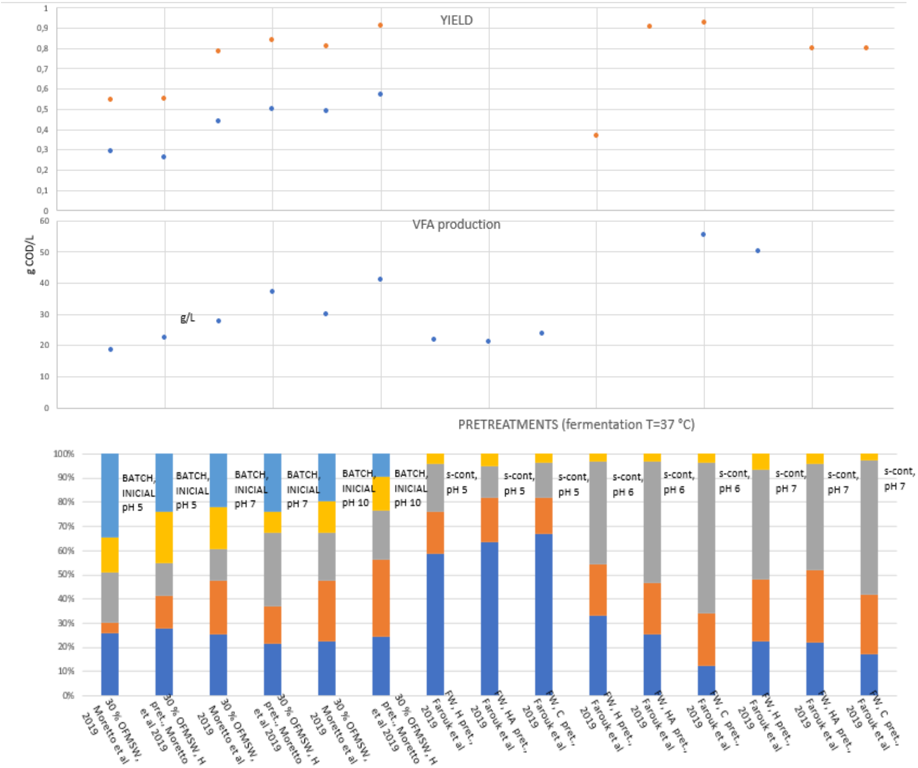

Figure 5 – Fermentation results of pretreated FW and WAS+OFMSW.

3.5. Parameters influencing VFAs production

There are several operational parameters that affect VFA production in the anaerobic fermentation of a reactor, among them: pH, temperature (T), organic loading rate (ORL), hydraulic retention time (HRT). Many researchers are investigating the production of VFA by anaerobic fermentation, but the fermentation of organic wastes for the production of VFA is still a little systematized field and a review of all the research done to see which are the optimal operational parameters for the different substrates is still lacking. This work aims to contribute to this end, by comparing the results of different investigations. A comparative analysis has been made for different temperature ranges (20-25 °C, 30-40 °C, 45-55°C) and pH (5-6.5, 6.5-8, 8-10).

Effect of pH and temperature for mono-fermentation and co-fermentation of PS, WAS and FW at working temperature of 20-25 °C

The pH is one of the operating parameters with the most influence on the yield of the acidogenic process. The influence of pH is not independent of the other variables, but also depends on the raw material and temperature. In Figure 6, the results of various researchers at different pH and at room temperature (20-25°C) are shown.

Figure 5 shows that VFA production is higher in the PS than in the WAS, as expected given the higher biodegradability of the PS. It can also be seen that VFA production is higher at pH=5 (0.8027 g/L) than at pH=6 (0.55306 g/L) for the fermentation of WAS. In contrast, in the case of PS the production of VFA is similar in both pH values (namely 3.390 and 3.361 g COD/L for pH=5 and 6, respectively). Regarding the distribution of the produced VFAs in WAS fermentation, at pH 5 there is a high proportion of valeric acid which, at pH=6 decreases to increase the proportion of acetic and propionic acid. In PS fermentation, at pH=5 the main product is acetic acid and at pH=6 the main product is butyric acid.

The fermentation of FW gave a higher production of VFA due its high biodegradability, but it is not possible to compare the results between several references because of its heterogeneity and the different conditions and reactor configuration (for the selected references in Figure XXX working with FW mono-fermentation, semi-continous and leach-bed reactors). In the references selected, the VFA yield of the leach-bed reactor (Xiong et al., 2019) were higher than the yield of the fermentation of the semi-continous reactor (Lim et al., 2008). Also, the fermentation in the leach-bed reactor produced a huge amount of butyric acid and did not produce valeric acid, while the fermentation in semi-continous produced principally propionic acid.

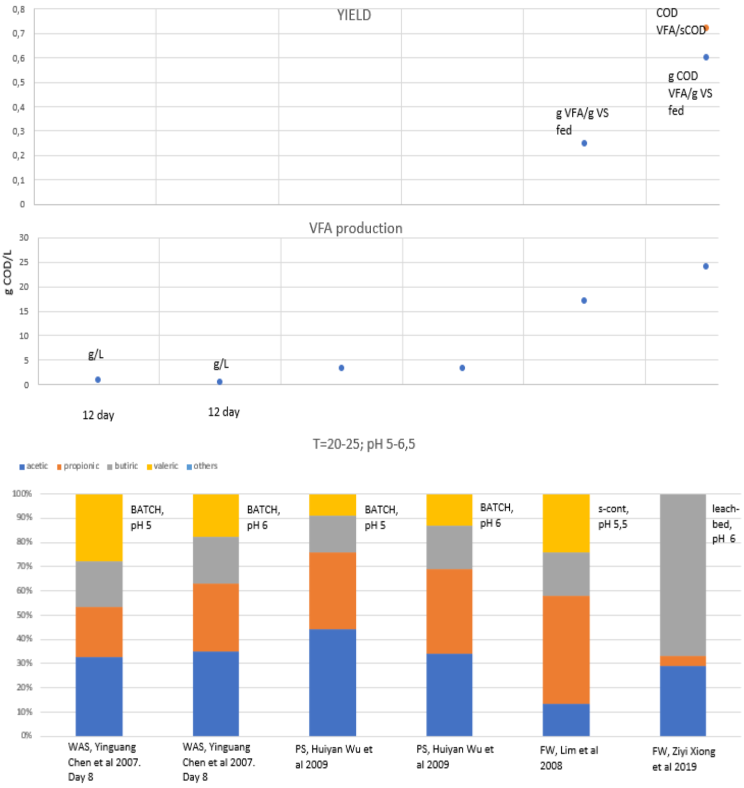

Figure 5- VFA production and composition when using WAS, PS and FW mono-fermentation at a working temperature of 20-25 °C and pH in the range of 5.0-6.5. Units are indicated on the graph when they are different from each other.

In Figure 6, the results of different reports between pH 6.5-8 and temperature 20-25 °C are represented, where it is observed that VFA production at pH=8 is higher than at pH=7, for the case of WAS. On the contrary, in the case of PS it is the other way around. Moreover, it is also clear that PS produces a higher amount of VFA as it is more biodegradable. Regarding the composition of VFAs, acetic acid predominates in the fermentation of WAS. In contrast, according to Wu et al. (2009), propionic acid predominates in PS fermentation whereas according to Ahn et al. (2006) recorded that the predominant VFA is acetic acid. This difference could be because the characteristics of primary sludge depend on seasonal variation due to the degree of refractory materials flowing in the sewage system (Ahn and Choi, 2004).

The FW fermentation (Xiong et al., 2019) gave a lower yield in terms of COD_{VFA}/sCOD than the fermentation of PS (Ahn et al., 2006). Other difference is that the FW fermentation produced a higher percentage of butyric and a lower percentage of propionic in the total distribution of VFA, and it did not produce valeric acid.

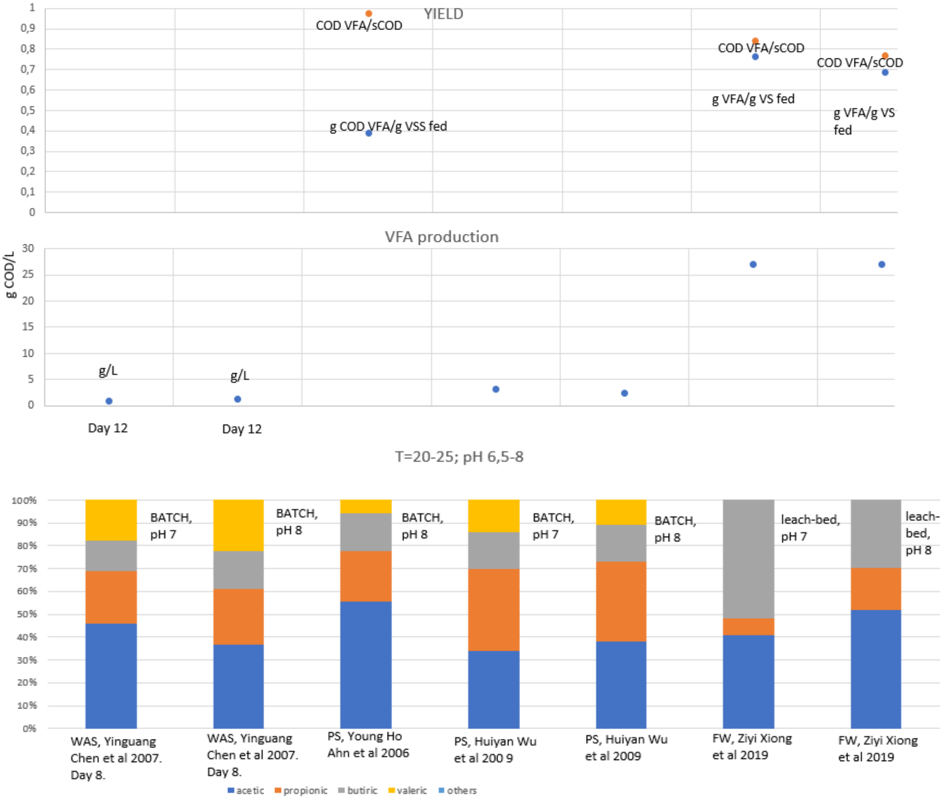

Figure 6- VFA yield, production and composition for WAS, PS and FW mono-fermentation at T=20-25 °C and pH=6,5-8. Units are indicated on the graph when they are different from each other.

In Figure 7, it is observed the effect of pH in the range of 8-10 when working with WAS or PS mono-substrate at temperatures between 20 and 25 °C. On the one hand, this Figure shows that the production of VFA in WAS fermentation is higher at pH 10 (2.770 g/L) than at pH 9 (1.465 g/L). On the other hand, it is seen that VFA production in PS fermentation is higher at pH 9 (2.536 g COD/L) than at pH 10 (1.693 g COD/L). Moreover, contrary to the other pH, it is seen that at this pH the production of VFAs is higher for WAS fermentation than for PS fermentation. This can be concluded, even if the data are in two different units (g VFA/L and g COD/L), because in fact, if we could convert from g/L to g COD/L the VFA production in g COD/L would be even higher than in g/L since all VFAs have the relation (COD substrate/substrate mass) higher than the unity.

In the case of PS, the composition of VFAs remains practically constant in both pH, whereas in WAS fermentation, at pH 10 acetic acid increases and butyric and valeric acid decreases.

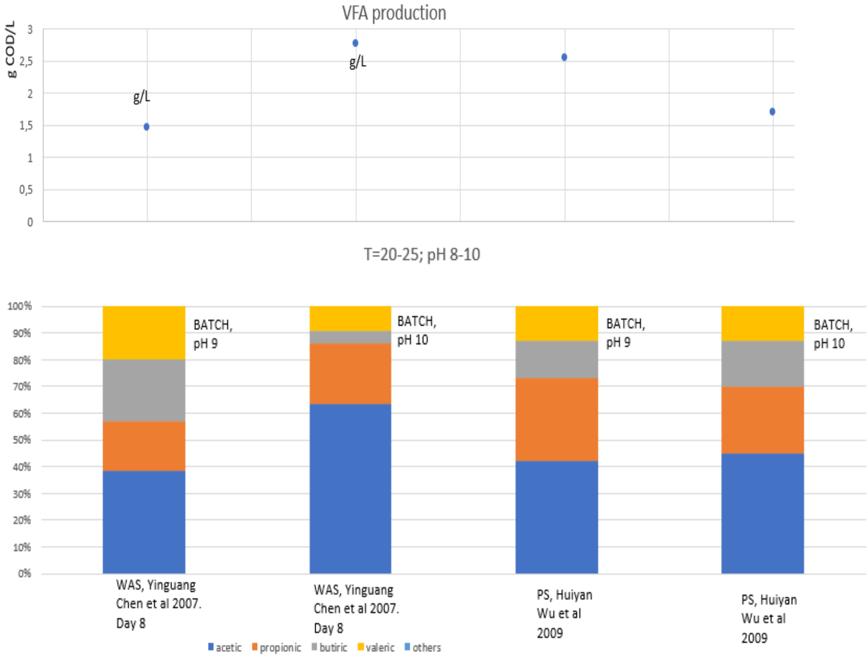

Figure 7- VFA production and composition for PS and WAS mono-fermentation at T=20-25 °C and pH=8-10. Units are indicated on the graph when they are different from each other.

Making an overall assessment of how pH influences the temperature range 20-25 °C; we can say that in the case of PS fermentation the optimum pH is the acidic. In fact, Wu et al. (2009) performed experiments from pH 3 to 11 and it turned out that the optimum pH is 3. They also saw how as the pH increased, the production of VFA decreased. On the other hand, in WAS fermentation, according to Chen et al. (2007), the optimum pH is 10. As the pH increases, production increases. Although at pH 11 the production decreases with respect to pH 10, because are unfavorable condition for the bacteria. Also, these authors demonstrated that the sludge hydrolysis was in the following order: alkaline>acidic>(neutral and blank test), and between pH 6.0 and 11.0 the sludge hydrolysis increased with pH. In both studies, the main VFA was acetic acid and as the pH increased, it also increased (except for pH=8 and pH=9 in the WAS fermentation, which decreases). Regarding the composition of the other VFA a not clear trend can be pointed out.

Yuan et al. (2016) also reported that pH 10 improved VFA production, but they stated that this was not so much due to better hydrolysis but because of better microbial activity at that pH. In contrast, Yu et al. (2008) demonstrated that the biotic effect was not the leading cause of the VFA improvement. Indeed, their result showed that during 20 days of fermentation, the total VFA production at pH 10.0 was from 2 to 34 times higher than that at pH 5.5. However, the enzyme activities (i.e. protease, a-amylase, alkaline phosphatase and acid phosphatase) at pH 10 were all lower than pH 5.5. Therefore, the VFA improvement may be mainly driven by the quantity of soluble organic matters which were easily degradable and available to the VFA producers.

In the case of FW fermentation on a leach bed reactor, Xiong et al. (2019) concluded that pH 7 (0,76 g COD_{VFA}/g VS fed) and 8 (0,69 g COD_{VFA}/gVS fed) increased the production of VFA respect pH 6 (0,60 g COD VFA/g VS fed). This is congruent with Li et al. (2018) that reported that pH 7 (0,42 g COD_{VFA}/gV S fed) and 8 (0,46 g COD_{VFA}/gVS fed)also enhanced the production of VFA respect pH 6 (0,36 g COD_{VFA}/g VS) fermentation of potatoe waste in a leach-bed reactor.

Feng et al. (2009) co-fermented WAS and rice at 21 °C at different pH (from 4 to 11). They reported that the optimal pH to produce VFA was pH 8. At this pH, the consumption of the substrate protein, the activity of protoase and the VFA production was better for the co-fermentation than the mono-fermentation of either WAS or rice. It was reported that co-fermentation increased the activity of the protease hydrolyzing more protein of the sludge and enhancing the production of VFA. Regarding the distribution of VFAs, these authors detected that the acetic was the main component in each pH except at pH 6-9 that was propionic.

Effect of pH for mono-fermentation and co-fermentation of PS and WAS at working temperature of 30-40 °C

In Figure 8, are represented the different data of literature between the pH range of 5-6.5 and T range of 30-40 °C. For mono-fermentation of sludge, there are only the results of Conca et al. (2020) fermenting cellulosic primary sludge.

In this Figure, it is clearly seen that the VFA yield of co-fermentation increased with the addition of FW and it reached the maximum at 81.3% FW and 16.7% WAS (Wu et al., 2016) (0.867 g COD VFA/ g VS fed) compared to the others. This is because they optimized the VFA production following an experimental equation (Eq. 14), where Y is the yield of VFA (mg COD/g VS fed), A is the relation FW/ES; B is the solid retention time (SRT (d)), C is the organic loading rate (OLR (g VS/L d)) and D is the temperature T (°C).

$$Y = -5672.72 + 931.10A + 262.83B + 233.67C + 104.19D - 6.45AB + 3.38AC + 2.17AD - 2.56BC + 0.54BD + 0.52CD - 95.78A^2 - 15.58A^2 - 14.88C^2 - 1.50D^2 \tag{14}$$

This maximum yield of 0.867 g COD VFA/ g VS fed was recorded at the following conditions: FW/WAS 5, SRT 7 d, OLR 9 g VS/L d and T 40 °C. The pH was uncontrolled and the buffer capacity of the system maintained the pH between 5.2 and 6.4. Moreover, Wu et al. (2016) reported that co-fermentation system enriched hydrolytic and acidogenic bacteria by 44.32% and inhibited the growth of methanogens simultaneously.

For the mono-fermentation of FW or OFMSW, Jiang et al. (2013) saw how from pH 5 to pH 6, VFA productivity increased greatly (from 17.08 g/L to 39.46 g/L). Moreover, these authors also concluded that at pH 6 there is a slightly higher yield than at pH 7 (37.09 g/L). Regarding the distribution of VFAs, operating at pH 6 drastically decreases acetic acid and increases butyric acid. Lim et al (2008), when fermenting FW with a reactor operating in semi-continous conditions, obtained similar results: VFA production and yields at pH 6 (25 g/L; 0.365 g VFA/ g VS fed) were higher than at pH 5.5 (23.5 g/L; 0.345 g VFA/ g VS fed) and pH 5 (16.5 g/L; 0.24 g VFA/ g VS fed). These results are also congruent with Bermúdez-Penabad et al. (2017), who reported that the production of VFA from the fermentation of tuna waste was higher at pH 6 than 5.

Regarding the VFA composition, Lim et al (2008) results are neither consistent with Jiang et al. (2013) or Bermúdez-Penabad et al. (2017). Lim et al. (2008) concluded that as pH increased, succinate and caproate (others) decreased drastically, while butyric and acetic acid increased. While Bermúdez-Penabad et al. (2017) concluded that at pH 6 the acetic and butyric acid percentage decreased and the valeric and other VFA percentage increased. These differences regarding the distribution of VFA could be related to the specific composition of each substrate and the reactor configuration.

In the study of Yin et al (2016) three substrates (glucose, peptone, and glycerol, representing carbohydrates, proteins, and lipids, respectively) were acidogenically fermented. As we can see, the one that gave the highest VFA production was the mixture (38.2 g COD/L), then glucose (38.2 g COD/L), peptone (32.2 g COD/L), and glycerol (31.1 g COD/L). The yields follow a similar order: mixture (0.665 g COD VFA/ g COD VS_0), glucose (0.659 g COD VFA/ g COD VS_0), peptone (0.596 g COD VFA/ g COD VS_0) and glycerol (0.510 g COD VFA/ g COD VS_0). The fact that mixing the substrates increased VFA production, indicates that synergistic effects between microorganisms improved acidogenic fermentation. Regarding the distribution of VFAs, butyric, acetic, and propionic acids were the main products for glucose, peptone, and glycerol, respectively. In contrast, the mixture has about the same composition of each.

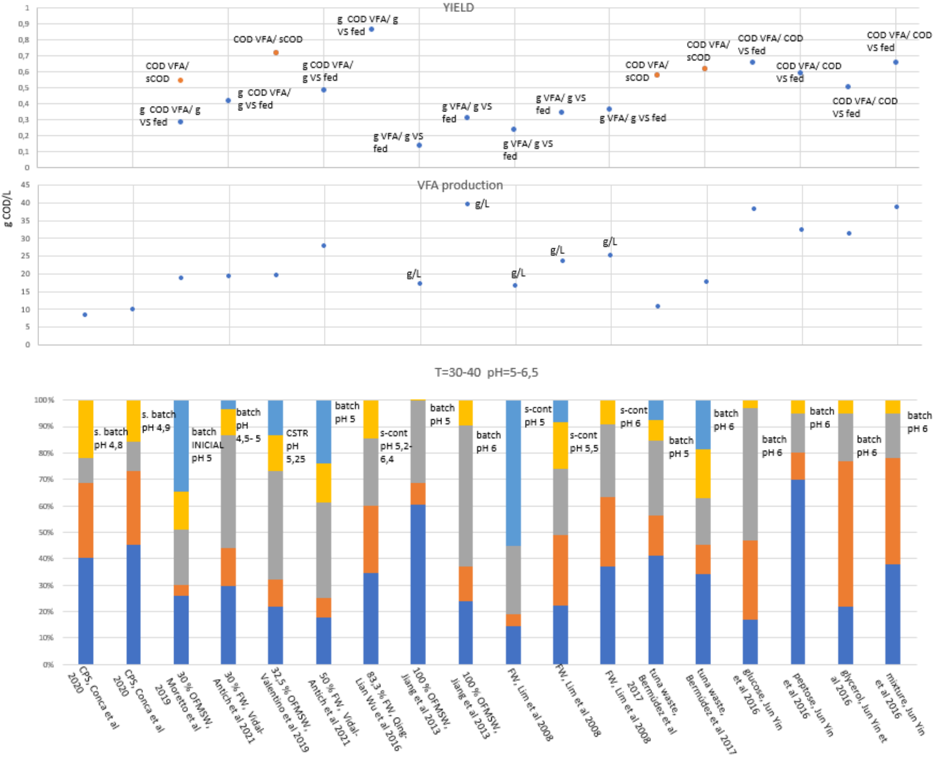

Figure 8 - VFA yield, production, and composition at T=30-40 °C and pH=5-6,5 for WAS, PS, FW mono-fermentation and co-fermentation. Units are indicated on the graph when they are different from each other.

In Figure 9 are represented the results of various research articles between pH 6.5-8 and T 30-40 °C. As stated in this figure, co-fermentation of 30% OFMSW and 70% WAS (Moretto et al, 2019) gaves much better results in VFA yield basis (g $\mathrm{COD_{VFA}}$/ g VS fed) with respect to only WAS or only FW or OFMSW. The optimal percentage of OFMSW at this range of pH and T is about 30% because increasing the percentage to 50% of FW + 50% THS (thermal hydrolyzed sludge) (Gong et al., 2021), lowered VFA production and yield.

Regarding VFA composition, it is observed that acetic acid composition decreases in co-fermentation compared to mono-fermentation of WAS or FW. Although, when the percentage of FW is increased in WAS, the fermentation produces more acetic acid. Similarly, butyric acid tends to increase its proportion in the VFA mixture as the percentage of FW in the raw material increases. Valeric acid, unlike the previous two, remains constant regardless of the feedstock. Finally, no clear trend is observed for propionic acid.

Batch fermentation of PS (Ahn et al., 2015) provided a higher VFA yield than WAS fermentation. It could also be observed that when the same PS was femented in a semi-continous reactor, the VFA composition changed. Indeed, PS in semi-continuous conditions did not produce valeric acid and instead produced more butyric acid (Ahn et al., 2015).

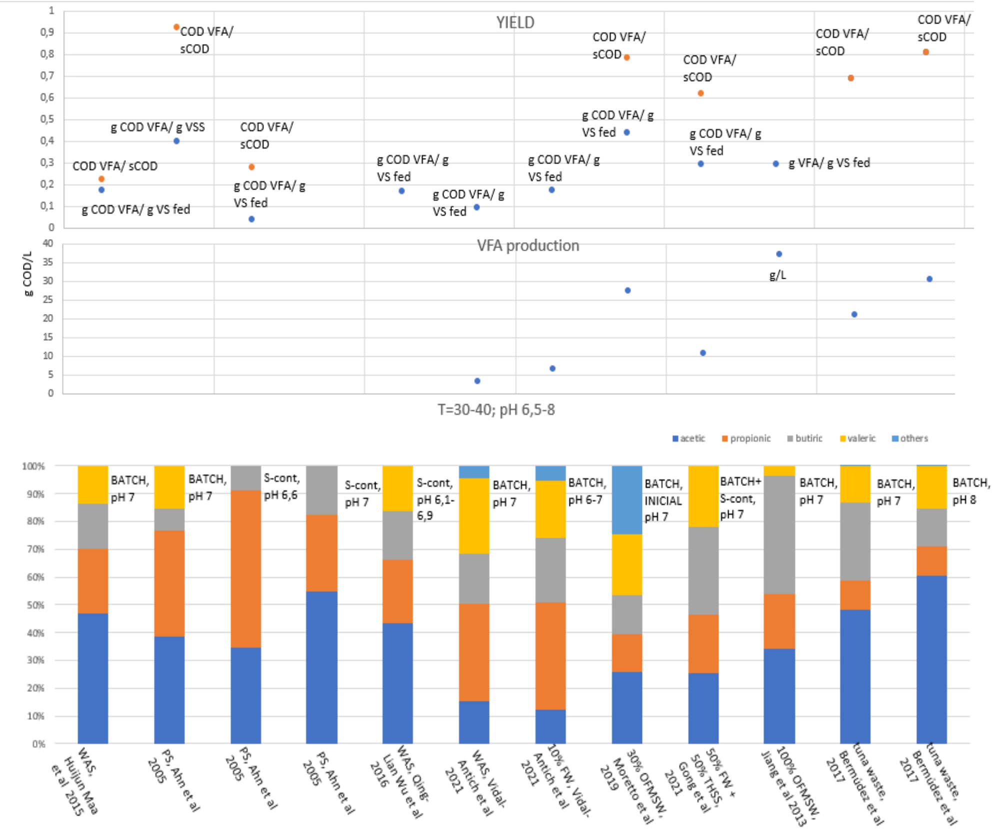

Figure 9- VFA yield, production and composition at T=30-40 °C and pH=6,5-8. Units are indicated on the graph when they are different from each other.

Figure 10 summarizes the fermentation results on the range of pH 8-10 and T 30-40 °C. Again, we can see how co-fermentation has a higher yield and a higher VFA production than FW or WAS fermentation. The optimum value is around 30 % OFMSW, since from 50% FW the yield and VFA production already decreased.

It should be noted that the difference between WAS, and PS fermentation was more pronounced for the VFA distribution (PS does not produce valeric acid and produced more butyric acid) than in the yields, that are similar.

Regarding the composition of the VFA, acetic acid predominates except for the 30% OFMSW mixture. In this one, propionic acid and the other minority VFAs increased.

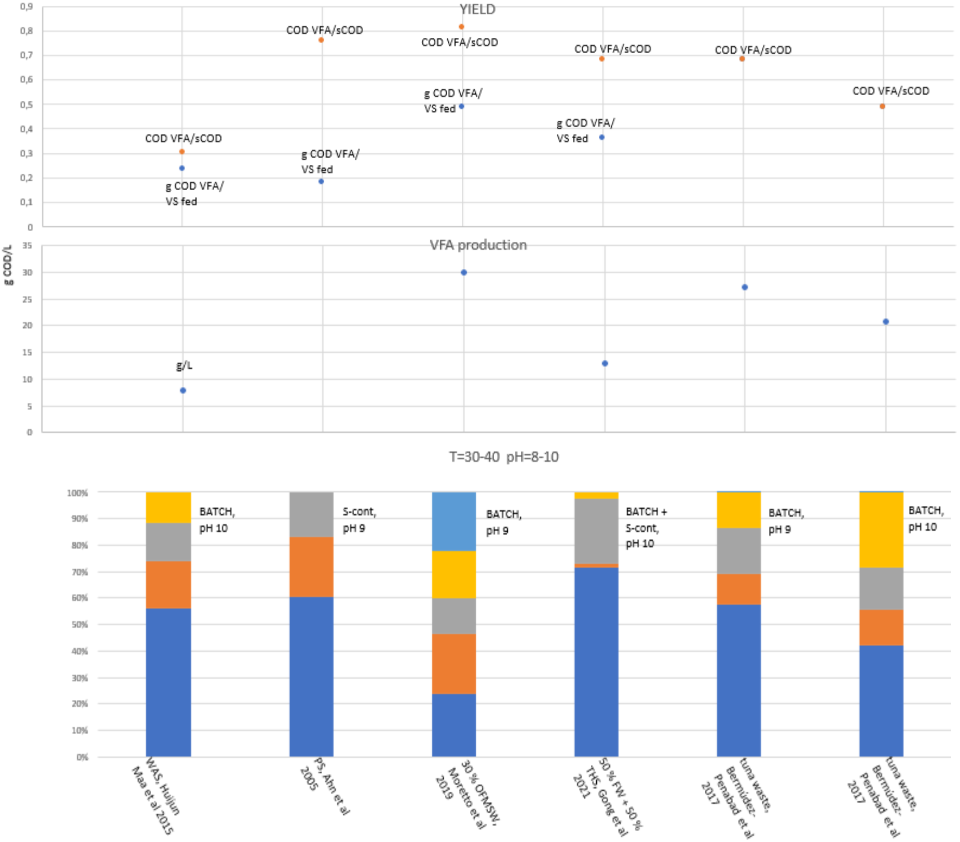

Figure 10. VFA yield, production and composition at T=30-40 °C and pH=8-10 for mono-fermentation and co-fermentation of WAS, PS and FW. Units are indicated on the graph when they are different from each other.

Making an overall assessment of the influence of pH at this temperature range (30-40 °C), for WAS the optimum pH value is 9-10. This is also congruent with other reports like Liu et al. (2012) that reported that among pH 3, 5, 7, 9, 11 and 12; pH 9 was the one that presented higher solubilitzation, hydrolysis and VFA production of protaicous sewage sludge while it inhibited the activity of methanogens. Also, Jie et al. (2012) concluded that pH 10 was the best for WAS fermentation. Moreover, Ma et al. (2019) reported that dissolved organic matter at pH 10 results had the highest molecular diversity and generation or degradation of easily degradable substrates for the sewage sludge fermentation.

However, when WAS was pretreated with an HA pretreatment, pH=7 is the optimum (Ma et al 2015), because the optimum pH for acidogenesis is 7 and the optimum pH for the hydrolysis is 10 (Ma et al 2015). For the mixed sludge (50% PS + 50% WAS), Jankowska et al. (2015) reported that pH 10 was the one with higher VFA yield at long retention time while at low retention time, the pH with higher VFA yield was pH 4.

In the Figures, it can also be seen that the production of VFA is higher for the fermentation of WAS at mesophilic temperature than at room temperature (7,78 g/L at pH 10 and 35 °C (Ma et al., 2015) and 2,77 g/L at pH 10 and 21°C (Chen et al., 2007) both in batch mode). This is congruent with Feng et al. (2009), that studied the effect of temperature on the VFA production from WAS under alkaline condition (pH = 10). They observed significant VFA increase when temperature was raised from 10 to 30 °C. Also, Yuan et al. (2011) reported an increase of VFA yield on the fermentation of WAS when increasing the temperature from 4 °C to 14 °C and 24,6 °C.

For a WAS and OFMSW mixture of 30% in OFMSW, the optimum pH is again basic (pH 9). If the mixture is 50% FW and the sewage sludge (SS) has undergone thermal pretreatment, the optimum pH is no longer basic but neutral (pH=7) (Gong et al 2021), that is congruent with the results of Ma et al. (2015). But the best yield reported was at pH uncontrolled (5.2-6.4) and 81,3 % of FW and WAS (Wu et al., 2016), that was incredibly high (0.867 g COD VFA/ g VS fed). Finally, for 100% FW comparing between pH 5, 6 and 7 it has been shown that the optimum pH value is 6 (Lim et al., 2008; Farouk et al., 2019; Jiang et al 2013). And in the case of tuna waste, the optimum pH is basic (pH 9). Regarding the composition of VFAs, it can also be observed that as the pH rises, the general tendency is to increase the acetic acid composition.

Effect of pH for PS, WAS and FW mono- and co-fermentation at working temperature of 50-55 °C

Figure 11 depicts the VFA yield, ratio of VFA with respect to soluble COD, VFA concentration at the effluent and VFA distribution for acidogenic fermentation or co- fermentation studies under thermophilic temperature (50-55 °C) at acid pH (5-6.5). As stated in this Figure, the yield of 30% OFMSW (0.59 g COD VFA/ VS fed) is higher than OFMSW fermentation, as it occurred in mesophilic temperature. Moretto et al. (2019) gives an extremely low yield since pH 5 is the initial pH and then it changes to pH 4.2 and at this T and pH the fermentative bacteria were strongly inhibited (Moretto et al., 2019). Regarding VFA composition, butyric acid is the major component in all cases except for Moretto et al. (2019).

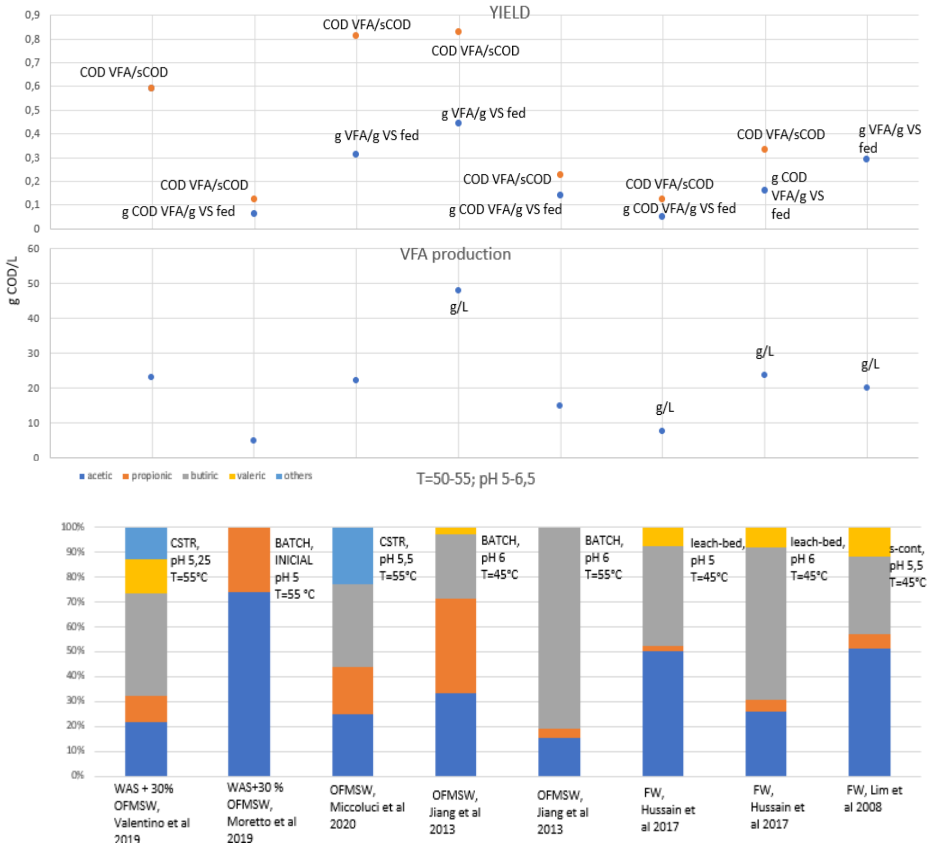

Figure 11. VFA yield, production and composition for WAS and FW mono-fermentation and co-fermentation at T=50-55 °C and pH=5-6.5. Units are indicated on the graph when they are different from each other.

Figure 12 represent the VFA yield, ratio of VFA with respect to soluble COD, VFA concentration at the effluent and VFA distribution for acidogenic fermentation or co- fermentation studies under thermophilic temperature (50-55 °C) at neutral pH (6.5-8). As it can be seen in this Figure, the highest yield at this temperature is that of 50% FW (0.305 g COD VFA/ g VS fed) and not that of 30% in OFMSW (0.22 g COD VFA/ g VS fed).

In addition, it is also seen how the batch operation gives better yields than the same conditions in semi-continuous (pH=7, the same PS). Even the compositions of VFAs change: in semi-continuous, acetic acid increases a lot and the other VFAs decrease. Moreover, we can deduct from the figure that butyric acid increases and acetic acid decrease as the proportion of FW and/or OFMSW in the fermentation feedstock increases.

Regarding OFMSW or FW fermentation, it gave a low proportion of acetic acid while the proportion of butyric and valeric acid increased.

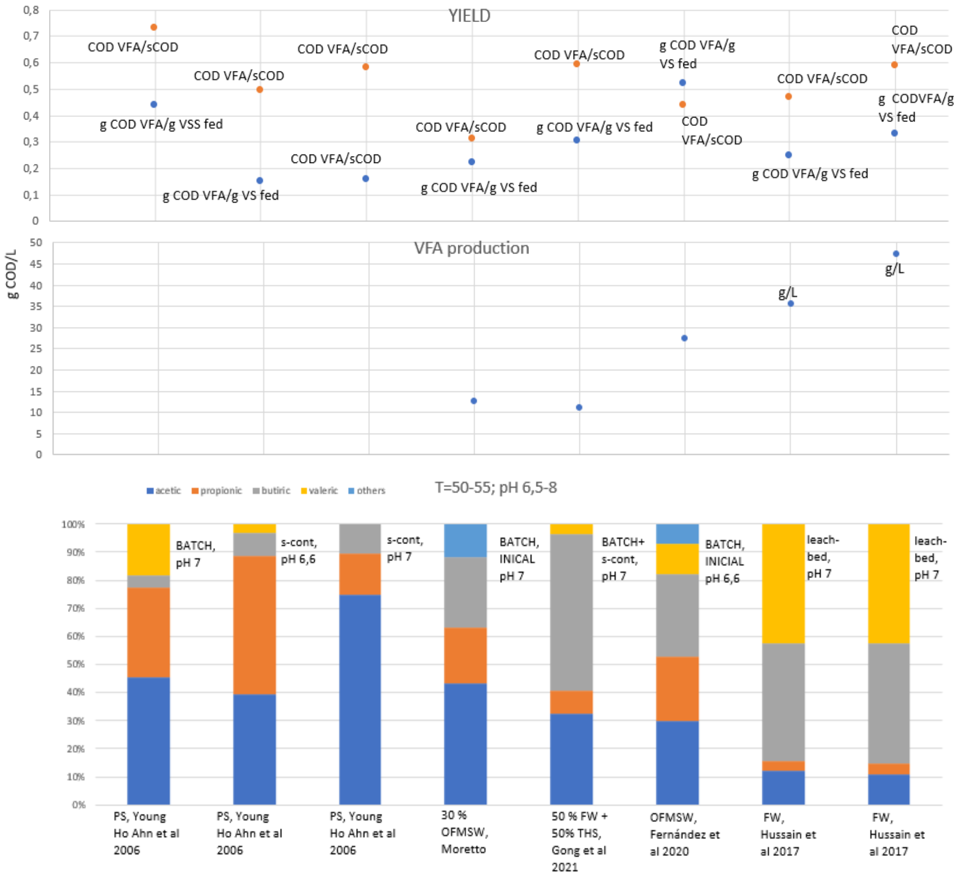

Figure 12 -VFA yield, production and composition at T=50-55 °C and pH=6.5-8. Units are indicated on the graph when they are different from each other.

In Figure 13 there are the fermentation results on the range of pH 8-10 and temperature 45-55 °C. As we can see in this figure, in this basic pH range, the optimum yield is 30% OFMSW. In fact, the yield of 50% OFMSW is even lower than PS fermentation. This is since strong alkaline pH conditions have the opposite effect on VFAs/SCOD at different temperatures, enhancing acidogenic efficiency at 35 °C and decreasing acidogenic efficiency at 55 °C (Gong et al., 2021).

Regarding the compositions of VFAs, acetic acid predominates except for Moretto et al. (2019) and this is because pH 9 is the initial pH and then the same system lowers the pH when producing VFA given its acidity.

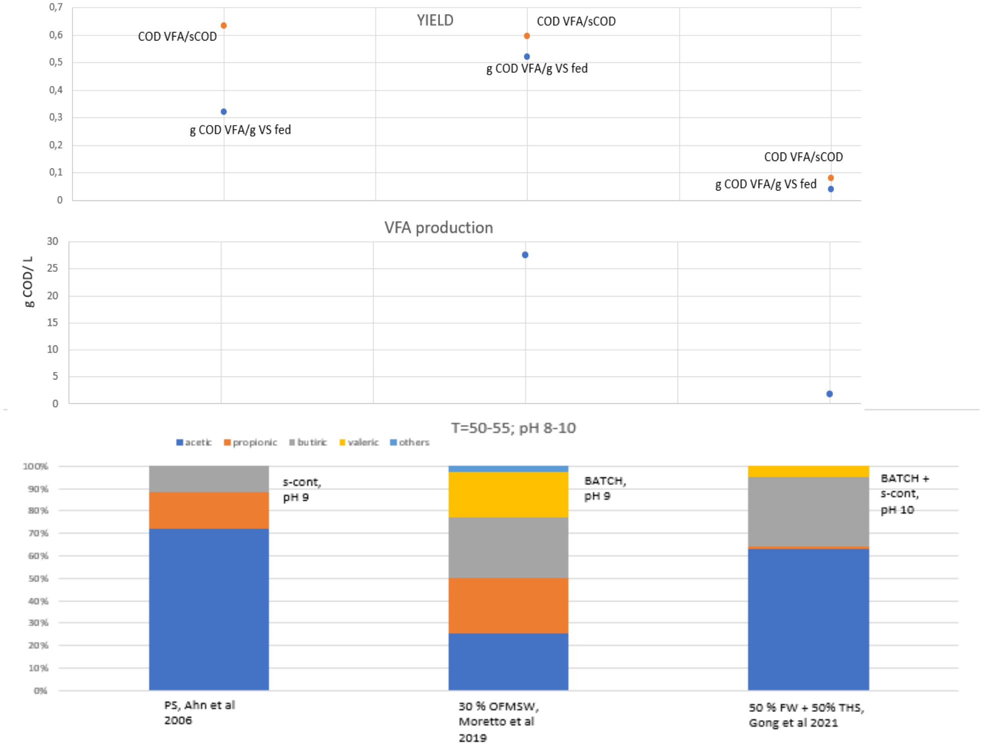

Figure 13 -VFA yield, production and composition at T=50-55 °C and pH=8-10. Units are indicated on the graph when they are different from each other.

In Ho Ahn et al. (2006) the results clearly showed that the thermophilic and higher pH conditions allow higher solubilization of PS in the reactor. However, the higher alkaline condition at pH 11 -that is not represented in this Figures because has low yields- prevents solubilized products from converting to organic acids, showing that the optimal pH condition for acidogenesis in the system was pH 9 at thermophilic conditions (0.32 g COD VFA/ VS fed). In mesophilic conditions, the best yield was obtained at pH 7 (0.18 g COD VFA/ VS fed). With respect to VFA compositions, in both mesophilic or thermophilic fermentation propionic acid was the largest VFA. Also, they observed that with increasing temperature the proportion of propionic acid decreased while acetic acid increased.

Zhang et al. (2009) also reported that thermophilic temperature increase the production of VFA in alkali pH. In fact, they did several experiments with different pH (from 4 to 11) under both thermophilic and mesophilic conditions. They concluded that in both temperatures the alkali pH enhanced the production of VFA. The best yield at 35 °C was 0.298 g CODVFA/g VS fed at pH 9, while the best yield at 55 °C was 0.368 CODVFA/g VS fed at pH 8. This might be due to the fact that hydrolysis is enhanced at thermophilic temperature and alkali pH, while acidogenesis might be enhanced at mesophilic temperature and neutral pH. The fermentation time for the mesophilic and thermophilic reactor were 5 d and 9 d, respectively in Zhang et al. (2009). So, although thermophilic temperature increases the production of VFA the fermentation time is almost the double of the one for mesophilic temperature. Regarding the distribution of VFA, they saw that at both thermophilic and mesophilic temperatures the acetic acid was the main VFA, and as the pH increased its proportion in the total VFA also increased.

These results are in line with Chen et al. (2019), because their study revealed that at thermophilic temperature (55 °C) the maximum acidification was obtained at pH 8.9 with the best production of VFAs recorded (423.22 ± 25.49 mg COD/g VSS), while the maximum hydrolysis efficiency was observed at pH 9.9 (an hydrolysis extent of 42%). In fact, pH 9.9 favored the hydrolysis of high molecular weight (HMW) dissolved organic matter (DOMs) while it did not enhance the acidogenesis of low molecular weight (LMW) DOMs.

In Hao et al. (2015) study, the total production of VFA greatly increased at 55 °C (4,2679 g COD/L) in the fermentation of dewatered sludge compared to 35 °C (0,4201 g COD/L). They also observed that the activities of both α-glucosidase and protease increased during acid-forming phase in thermophilic batch test. In addition, temperature increased the abundances of Clostridiaceae, Microthrixaceae and Thermotogaceae, which could facilitate the process of hydrolysis and acidification. The relative abundance of homoacetogens also increased in thermophilic condition, which explain that in the distribution of VFAs, thermophilic fermentation led to higher percentages of acetic acid and iso-valeric acid compared to mesophilic fermentation.

Xiong et al. (2012) studied the influence of the temperature (40 °C, 50 °C and 60 °C) in WAS hydrolysis and solubilization. They observed that the hydrolysis can be well adjusted to an exponential equation at 40 °C and 50 °C, but not at 60 °C. This is due to the fact that this adjustment can only be done when hydrolysis is the rate-limiting step (Mahmoud et al., 2014). At 60 °C, there were a huge amount of sludge hydrolyzed but the acidogenic bacteria were strongly inhibited, so the rate limiting step were the acidogenesis. For this reason, the maximum production of VFA were at 50 °C.

With the feedstock with a volume percentage of 65-70 % in WAS and 25-30 % in OFMSW, Moretto et al. (2019) performed several experiments at different temperatures and pH. At both mesophilic (37 °C) and thermophilic (55 °C) temperatures, they worked at different initial pH: namely, 5, 7 and 9. The conclusion of their study was that at 37 °and at 55 °C the pH with the best results was basic (pH=9). Thermophilic operation did not significantly improve the yield and the production of VFA (27.5 g COD VFA/L; 0.52 g COD VFA/ g VS fed) with respect to mesophilic conditions (30 g COD VFA/L; 0.49 g COD VFA/ g VS fed). The optimum conditions were the feedstock thermically pretreated operating at pH=9 and T=37 °C (41 g COD VFA/L; 0.57 g COD VFA/ g VS fed). With respect to the compositions, at 55 °C the proportion of valeric and other minority VFA decreased significantly, while the others (acetic, propionic and butyric) increased.

Valentino et al. (2019) demonstrated that at pH 5-5,5 thermophilic conditions slightly increased the yield and production of VFA (23 g COD VFA/L; 0.59 g COD VFA/VS fed), but only the mesophilic fermentation process provided a VFA-rich stream (19.5 g COD VFA/L; 0,50 g COD VFA/VS fed) with stable physical-chemical features, with no need of chemicals addition for pH control due to the buffering capacity of the system. Regarding VFA compositions, they remained constant regardless of temperature.

For co-fermentation of 50% FW and 50% SS, Gong et al. (2021) demonstrated that 35 °C+pH 7 (10.62 g COD VFA/L; 0.294 g COD VFA/ g VS fed) , 35 C+pH 10(12.93 g COD VFA/L; 0.36 g COD VFA/ g VS fed), and 55 °C+pH 7 (10.99 g COD VFA/L; 0.305 g COD VFA/ g VS fed) were the optimum conditions for VFA production from co-fermentation of FW and THSS. The basic pH at thermophilic temperature resulted in very low production and yield (1.73 g COD VFA/L; 0.04 g COD VFA/ g VS fed). In fact, when the temperature or the pH was changed, the bacteria associated with VFA production also changed of composition. Regarding VFA compositions, a significant increase of butyric acid can be seen at thermophilic temperature.

Hussain et al. (2017) investigated the effect of pH (5, 6 and 7) in the fermentation of FW at 50 °C. They found that pH 7 gave the best yield (0.247 g CODVFA/g VS fed) than the others (0.158 g CODVFA/g VS fed at pH 6 and 0.047 g CODVFA/g VS fed at pH 5). Once the optimum pH condition was established, an additional leachate recirculation rate of 9 L/d was tested (LBR-7R) and even gave a better result than the previous one (LBR-7, with a recirculation rate of 6 L/d).

Fernández-Domínguez et al. (2020) performed various batch test of fermentation of OFMSW at different temperatures (20, 35, 45, 55, 70 °C) and the initial pH was about 6.5. They found that the best results in terms of yield of VFA were obtained at mesophilic temperature. In fact, the VFA yield at 35 °C was 2%, 6%, 10% and 14% higher than at 55, 45, 20 and 70 °C, respectively.

Finally, with 100% OFMSW as feedstock, Jiang et al. (2013) worked at pH=6 and different temperatures of 35, 45 and 55°C obtaining the following VFA yields, respectively: 0.379 g VFA/ g VS fed; 0.440 g VFA/ VS fed; 0.137 g VFA/g VS fed. So, the optimum temperature found was 45 °C, a middle point between thermophilic and mesophilic temperature. Like Gong et al. (2021), a large increase in butyric acid was also observed along with increasing temperature. These results are congruent with Komemoto et al. (2009) who reported a solubilization rate of food waste 47.5%, 62.2%, 70.0%, 72.7%, 56.1% and 45.9% at 15, 25, 35, 45, 55 and 65°C, respectively. Also, the production of biogas was better under 45°C and 35°C.

To sum up, it could be concluded that thermophilic conditions significantly increase the solubilization and hydrolyzation of the substrate (Moretto et al., 2019; Gong et al., 2021), although not the production of VFA in all the cases. The VFA yield in PS or WAS fermentation is clearly increased at basic pH (pH=9). In contrast, for the mixture of WAS and OFMSW or the mono-fermentation of OFMSW it is not efficient to work at this temperature. Jiang et al. (2013) showed that an intermediate temperature between mesophilic and thermophilic (45 °C) does increase the VFA yield of OFMSW fermentation. It remains to be seen whether this also applies to the WAS-OFMSW mixture. Regarding the distribution of VFAs, the changes can perhaps be explained by the operational conditions affecting the types of particulates being hydrolyzed and consequently the types of VFA produced (Eastman and Ferguson, 1981). Another possible reason for this VFA distribution change could be the change in microbial population and a shift in yield and the reaction pathways (IWA (2002)).

ORL and HRT

Organic loading rate (OLR) shows the amount of waste, which can be expressed in terms of COD, VS, VSS or DOC, fed into the reactor daily per unit reactor volume. It can be increased by reducing the hydraulic retention time or increasing the substrate concentration.

If the ORL is increased, the sCOD and the VFA production might increase due to the higher content of volatile solid fed. However, the yield in terms of g COD VFA/ g VS fed is always lowered because less VFA is produced for the same amount of VS fed (see Table 5, Table 6 and Table 7). For example, Lim et al. (2008) reported that the VFA concentration produced from food waste increased with the increase of the OLR from 5 g/L/d to 13 g/L/d, but the yield (g VFA/ g VS fed) decreased (see Table 5). Moreover, the operation of fermenter at 13 g/L/d was unstable because the fermentation broth became very viscous and, in addition, it was the only fermenter operation that produced lactic acid.

At a higher OLR achieved through decreasing HRT, lower VFA production could be observed due to lower hydrolysis efficiency (Fang et al., 2020). For example, Banerjee et al. (1999) demonstrated that the total VFA production from PS fermentation decreased from 0.4 to 0.3 g/L, when the OLR increased from 4 to 7 g TS/(L day) by decreasing HRT from 30 to 18 h. Also, in Moretto et al. (2019), when the ORL increased from 7.7 to 11.3 kg VS/m3 d by decreasing the HRT from 6.1 to 4.0 d, the VFA production from the co-fermentation of OFMSW and WAS decreased from 39 to 31 g COD/L. At the same time, the sCOD decreased from 43 to 41 g COD/L.

In addition, if the OLR increases further beyond a certain limit, the fermentation system could collapse due to lactate accumulation and pH drop (Jouany, 2006). For this reason, high rate processes for OFMSW fermentation are considered unsuitable since the easy putrescible matter may lead to a quick VFA production, not properly buffered unless an efficient pH control system is applied (Valentino et al., 2018).

Regarding the distribution of VFA, Lim et al. (2008) reported that the propionic acid; caproate and succinate increases as the ORL increased while the acetic acid decreased. These results are in line with Yu et al. (2002, that reported that under mesophilic conditions, when the OLR increased from 4 to 24 kg COD/(m^3 day), the percentage of propionate increased from 13% to 41% of the total VFA, while the percentage of acetate declined from 40% to 17% in the fermentation of waste water. In contrast, in Jiang et al. (2013) the acetic proportion increased while the propionic acid proportion decreased when the ORL was increased. So, there might not be a clear correlation between the ORL and the VFA distribution. Moretto et al. (2019) also reported that the higher yield of VFA for the cofermentation of WAS and OFMSW was at low ORL (see Table 8).

In conclusion, the optimum of the ORL and HRT parameters is thought to be at low ORL and high HRT.

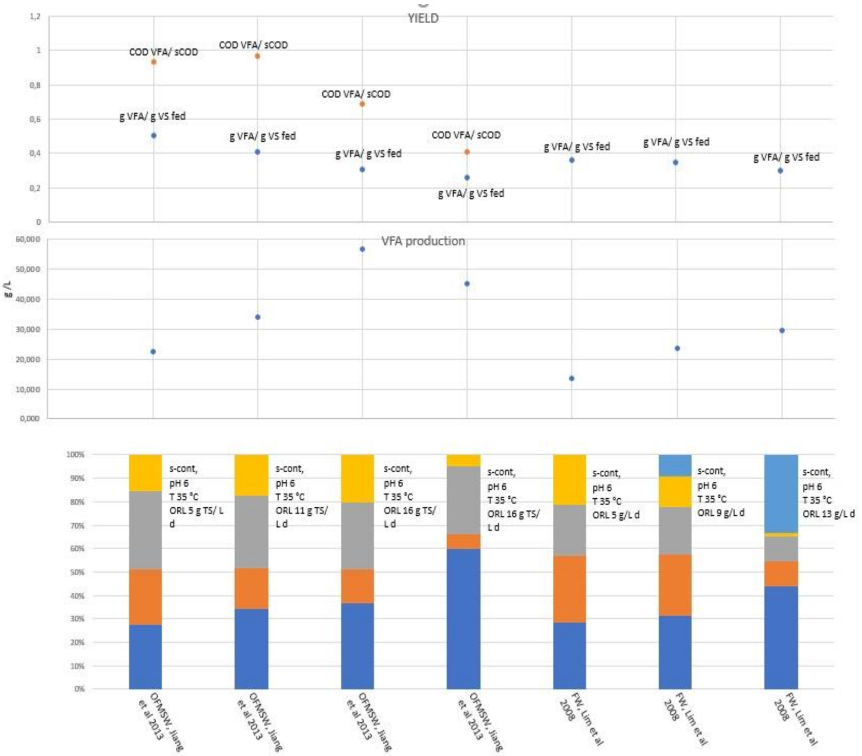

Figure 14 – VFA production, yield and distribution for diferent ORL.

Table 5- Influence of the ORL on the sCOD, VFA production and nitrogen realease (Lim et al., 2008)

OLR (g/L d)	5	9	13
SCOD (mg/L)	26,000–28,000	37,000–38,000	49,500–51,500
TVFA (g/L)	13.0–14.0	23.0–24.0	28.9–30.0
Yield (g VFA/g VS0)	0.34–0.37	0.34–0.35	0.29–0.30
NH_4^+–N (mg/L)	0	7.2–20.2	0–17.2

Table 6- Influence of the ORL on the sCOD, VFA production and nitrogen realease (Lim et al., 2008)

OLR (kg VS/(m3 d))	7.7	9.3	11.3
SCOD (g COD/L)	43	43	41
VFAs (g COD/L)	39	38	31
VFAs (g COD/g VSfed)	0.74	0.71	0.60
VFA/SCOD (%)	91	88	76
NH4+–N (g/L)	1.02	0.87	0.82

Table 7 - Influence of the ORL on the sCOD, VFA production and nitrogen realease (Moretto et al., 2019)

OLR (g TS/L d)	5	11	16 (11th day)	16 (30th day)
SCOD (g COD/L)	22.37	34.22	56.62	45.27
VFAs (g/L)	13.27	21.44	24.93	12.98
VFAs (g/g VSfed)	0.504	0.411	0.306	0.261
VFA/SCOD (%)	93.4	96.8	68.9	40.8
NH4+–N (mg/L)	468.6	750.8	841.67	423.5

REACTOR CONFIGURATIONS

In Figures of section 3.4, results from anaerobic fermentation carried out in different types of reactors have been considered: batch tests, CSTR, semi-continuous and leach-bed reactors. The type of reactor used affects the yield of VFA obtained and the distribution of VFA in the outflow.

Batch tests are usually performed in glass bottles of small volume (about 1 L) that are fed with substrate and sometimes with inoculum before sealing. Then the pH, VFA concentration, sCOD and relevant variables are then monitored by drawing a small sample from the reactor (about 15 mL) each day. Also, the pH can be adjusted if desired.

The CSTRs monitored in this study, are small or medium volume tanks (from 6 L in Moretto et al. (2019) to 200 L or 380 L in Micolucci et al. (2020) and in Valentino et al. (2019), respectively) working at specific operational parameters (pH, T, HRT and ORL). The semi-continous reactors are also tanks but they are fed and drawn-off once a day and not continuously like in the CSTR.

The leach-bed reactor is divided in two parts: the containtment vessel, where are the food wastes (see Figure 15) and the leachate bed where is the inoculum. To ensure a consistent contact between inoculum and food waste, the reactor receives an intermittent leachate recirculation each defined time (for example, it was 10 min in Xiong et al. (2019)). For this reason, the leachate recirculation is a fundamental parameter to optimize the production of this reactor. Hussain et al. (2017) found that the leachate recirculation rate of 9 L/d performed better in terms of VFA yield than a rate of 6 L/d. However, leachate recirculation rates higher than 9 L/d resulted in accumulation of leachate on top of the food waste.

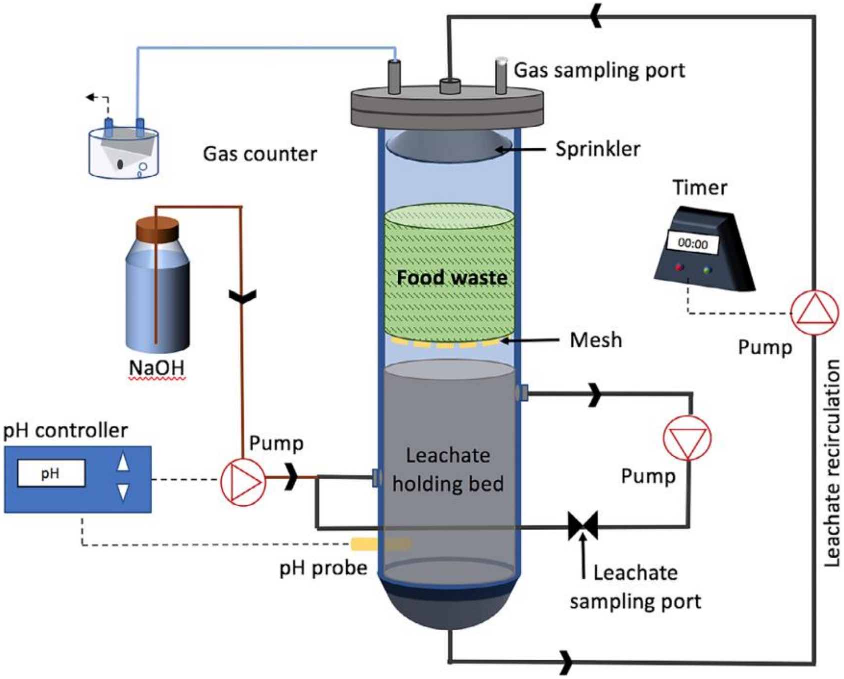

Figure 15 – Operation of a leach-bed reactor (Xiong et al., 2019)

So, depending on the reactor, the performance of the anaerobic fermentation could change. Moretto et al. (2019) did several batch test at different inicial pH to find the optimal pH for their substrate (thermal pretreated mix of 27.5% OFMSW and WAS) at mesophilic temperature (37 °C). The best batch test worked in an initial pH 9. Then, these authors applied these conditions to CSTR with different ORL and HRT (7.7, 9.3 and 11.3 kg VS/m^3 d with an HRT of 6.1 d, 5.0 d and 4.0 d, respectively). The results are shown in Table 3.X, where it is observed that the yield in terms of g COD$_{VFA}$/g VS fed for CSTR operation are better than in batch mode. Moreover, the yields in terms of COD$_{VFA}$/sCOD are equal to the batch test at the optimum ORL (7.7 kg VS/m^3 d). Therefore, this data suggests that the operation in CSTR reactor produces more VFA for VS fed in the reactor. This might be due since CSTR once that has reached the estacionary state, the operational parameters constant does not change during the fermentation.

Table 8 – Results of the acidogenic fermentation in CSTR reactor or batch test (Moretto et al., 2019)

	OLR (kg VS/(m^3 d))	pH	T (°C)	% OFMSW	VFA yield (g COD_{VFA}/g VS fed)	COD_{VFA}/sCOD (g/g)
Batch	-	9 inicial	37	27,5	0,57	0,91
CSTR	7.7	9 inicial	37	27,5	0,74	0,91
CSTR	9.3	9 inicial	37	27,5	0,71	0,88
CSTR	11.3	9 inicial	37	27,5	0,6	0,76

Ahn et al. (2008) performed some batch tests for the acidogenic fermentation of primary sludge and then also operated in a semi-continous reactor. Their results are summarized in Table 9. As stated in this table, both the yield in terms of COD_{VFA}/sCOD and in terms of g COD_{VFA}/ g VSS_{COD} were better for the batch test than for the semi-continuous reactor. So, this data suggests that the operation in semi-continuous could decrease the VFA yield.

Table 9 – Results of the acidogenic fermentation in semi-continous reactor and batch test (Ahn et al., 2008)

	pH	T(°C)	HRT (d)	VFA yield (g COD_{VFA}/g VSS_{COD})	COD_{VFA}/sCOD (g/g)
Batch	7	35	5	0,40	0,926
Batch	7	55	5	0,44	0,731
Semi-continous	7	35	5	-	-
Semi-continous	7	55	5	0,10	0,58

Moreover, the leach-bed reactor results reported in this study are very promising, especially the results of Xiong et al. (2019) that reported remarkably high VFA yields at 22° C.

4. FINAL CONSIDERATION FOR THE IMPLEMENTATION OF A VFA PRODUCTION UNIT IN A CONVENTIONAL MUNICIPAL WASTEWATER TREATMENT PLANT.

Some recent works are focused in the production of VFA from sewage sludge than to produce methane, since VFA is a higher value-added product. And this VFA can be used to produce PHA, that has an even higher value than VFA. Liu et al. (2018) made an economic balance comparing both the production of VFA and methane for WAS fermentation and saw how it is better to produce VFA. In fact, the profit for VFA production for the same fermentation would be 9.12 (USD/m^3 sludge), while for methane production it would be 3.71(USD/m^3 sludge). Also, Moretto et al. (2020) did a preliminary economical evaluation of a biorefinery that produces PHA from OFMSW and WAS their result was -assuming an average market price of 4 €/kg PHA (Rodriguez-Perez et al., 2018)- that the total estimated revenue would be 552,031 €/y, given by PHA production (324,543 €/y) and net electrical energy (125,898 V/y). These revenues from their scenario were 23% higher than revenues from simply co-digesting organic waste, giving an interesting perspective for possible investments.

However, Miccoluci et al. (2020) demonstrated that methane production is not remarkably reduced if a part of the VFA-rich liquid stream is separated before anaerobic digestion or not. These authors tested two different configurations (see Figure 16): in the first configuration (A), the entire mass flow produced in the anaerobic fermentation was used as feed for the anaerobic digestor reactor; so, hydrogen and methane were the main mass flows produced in the whole system. In the second configuration (B), a part of the mass flow produced in the anaerobic fermentation was processed by the solid/liquid separation unit and, consequently the anaerobic digestor reactor was fed with a mixture of fermentation liquid (from the first phase of the process), and the solids-rich mass flow produced by the solid/liquid separation unit. The configuration B, led to a VFA yield of 0.31 kg COD VFA/ kg COD fed, a specific methane production of 0.48 m^3 CH_4/kg TVS_{fed} and a specific hydrogen production of 0.074 m^3 H_2/kg TVS_{fed}. The mass balance for configuration B can be seen in Figure 3.XXX.

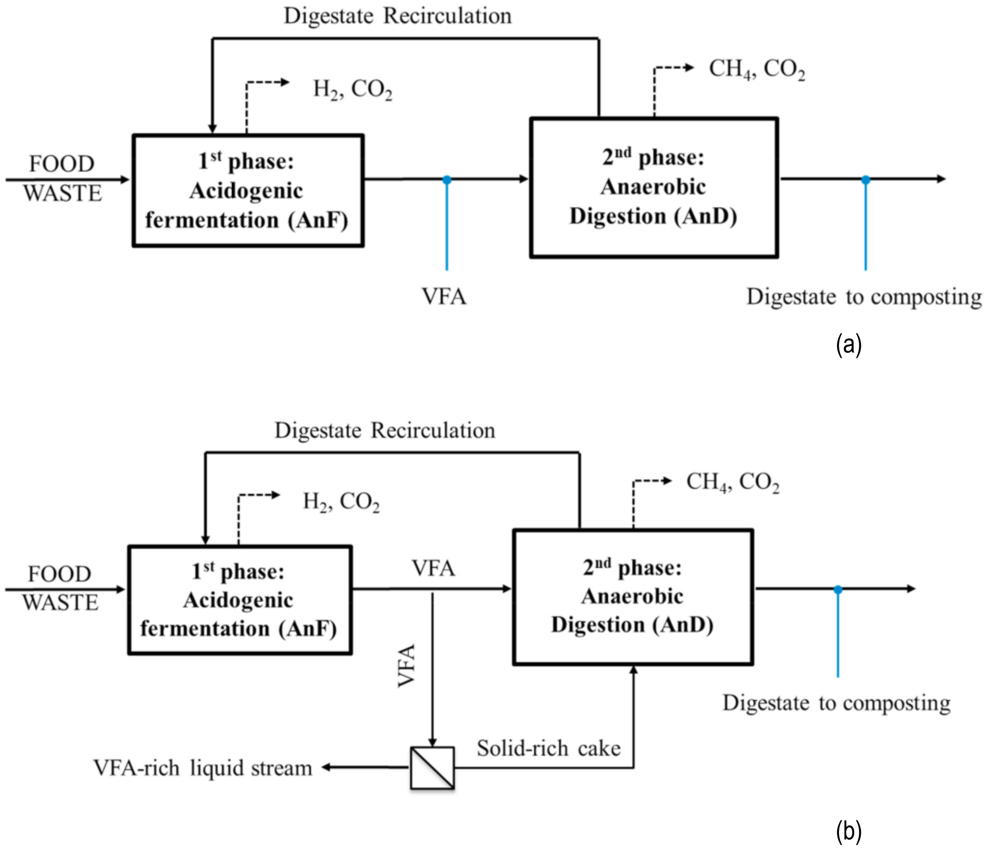

Figure 16 - Process configuration A (a) and B(b) for the Production of VFA, bio-hydrogen and bio-methane from food waste valorisation. (Micolucci et al., 2020)

As mixed sludge (PS+WAS) produces higher VFA than WAS fermentation, as reported by Usicik et al. (2008) who obtained a yield of 270, 62 and 114 mg COD_{VFA}/ VSS for PS, WAS and mixed sludge fermentation, respectively, if a VFA production unit should be implemented in a municipal WWTP, the author of this Final project proposal is to use a CSTR to ferment the mixed stream of primary sludge and WAS traditionally used to produce methane in order to produce VFA by co-fermentation with food waste.

Also, the configuration B used in Micolucci et al. (2020) would be proposed by optimizing the economic benefit with the ratio of the flow liquid rich in VFA that is separated before the anaerobic digestion. The percentage and operating conditions would be the same as Wu et al. (2016) since they obtained the highest yield reported in this study (0.867 g COD_{VFA}/ g VS fed): substrate with a composition of 81.3% in FW and 16.7% WAS (Wu et al., 2016), pH uncontrolled (5.2-6.4, due the buffer capacity of the system), SRT 7 d, OLR 9 g VS/L·d and a temperature of 40 °C. Since Wu et al. (2016) worked at batch mode and at CSTR mode operation the pH might need some control, the recirculation of digestate that did Micolucci et al. (2020) is a cheap way to control the pH. This method operates through two control levels: the first control level manages the pH in the fermentation reactor while the second control level manages the ammonia concentration in the digester reactor (Pavan et al., 2018).

The biogas (methane and hydrogen) produced it is proposed to be used to maintain the temperature of the system at 40 °C. In fact, Valentino et al. (2019) reported that the overall process was thermally self-sustaining if both anaerobic fermentation and anaerobic digestion were performed at mesophilic temperature (37 °C). Under this condition, the scaled-up system to industrial scale (170,000 PE) had the 36% surplus of thermal energy (21,360 MJ/d), and a potential economic income from the produced electricity (13.03 MWh/d) of 609,605 €/year.

If co-fermentation were not possible, it is the author believe that it would be best in terms of production of VFA and economic benefits to apply a pretreatment of 1.54 mg FNA/L + APG to the PS+WAS mixture since Liu et al. (2018) reported high yield (354.6 mg COD/g VSS) and a low fermentation time (5 d) with WAS fermentation. Moreover, their working conditions were room temperature (20 °C) and pH uncontrolled, so neither pH nor temperature need to be controlled lowering fermentation costs. Because, although working at alkaline pH improves VFA production, it would require a large amount of NaOH to maintain a constant pH in sewage sludge fermentation on an industrial scale.

5. CONCLUSIONS

In conclusion, co-fermentation of WAS with OFMSW or FW increases VFA production with respect to WAS mono-fermentation.

With respect to mono-fermentation, the best substrates are FW or OFMSW because they have a higher amount of biodegradable organic matter. After OFMSW comes primary sludge, which is more biodegradable than WAS. And finally, the worst mono-fermentation in terms of VFA production is with WAS as a substrate.

WAS fermentation has the highest VFA yield at neutral-basic pH (7- 8) and at thermophilic temperature (Zhang et al., 2009). This is because temperature of 55 C favors hydrolysis and acidogenesis (Hao et al., 2015), while neutral pH favors acidogenesis (Xiong et al., 2012; Ma et al., 2015). WAS hydrolysis is also enhanced at basic pH (9 or 10), but these conditions then impair acidogenesis (Ma et al., 2015) since the activities of the enzymes that carry out acidogenesis are lower under these conditions (Yu et al., 2008). Still, at room temperature the optimum pH is 10 (Yuan et al., 2006) because acidogenic bacteria have more hydrolyzed substrate available, while at mesophilic temperature the optimum pH is 9 (Zhang et al., 2009). In short, the optimal WAS fermentation operational conditions in order are as follows: pH 8+ thermophilic temperature (0.368 g COD VFA/ g VSS; Zhang et al., 2009), pH 9+ mesophilic temperature (0.298 g COD VFA/ g VSS; Zhang et al., 2009) and pH 10 + room temperature (0.256 g COD VFA/ g VSS; Yuan et al., 2006).

When WAS is pretreated, in mesophilic fermentation the best operating pH is neutral (Ma et al., 2015), since it is no longer necessary to go to high pH to increase hydrolysis. Within the pretreatments for WAS, it is the author's thought that the best pretreatment is the integration of FNA 1.54 mg/L and the presence of APG, since it allows to work at room temperature and without pH control a yield (0.355 g COD VFA/g VSS; Liu et al., 2018) almost equal to that at 55 C and pH 7 (0.368 g COD VFA/ g VSS), decreasing the operation costs. In addition, this pretreatment is eco-friendly and inexpensive, since FNA can be generated within the same WAS and APG is a cheap biodegradable biosurfactant.

On the other hand, PS fermentation shows the best VFA yield at basic pH (pH 9) and thermophilic temperature (0.32 g COD VFA/g VS fed; Ahn et al., 2006). In contrast, at room temperature, higher VFA yield was reported at acidic pH (3-4) (Wu et al., 2009). More data regarding PS fermentation are needed to elucidate the influence of pH and temperature on fermentation behavior. What is clear is that primary sludge fermentation reports higher yields than WAS fermentation. For this reason, also mixed sludge (PS+WAS) has a higher VFA yield than WAS fermentation, although lower than PS alone (Ucisik et al., 2008).

OFMSW or FW fermentation is optimized at mesophilic temperature (around 40 C) and pH 6-7 (Jiang et al., 2013; Fernández-Domínguez et al., 2020; Bermúdez-Penabad et al., 2017; Farouk et al. DATA; Lim et al., 2008). Both as at thermophilic temperature (Hussain et al., 2017) and room temperature (Fernández-Domínguez et al., 2020) VFA yields decreased. At room temperature the optimal pH was also reported to be neutral (Xiong et al., 2019; Li et al., 2018); as well as at thermophilic temperature where Hussain et al. (2017) obtained the best VFA yield at pH 7.

Co-fermentation of WAS and OFMSW can produce higher VFA yields than their respective mono-fermentation (Vidal-Antich et al., 2021), as the data in this work suggest. At acidic pH (5-6.5), VFA yields were observed to increase in 50% FW and WAS substrate fermentation (Gong et al., 2021) relative to 30% OFMSW substrate fermentation (Moretto et al., 2019). In fact, substrate fermentation with 81.3 % FW (Wu et al., 2016) still increased relative to previous a. These authors reported the highest yield seen in this study (0.867 g CODVFA/ g VS fed). In contrast, at neutral and basic pH (6.5-10) co-fermentation with 30% substrate in OFMSW (Moretto et al., 2019) gave better results than substrate fermentation with 50% FW (Gong et al., 2021). Regarding the temperature, all the reported results suggest that mesophilic temperature is the better one to enhance VFA production (Moretto et al., 2019; Gong et al., 2021; Wu et al., 2016). The only study where the yield was increased in thermophilic conditions was Valentino et al. (2019), but they reported that at these conditions the operation was unstable, so it was better to work at mesophilic conditions.

Regarding the distribution of VFAs there is not a clear correlation with the operational conditions and the different substrates. The only conclusion that can be drawn from the data collected is that as pH increases, the distribution of acetic acid increases.

Finally, the author of this work proposed guidelines for implementing fermentation or co-fermentation of WAS and FW at a WWTP:

- In a WWTP, acidogenic fermentation is proposed to be carried out with the mixed sludge. In case of mono-fermentation of this substrate, the pretreatment of 1.54 mg FNA/L + APG is proposed given its efficiency and low price.
- If co-fermentation is possible, it is believed to be better than mono-fermentation.
- At the industrial level, acidogenic co-fermentation would be carried out with a CSTR in configuration B (see Figure XXX), optimizing the total revenues to decide which liquid fraction is separated from the effluent for PHA production.
- Neutral pH and a temperature of 40 C are belived to be the optimum conditions for acidogenesis, while alcali pH and thermophilic temperature are belived to be the best condions for hydrolysis.
- to obtain high yields of VFA in order to produce PHA, it is considered best to work at high HRT and low ORL.
- Optimal operating conditions are considered to be as follows: FW/WAS=5; acidic pH controlled by the buffer capacity of the system; T= 40 C; ORL 9 g VS/L and SRT of 7 d The pH control to be implemented would be done with digestate recirculation, since it is the most economical and easiest.
- It is estimated that the biogas produced will be sufficient to cover the heat demand to keep the system at temperature and there will even be an income due to the electricity produced with the biogas.

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ACRONYMS

Volatile Fatty Acids (VFAs), Polyhydroxyalcalonates (PHAs), Chemical Oxygen Demand (COD), soluble COD (sCOD), Total Solids (TS), Dry sòlids (DS), Total Suspended Solids (TSS), Volatile Solids (VS), Volatile Suspended Solids (VSS), Free Nitrous Acid (FNA), Rhamnolipid (RL), Zero Valent Iron (ZVI), Waste Water Treatment Plant (WWTP), Specific Metane Production (SMP), Food Wastes (FW), Waste Activated Sludge (WAS), Primary Sludge (PS), Mixed Sludge (MS), Organic Fraction of Municipal Solid Wastes (OFMSW), Acidogenic Fermentation (AF).

APPENDICES

APPENDIX 1: PRETREATMENTS APPLIED TO WAS

ACID AND ALCALI PRETREATMENT:

This pretreatment consists of maintaining an alkali or acid pH for a certain amount of time just before the acidogenic fermentation to enhance the hydrolysis of the substrate. Then the acidogenic fermentation can operate at any other pH.

Wu et al. (2017) investigated acid and alkali pretreatment applied to waste activated sludge (WAS) to enhance the anaerobic fermentation (AF) for phosphorus (P) and volatile fatty acids (VFAs) release into the liquid. In the acid and alkali pretreatment, 6 M hydrochloric (HCl) or 6 M sodium hydroxide (NaOH) was added to the 2 L of WAS for pH adjustment to 3.0 or 10.0, respectively. Then the samples were mixed on a magnetic stirrer at 250 rpm for 30 min. After the pretreatment, the sludge was used for batch anerobic fermentation (AF).

Compared with untreated WAS, the SCFAs production was increased after acid or alkali pretreatment, and reached the maximum after alkali treatment: 1464 mg/L (alkali pretreatment) > 1205 mg/L (acid pretreatment) > 96 mg/L (no pretreatment). So, Wu et al. (2017) observed that both acid and alkali pretreatment effectively promoted the hydrolysis and the release of polysaccharide and protein improving the production of VFA.

As stated in Figure 17, butyric and valeric acids were the main fermentation products. Acetic acid content in the alkali pretreatment was higher than that obtained in the other studies cases (acid pretreatment and without pretreatment). Also, the fraction of branched SCFAs (iso-butyric and iso-valeric) was higher than their corresponding straight SCFAs (n-butyric and n-valeric), which is in agreement with Yi et al. (2013) results. This could be attributed to the fact that the decomposition rate of straight-chain (C2-C5) VFAs was higher than that of their corresponding branched-chain isomer (Wang et al., 1999).

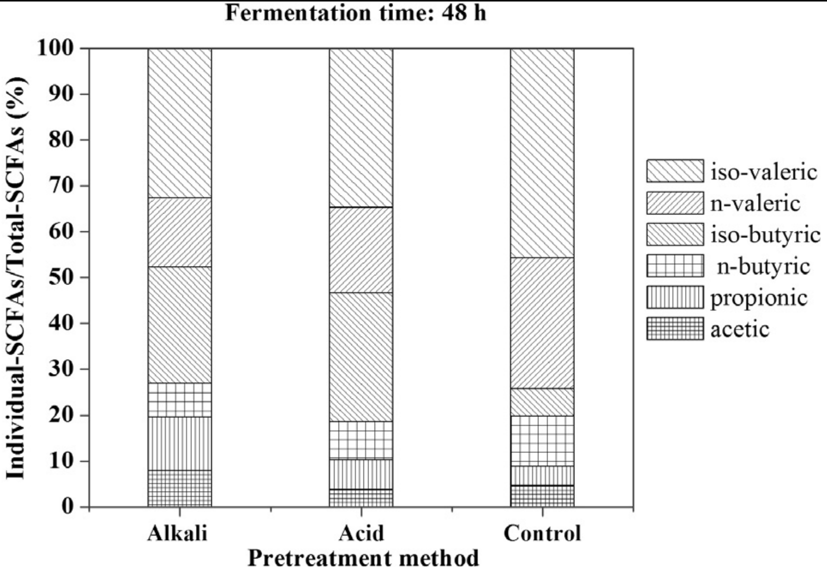


Fig. 17- VFAs composition in the fermentation supernatant (48h) when alkali and acid pretreatments are applied to WAS (Wu et al., 2017)

HEAT PRETREATMENT:

This pretreatment consists of maintaining the substrate at a high temperature (usually 70 to 90 °C) for a certain time before starting the acidogenic fermentation because the temperature increases its hydrolysis and solubilization.

Lu et al. (2008) compared a two-step process for treatment and stabilisation of primary sludge with a one-step process. On the one hand, the process with two steps consisted on a hyperthermophilic hydrolysis step that operated at 70°C (hydraulic retention time (HRT) of 2 days) followed by a thermophilic (55 °C) anaerobic digestion step at a HRT of 13 days. On the other hand, the one-step anaerobic digester was operated at 55°C and a HRT of 15 days. Both processes operated in CSTR.

The two-step process was characterized by a better pathogen reduction effect and a 12% higher organic suspended solids removal efficiency. The activity of the microbial community of the digester fed with pre-treated sludge was also higher compared to the one of digester treating raw sludge. Moreover, the pre-treatment of the primary sludge resulted up to 48% increase of the methane potential (20.09 and 13.56 mmolCH4 gVS^{-1} with and without pretreatment, respectively) and up to 115% increase of the methane production rate. In fact, the extra energy requirements for the operation of a pre-treatment step would be covered by the energy produced from the extra methane production, and yet there would be a significant energy surplus of 2.17 kJ d^{-1}.

As demonstrated, heat pretreatment improves the hydrolysis, but to find the optimal temperature and time, Dong et al. (2016) compared the effect of different pretreatment temperatures (60°C, 80°C, 100°C and 120°C) and pretreatment time (15, 30, 45, 60 and 75 min) on sludge hydrolyzation. These authors found that sludge pretreated at 100°C for 60 min could achieve the maximal hydrolyzation as stated in Figure 18.

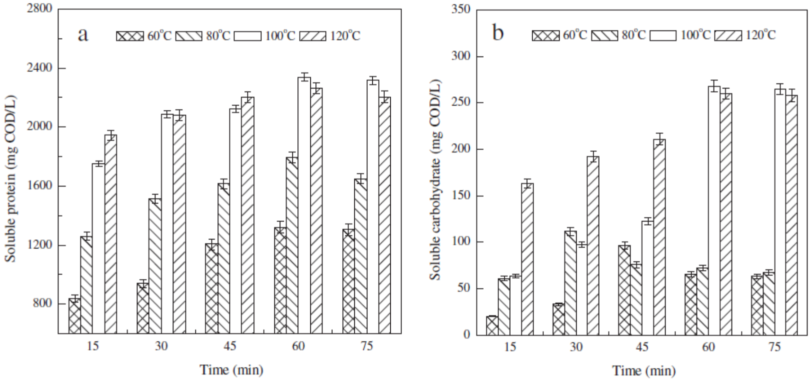


Figure 18- Effect of different pretretment temperatures and time of pretreatment on soluble protein (a) and carbohydrate (b) concentrations. Errors bars represent standard deviations of triplicate tests. (Dong et al., 2016)

COMBINED HEAT AND ALKALI PRETREATMENT:

The heat-alkaline pretreatment is the combination of the two previous pretreatments. So, it consists in maintaining the fresh sludge under a high temperature (for example, 70 or 90 °C) and an alkaline pH (pH 11 or 12) for a certain duration of time that usually is 2 h (Liu et al., 2018; Yu et al., 2018; Ma et al., 2016).

For example, Ma et al. (2016) pretreated WAS at 90 °C and pH 11.0 by the addition of 4 M NaOH for 2 h. In their study, it was compared the anaerobic fermentation at different pH (uncontrolled, 7.0 and 10.0) with untreated sludge and heat-alkaline pretreated WAS. The results are summarised in Table 3XX, where it is corroborated that the extent of hydrolysis was improved with the pretreatment. The best production of VFA on untreated sludge was recorded at pH 10, while it was at pH 7 for pretreated sludge. This result suggest that hydrolysis is enhanced at pH 10 while acidogenesis is enhanced at pH 7. For this reason, these authors studied the VFA production with glucose or bovine serum albumin as substrate, concluding that neutral pH produced a higher VFAs concentration than the alkaline pH condition. Also, they performed a terminal restriction fragment length polymorphism (T-RFLP) analysis that showed that the alkaline pH caused low microbial richness. So, it can be concluded that the alkaline pH improved the hydrolysis of organic matter in sludge while neutral pH improved the acidogenesis for the VFAs production from sludge.

Figure 19 (a and b) show how both VFA production and solubilization increase with a combined heat-alkali pretreatment, while the acetic acid content increases with pH in both pretreated and non pretreated sludge (see Figure 19 c). In addition, propionic acid composition decreases in all cases with pretreatment. Moreover Figure 19 c corroborates that the optimum pH to produce VFAs for untreated sludge is pH 10, while the optimum pH for pretreated sludge is pH 7.

Table 8. Hydrolysis, acidification rates and VFAs yields during the fermentation (Ma et al., 2016)

Parameter (units)	**Fermentation with untreated sludge**			**Fermentation with pretreated sludge**		
	R1 (uncontrolled pH)	**R2 (pH 7)**	**R3 (pH 10)**	**R4(uncontrolled pH)**	**R5(pH 7)**	**R6 (pH 10)**
Extent of hydrolysis (%)	9.11	18.98	54.37	73.39	75.98	70.18
Extent of acidification (%)	20.45	22.67	30.37	18.26	30.98	19.63
VFAs yield (mg COD/g VS)	158.55	175.77	235.46	141.53	240.14	152.14

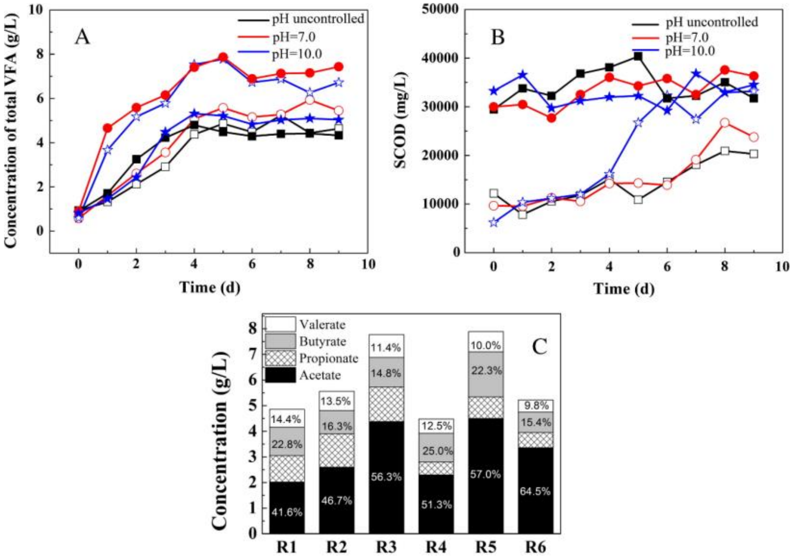


Figure 19 – VFA production from sludge at different pH values (A), SCOD profiles during anaerobic fermentation (B) and VFA distribution at the end of the fermentation tests (C).
(The open symbols in A and B represent un-pretreated sludge and the solid symbols represent pretreated sludge. The percentages in C represent the single VFAs proportion in the total VFAs)
(Ma et al., 2016)

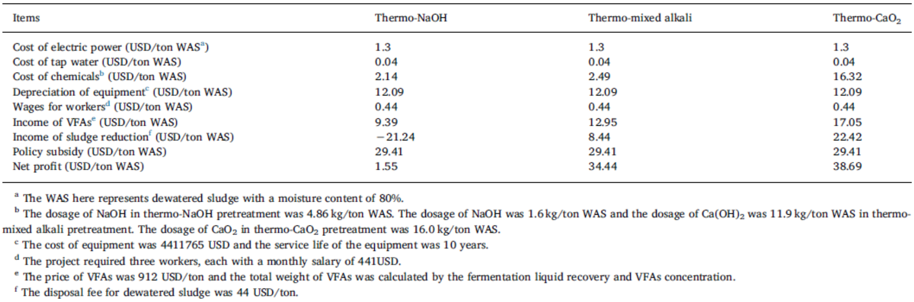
As the Heat-Alkaline pretreatment can be performed with different bases, Yu et al. (2018) investigated the effects of three kinds of waste activated sludge (WAS) thermo-alkali pretreatments: namely, thermo-NaOH, thermo-mixed alkali ($Ca(OH)_2$ and NaOH) and thermo-CaO_2 pretreatments. All the pretreatments consisted in adjusting the sludge pH to 12 with the corresponding solution and then maintain the temperature at 70 °C for 2 h.

After thermo-NaOH, thermo-mixed alkali and thermo-CaO_2 pretreatments, the average concentrations of sCOD in sludge were 21317.52 ± 2414.33, 14911.48 ± 1947.55 and 14662.04 ± 1845.03 mg/L, respectively. Therefore, the efficiency of thermo-NaOH pretreatment in sludge hydrolysis was much higher than the other pretreatments. Regarding the monitored VFA production, WAS pretreated with thermo-CaO2 (7.91 ± 0.56 g VFAs/L) and thermo-NaOH

(7.48 ± 0.64 g VFAs/L) were proved to be better than WAS pretreated with thermo-mixed alkali (6.93 ± 0.63 g VFAs/L). This might be since the addition of CaO2 increased the relative abundance of Firmicutes (Liu et al., 2015) Because some of these bacteria are correlated with propionate and acetate production through propionate pathway (Huang et al., 2016a).

Moreover, in the fermentation of WAS pretreated with thermo-CaO_2 VFAs are easier to recover than with thermo-NaOH. From the economic point of view, the pretreatments with thermo-NaOH, thermo-mixed alkali and thermo-CaO_2 for anaerobic fermentation of VFAs showed that the net benefits were 1.55, 34.44 and 38.69 USD/ton of dewatered sludge, respectively (see the detailed costs and benefits in Table 2XX). Therefore, thermo-CaO_2 pretreatment is the most promising method for the stuidied case of Yu et al. (2018).

Table 9 – Comparison of the economic feasibility of thermo-NaOH, thermo-mixed alkali and thermo-CaO_2 pretreatments for WAS anaerobic fermentation to produce VFAs (Yu et al., 2018)



Items	Thermo-NaOH	Thermo-mixed alkali	Thermo-CaO_2
Cost of electric power (USD/ton WAS[a])	1.3	1.3	1.3
Cost of tap water (USD/ton WAS)	0.04	0.04	0.04
Cost of chemicals[b] (USD/ton WAS)	2.14	2.49	16.32
Depreciation of equipment[c] (USD/ton WAS)	12.09	12.09	12.09
Wages for workers[d] (USD/ton WAS)	0.44	0.44	0.44
Income of VFAs[e] (USD/ton WAS)	9.39	12.95	17.05
Income of sludge reduction[f] (USD/ton WAS)	−21.24	8.44	22.42
Policy subsidy (USD/ton WAS)	29.41	29.41	29.41
Net profit (USD/ton WAS)	1.55	34.44	38.69

[a] The WAS here represents dewatered sludge with a moisture content of 80%.

[b] The dosage of NaOH in thermo-NaOH pretreatment was 4.86 kg/ton WAS. The dosage of NaOH was 1.6 kg/ton WAS and the dosage of $Ca(OH)_2$ was 11.9 kg/ton WAS in thermo-mixed alkali pretreatment. The dosage of CaO_2 in thermo-CaO_2 pretreatment was 16.0 kg/ton WAS.

[c] The cost of equipment was 4411765 USD and the service life of the equipment was 10 years.

[d] The project required three workers, each with a monthly salary of 441USD.

[e] The price of VFAs was 912 USD/ton and the total weight of VFAs was calculated by the fermentation liquid recovery and VFAs concentration.

[f] The disposal fee for dewatered sludge was 44 USD/ton.

CHEMICAL PRETREATMENT:

Chemical pretreatments consist of the addition of a chemical compound that accelerates the hydrolysis of the WAS. The most used chemical pretreatments are explained below.

ZERO VALENT IRON:

As a reducing material can enhance anaerobic process including the hydrolysis-acidification process, thus effectively improve the decomposition of protein and cellulose in WAS.

Zero-valent iron (ZVI) is a reducing agent that has been reported as an effective catalyst for the rapid conversion of a wide range of organic matters into benign hydrocarbons for later utilization (Zhang, 2003). For this reason, it might be able to create a favorable and stable anaerobic environment that may improve the performance of acidogenic fermentation (Phillips et al. 2010; Lin and Li 2018). Feng et al. (2014) reported that ZVI can enhance both the hydrolysis and the acidification step of the acidogenic fermentation. For this reason, Luo et al. (2014) studied how affected the presence of nano zero-valent iron in the production of VFA. The results of their study are shown in Figure 20.

These authors found that the presence of nano zero-valent iron, not only improved the VFA production, but also shortened the fermentation time when compared with those fermentation reactors without nano zero-valent iron. This is due to enhanced solubilization, hydrolysis and acidogenesis in the presence of ZVI, as demonstrated by investigations of the mechanism. In fact, the activities of key enzymes correlated with hydrolysis and acidification of organic matters were higher than those in the control. Also, the abundance of bacteria that are responsible of WAS hydrolysis and acidogenesis was greatly enhanced with nano zero-valent iron addition.

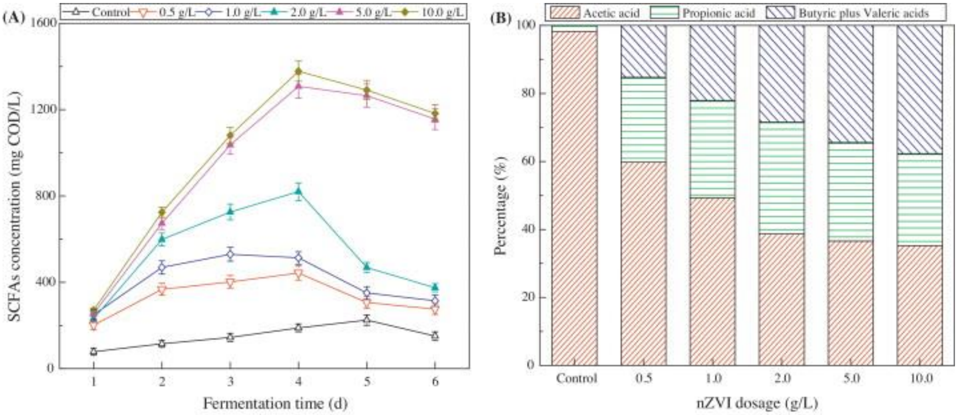


Figure 20 - nZVI dosage effects on SCFAs production (A) and composition (B). (Luo et al., 2014)

As we can see in Fig. 20, the production of VFA reached the maximum at a fermentation time of four days when the reactors had nZVI addition, while in the control was required five days. So, nZVI was a catalyst that shorted the fermentation time. The optimum nZVI dosage was of 5.0 g/L that reached a production of VFA of 154.8 mg COD/g VSS in a fermentation time of 4 d, 5.8-fold of the control with 5 d. When the dosage of nZVI was increased to 10 g/L no significant improvement were seen. Regarding the distribution of VFA, as nZVI dosage increased, the proportion of butyric increased while it decreased the proportion of acetic. Propionic acid proportion was constant independently of the dosage of nZVI, but there was just a little proportion in the control.

Free nitrous acid (FNA):

FNA is a renewable and cost-effective biocidal that can be produced in situ in WWTPs by partial nitrification of the anaerobic digestion liquor (Law et al., 2015; Wang et al., 2013b) that seems to be a promising pretreatment technique as it enhance WAS biodegradability (Wang and Yuan, 2015; Xu et al., 2018). This pretreatment, stimulate the activity of key enzymes that are responsible for hydrolysis and acidification, while it inhibits the activity of methanogens increasing VFA production (Li et al., 2016). Pijuan et al. (2012) reported that 50–90% of the sludge is mineralized after 24–48 h of FNA pretreatment.

In addition, this technique can be combined with other techniques to further improve VFA production. For example, Zhao et al. (2015) studied the combined effect of pretreatment with FNA and fermentation at pH 10. Fermentation at pH 10 can effectively inhibit the activities of methanogens (Yuan et al., 2006) and promote the abundance of Pseudomonas sp., the bacteria that excretes extracellular protease (Zheng et al., 2013) accelerating sludge solubilization. However, it takes approximately 8 days to reach maximum VFA accumulation at pH 10 (Wang et al., 2013a; Yuan et al., 2006). But, even under this fermentation time, more than 60% of volatile suspended solids (VSS) could not be degraded (Zhang et al., 2009).

The result of the experiments carried out by Zhao et al. (2015) are presented in Figure 21, where it is shown that sole fermentation at pH 10 takes 8 days to reach the maximum VFA production, which is consistent with the studies of Wang et al. (2013a) and Yuan et al. (2006). In addition, both production and fermentation time improved with respect to the control test (uncontrolled pH). Sole-FNA also increases VFA production respect the blank and reduces the fermentation time to 6 d. FNA pretreatment combined with pH 10, further increased the production of VFA and reduced to 4 d the fermentation time. In effect, the combination of both methods produces a synergistic effect by increasing hydrolysis and acidogenesis. Indeed, the activities of key enzymes relevant to hydrolytic and acid-forming microbes were found to be optimum at 1.54 mg/L FNA, which yield was 370.1 mg COD VFA/g VSS, as it can be seen in Table 10.

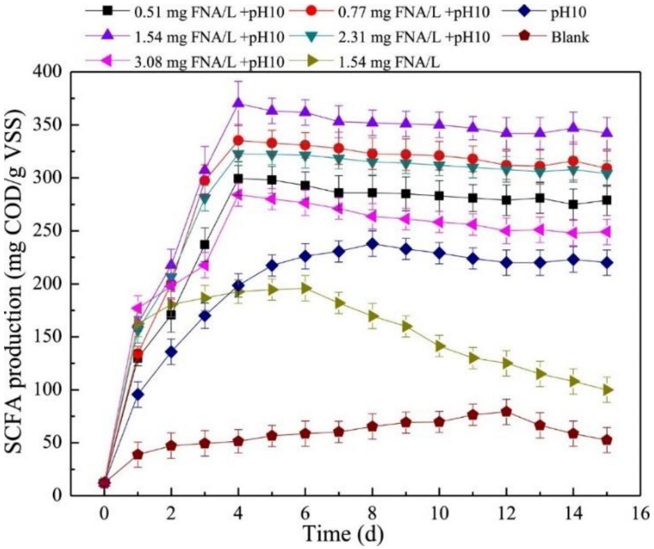


Figure 21- Total SCFA production under different operational conditions of pH and free nitrous acid concentration during sludge anaerobic fermentation. Error bars show 95% confidence intervals (Zhao et al., 2015)

Table 10 – Comparison of specific activities of key enzymes involved in SCFA production among different fermentation reactors under their optimal conditions. The specific enzyme activity was defined as unit of enzyme activity per milligram of VSS. Error bars show 95% confidence intervals. (Zhao et al., 2015)

Reactor	Protease	α-glucosidase	AK	PTA	BK	PTB	OAATC	COA transferase
0.51 mg FNA/L + pH10	7.12± 0.04	0.224 ± 0.012	2.68 ± 0.04	0.237 ± 0.012	0.173 ± 0.008	0.018 ± 0.008	0.682 ± 0.016	0.754 ± 0.008
0.77 mg FNA/L + pH10	7.28± 0.08	0.241 ± 0.008	2.71 ± 0.12	0.251 ± 0.004	0.182 ± 0.012	0.019 ± 0.004	0.716 ± 0.008	0.815 ± 0.012
1.54 mg FNA/L + pH10	7.56 ± 0.08	0.255 ± 0.004	2.94 ± 0.08	0.264 ± 0.008	0.195 ± 0.008	0.020 ± 0.012	0.849 ± 0.004	0.836 ± 0.012
2.31 mg FNA/L + pH10	6.84± 0.12	0.228 ± 0.016	2.76 ± 0.12	0.243 ± 0.012	0.175 ± 0.008	0.016 ± 0.004	0.728 ± 0.008	0.726 ± 0.012
3.08 mg FNA/L + pH10	6.15± 0.08	0.215 ± 0.008	2.62 ± 0.08	0.231 ± 0.008	0.156 ± 0.004	0.015 ± 0.016	0.658 ± 0.012	0.628 ± 0.008
pH10	5.56± 0.08	0.196 ± 0.016	2.61 ± 0.08	0.229 ± 0.008	0.141 ± 0.012	0.011 ± 0.008	0.543 ± 0.004	0.481 ± 0.004
Blank	3.99 ± 0.04	0.133 ± 0.012	1.56 ± 0.08	0.124 ± 0.004	0.089 ± 0.008	0.005 ± 0.004	0.140 ± 0.008	0.086 ± 0.012

Similar results were obtained by Li et al (2016). Indeed, among the different batch tests with different concentrations of FNA (blank; 0.8 mg/L; 1.8 mg/L; 2.7 mg/L; 3.6 mg/L), the one that gave the best VFA productivity was the one with 0.8 mg FNA/L with a fermentation time of 6 days, which was 3.7-fold of the blank at fermentation time of day 12. Also, they found that methane production in 1.8mg FNA/L reactor was 68.9% of that in the blank at the fermentation time of day 15, which indicated that the activities of methanogens were seriously inhibited by the FNA pretreatment.

These results are in line with the results of with Zhaedi et al. (2016), who explored the effectiveness of four different FNA concentrations (0, 2.49, 3.55 and 4.62 mg $N\text{-}HNO_2/L$) and three exposure times (2, 5, 9 h). The results showed a significant increase (up to 25%) on SMP when the sludge was pretreated with the lowest FNA concentration (2.49 mg N-HNO_2/L) during 2 and 5 h but did not show any improvement at longer exposure times or higher FNA concentrations.

Zahedi et al., 2019 explorated alternatives to reduce costs of FNA pretreatment on WAS and they found that reusing NO_2^- from pre-treated WAS for other pre-treatments was not viable. However, they reported that an increase in the TS content of WAS does not affect the efficiency of the FNA pre-treatment. So, concentrating the sludge before the FNA pre-treatment would be a good strategy to reduce costs related to nitrite.

Bio-surfactants:

Surfactants can improve the organic matter and EPS solubilization and hydrolysis in WAS due to the high surface activity, which will also improve the VFA production. However, the chemically synthesized surfactants are not biodegradable and have a negative impact on environment. Biosurfactants are biodegradable and ecofriendly, so are a good substitute for chemical surfactants.

Huang et al. (2015) studied the effects of three types of bio-surfactants (namely, surfactin, rhamnolipid and saponin) on VFA production during sludge acidogenic fermentation. Figure 22 shows the results of Huang et al. (2015), where it could be observed that VFA production was substantially promoted after the addition of bio-surfactants and the fermentation time was reduced to 4 or 5 days. The highest VFA concentration was 3300 mg COD/L (311 mg COD/g VSS) for surfactin and rhamnolipid bio-surfactants at 0.05 g/g dry sludge, since no significant improvements were observed when this dosage was increased to 0.10 g/g dry sludge. Regarding saponin, the maximum concentration of VFAs was about 3100 mg COD/L (292 mg COD/g VSS) for a chemical dosage at 0.1 g/g dry sludge.

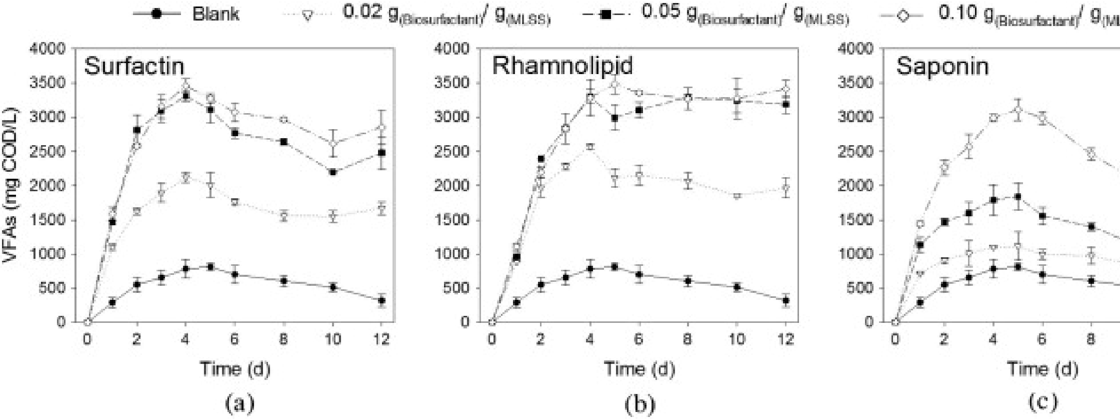
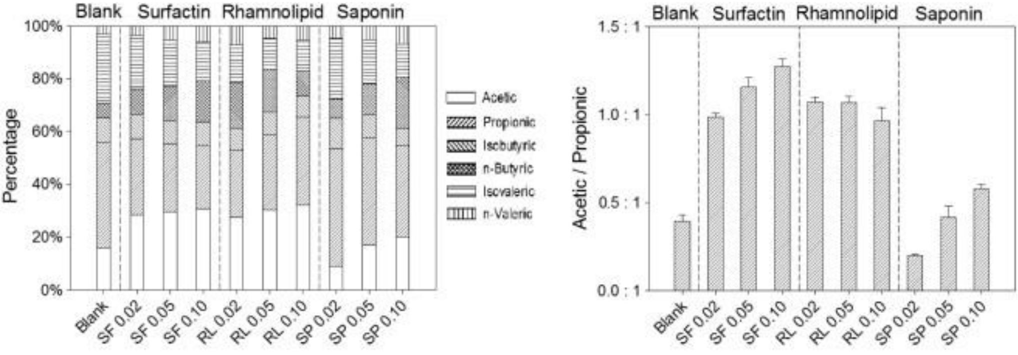


Figure 22 – VFA production for the three different bio-surfactants (Huang et al., 2015)

In Figure 22 it can be seen that the distribution of VFAs was affected by the type and the dosage of the bio-surfactants. The surfactin or saponin addition increased the ratio of acetic acid to propionic acid and the percentage of n-butyric acid. Regarding rhamnolipid, the percentage of acetic acid and propionic acid increased simultaneously when increasing the dosage of rhamnolipid. Also, the ratio of acetic acid to propionic acid in the tests with rhamnolipid or surfactin was higher than that with saponin and the blank. As a conclusion of this study, the best surfactant was rhamnolipid because it gave the best yield and, in addition, it was the only one that inhibited the methanogens activity.



(a) (b)

Figure 23 – VFA distribution for the three different bio-surfactants (Huang et al., 2015)

So, Rhamnolipid (RL) could be a suitable replacement of traditionally chemical surfactants. Moreover, RL can be produced in situ because an external addition of RL can trigger this self-synthesis process in WAS by some microorganisms such as Pseudomonas, during WAS anaerobic fermentation (Yang et al., 2015; Zhou et al., 2013).

He et al. (2016) studied the use of Rhamnolipid (RL) and their results showed that the integration treatments of RL + operation pH 10 performed significant enhancement of VFA production and reduction of the fermentation time as it can be seen in Fig 24. The maximum VFA production was 378 mg COD/g VSS at 3-day fermentation time with a dosage of 0.2 g RL/g TSS and initial pH 10 treatment, which was 4.31, 1.32 and 1.24 times higher than that of control, initial pH 10 and 0.2 g RL/g TSS treatment, respectively. The hydrolysis rate constant was also improved greatly in the integrated treatment: 8.68, 2.14 and 1.55 times higher than that of control, sole RL and initial pH 10 treatments, respectively (He et al., 2016).

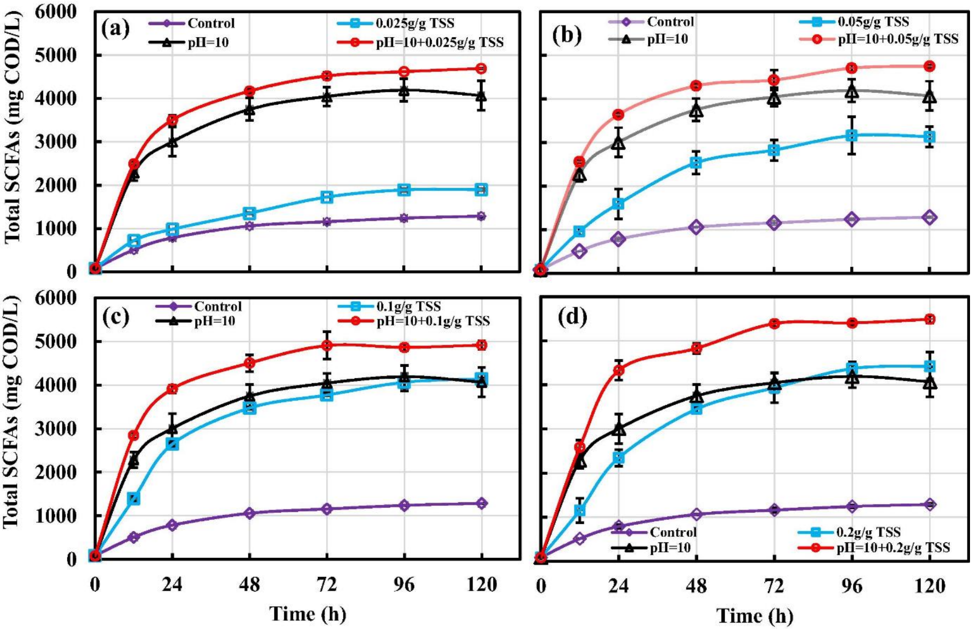


Figure 24- Effects of RL dosage on VFA production at initial pH 10 anaerobic fermentation: (a) 0.025 g RL/g TSS, (b) 0.05 g RL/g TSS, (c) 0.1 g RL/g TSS, and (d) 0.2 g RL/g TSS. (He et al., 2016)

Regarding the distribution of VFAs, the main VFA in all the tests was acetic acid, followed by propionic acid, iso-valeric acid, n-butyric acid, n-valeric acid and iso-butyric acid. And, when the inicial pH was 10, the percentage of acetic acid increased more.

Rhamnolipid can be combined with other pretreatments. For example, Wu et al. (2017) combined the FNA pretreatment with rhamnolipid presence. The dosage selected by these authors of rhamnolipid was 0.04 g/g TSS according to previous literature (Zhou et al., 2015). Also, the FNA pretreatment lasted 2 days according to other previous reports (Wang et al., 2013). The results of this experiment are summarized in Fig. 25.

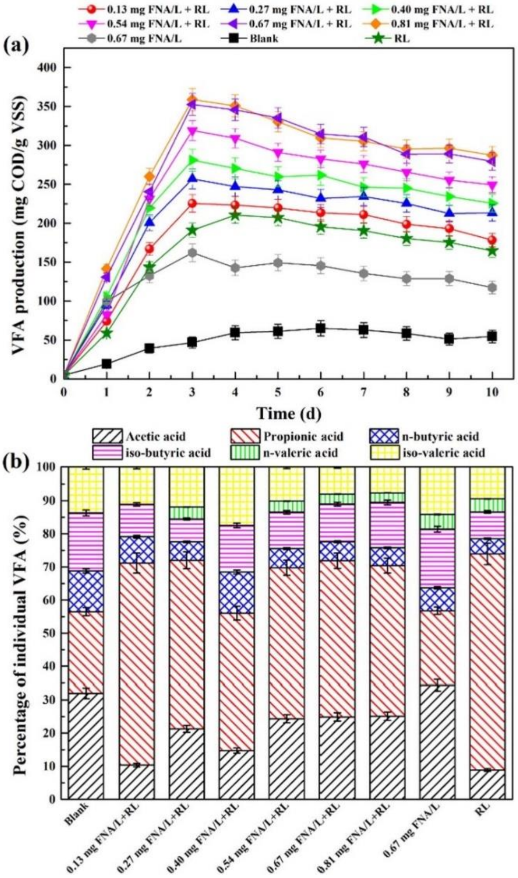


Fig. 25. Comparisons of VFA production and distribution at different pretreatment application. (Wu et al., 2017)

As stated in Fig. 25, the highest VFA production was 352.62 mg COD/g VSS at 0.67 mg/L FNA + 0.04 g RL/g TSS; that was 2.13, 1.64 and 5.42 times higher than sole FNA, sole RL and blank, respectively. At 0.81 mg/L FNA the VFA production did no improve because excess FNA inhibits the hydrolytic and acidogenic enzymes due to its toxicity (Pijuan et al., 2012; Zhao et al., 2015). However, previous studies reported that the optimal FNA concentrations for VFA production were 1.54 and 1.80 mg/L (Zhao et al., 2015, Li et al., 2016). This might be due to the positive effect of RL, that accelerated the solubilization, hydrolysis and acidogenesis and inhibited the methanogenesis (Huang et al., 2015). Regarding VFA distribution, RL improves the propionic acid composition while lowers the composition of acetic acid.

Sodium dodecylbenzene sulfonate (SDBS) is another bio-surfactant that can be easily found in waste-activated sludge (WAS). Jiang et al. (2007) investigated the effect of SDBS on VFAs production from WAS.

The results (see Figure 26) showed that VFAs production increased significantly in the presence of SDBS. At fermentation time of 6 days, the concentration of VFAs was 2599.1 mg COD/L with a SDBS dosage of 0.02 g/g dry sludge, whereas it was 339.1 mg COD/L without SDBS. In high concentrations of SDBS the production of VFAs increased to 3285.2 at 0.1 g/g dry sludge with a fermentation time of 12 days, and 2674.5 at 0.2 g/g and a fermentation time of 15 days. However, as a longer fermentation time was required, the optimum conditions for VFAs production were a SDBS dosage of 0.02 g/g and fermentation time of 6 days, that produced VFAs with the following distribution: acetic acid (27.1%), propionic acid (22.8%), iso-valeric acid (20.1%), iso-butyric acid (11.9%), n-butyric acid (10.4%) and n-valeric acid (7.7%).

These authors also observed that during fermentation, the solubilization of particulate organic carbon in the sludge along with its hydrolysis and acidification of the solubilized particles increased with the presence of SDBS, while methane formation decreased. This was due to the presence of SDBS, that enhanced the activity of enzymes related to hydrolysis and acidogenesis.

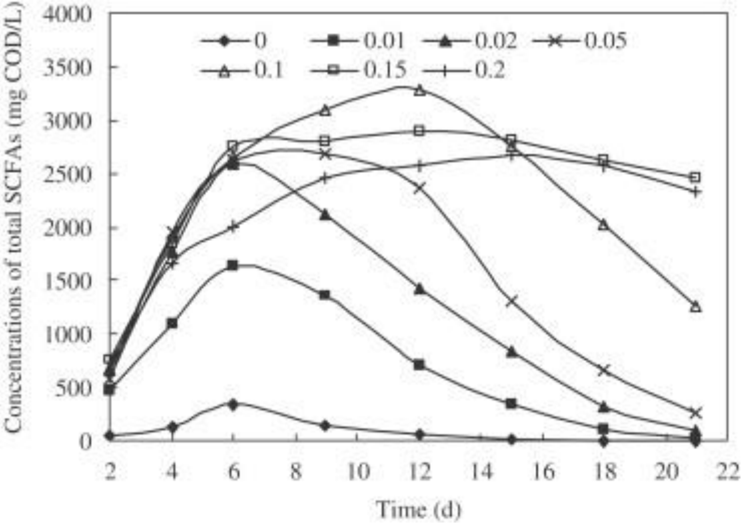


Figure 26 - Effects of SDBS dosage and fermentation time on VFA production. (Jiang et al., 2007)

Zhao et al. (2016) studied the combined effect of FNA pretreatment (that was maintained for 2 days) and the presence of SDBS. The SDBS concentration selected was 0.02 g/g dry sludge (DS) according to the previous literature (Chen et al., 2013; Jiang et al., 2007). The results of their experiments are summarized in Fig. 27.

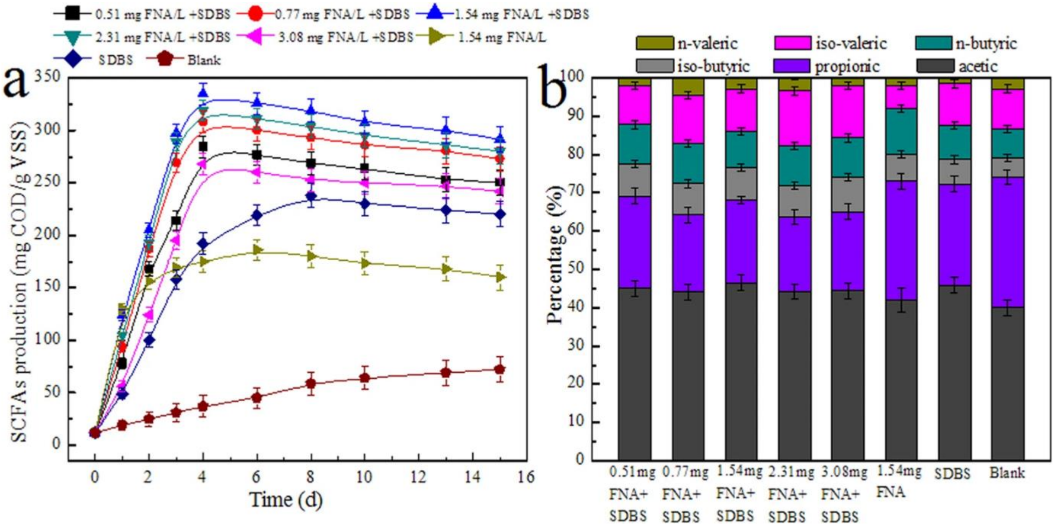


Figure 27- Effect of FNA concentration on VFA production and distribution from WAS anaerobic fermentation with the combined FNA and SDBS treatment. (Zhao et al., 2016)

As stated in Figure 27, the highest VFA yield is 334.5 mg COD/g VSS and it was achieved at 1.54 mg FNA/L pretreatment for 2 d with the presence of SDBS, which was much higher than those from their individuals. This was due to the fact that FNA pretreatment and SDBS presence accelerated the solubilization, hydrolysis, and acidification steps, but inhibited the methanogenesis step. Additionally, the fermentation time for the maximum VFA production in the combined reactor was only 4 d, whereas 6, 8, and 15 d in the sole FNA reactor, the sole SDBS reactor, and the blank, respectively. Regarding the VFA distribution, it remained constant regardless of pretreatment with FNA and the presence of SDBS. The main VFA was acetic acid, followed by propionic acid, iso-valeric acid, n-butyric, iso-butyric and n-valeric, similar to Luo et al. (2011)

Wan et al. (2020) studied another combined pretreatment: the combined effect of the heat pretreatment (70 °C, during 1 h) with the presence of SDBS (with a concentration of 0.01 g/g total solid). Their results are represented in Figure 28.

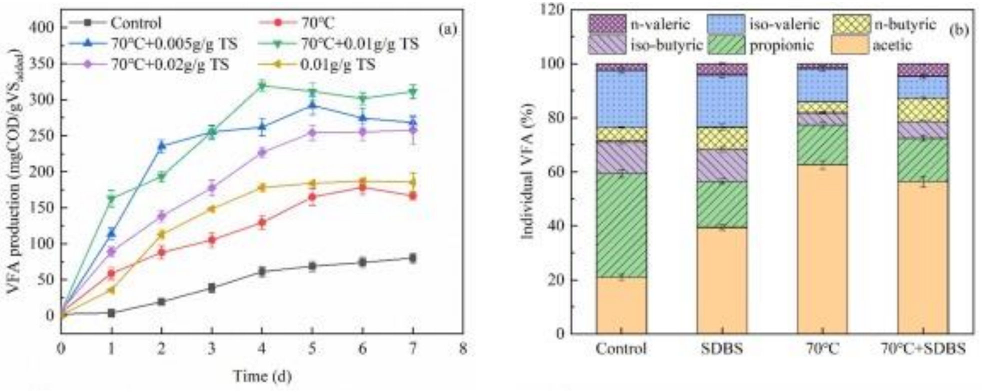


Figure 28- Effect of combined thermal pretreatment with 0.01 g/g TS of SDBS on VFA production and distribution. (Wan et al., 2020)

The maximal VFA yield (320 ± 7.7 mg COD/g VS) was from combined pretreatment (70 °C + SDBS of 0.01 g/g TS), which was 1.8, 1.7 and 4.0-fold of the sole low-thermal, the sole SDBS, and the control. Also, the combined presented a shorter fermentation time (4 days) respect all the other tests. However, when the SDBS concentration increased to 0.02 g/g TS, the VFA yield decreased. This might be due to the toxic effects of higher surfactant concentration to acidogenic bacteria (Feitkenhauer, 2003).

Regarding the distribution of VFAs, the percentage of acetic acid was 21.0% in the control, 39.4% in the sole-SDBS test (0.01 g/g TS), 62.6% in the low-thermal pretreated test (70 °C) and 56.4% in the integrated system test (70 °C + 0.01 g SDBS/g TS). Regarding the propionic acid composition, it decreased in all tests that a pretreatment was applied.

From the economic point of view at a laboratory scale, the cost of the combined pretreatment was 112.5 USD/ton TS, that is much lower than other pretreatment methods: CaO_2 addition (that has a cost of 188.5 USD/ton TS) or APG additions (with a cost of 250 USD/ton TS). Moreover, the maximal VFA yield (320 ± 7.7 mg COD/g VS) obtained in Wan et al. (2020) is significantly higher than that reported in other studies from CaO2 (about 262 mg COD/g VS) or

Alkyl polyglucose addition (about 157 mg COD/g VS), respectively (Li et al., 2015, Luo et al., 2015).

Sodium dodecyl sulfate (SDS) is another surfactant like sodium dodecylbenzene sulfonate (SDBS). Luo et al., 2011 studies the combined effect of mixed enzymes and SDS. Because hydrolytic enzymes can enhance the hydrolysis (among them glucosidases, lipases and proteases). The mixed enzymes (ME) were used in their study were commercial neutral protease and α-amylase in a ratio 3:1 and a total dosage of 0.06 g/g DS. The activities of neutral protease and α-amylase were 6000 and 3700 U g^{-1}, respectively. The optimum temperature of neutral protease was 40-50 °C, whereas it was 50-70 °C for α-amylase. Thus, they worked at 50 °C because it was the optimal temperature to enhance sludge hydrolysis by the mixed enzyme system (Yang et al., 2010). They also studied whether the combination of surfactant and enzyme produced a synergistic effect on the hydrolysis of waste solids. The dosage of sodium dodecyl sulfate (SDS) was 0.10 g/g DS. The results of their experiments are summarized in Figure 29.

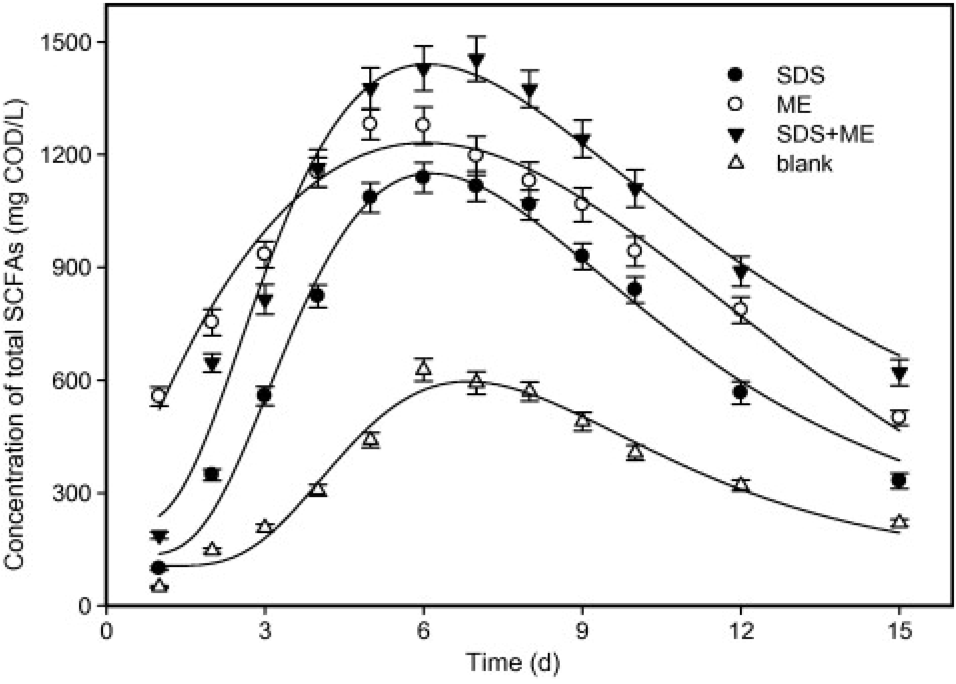


Figure 29- VFA accumulation during anaerobic fermentation. (Luo et al., 2011)

As shown in Figure 29, the maximum VFA concentration in the blank was 628.07 mg COD/L at a fermentation time of six days. And, in the SDS, ME and SDS + ME system the maximum VFA concentration increased 1.82 (day 6), 2.04 (day 5), 2.32 (day 7) times, respectively. This was due to the fact that soluble protein and carbohydrate concentrations increased from 362.5 and 90.0 mg/L (blank) to 1486.0 and 455.8 mg/L for the SDS + ME system. As for the distribution of VFA, as indicated in Figure 6, the main VFA was acetic acid, followed by propionic acid, iso-valeric, iso-butyric, n-butyric and n-valeric acid.

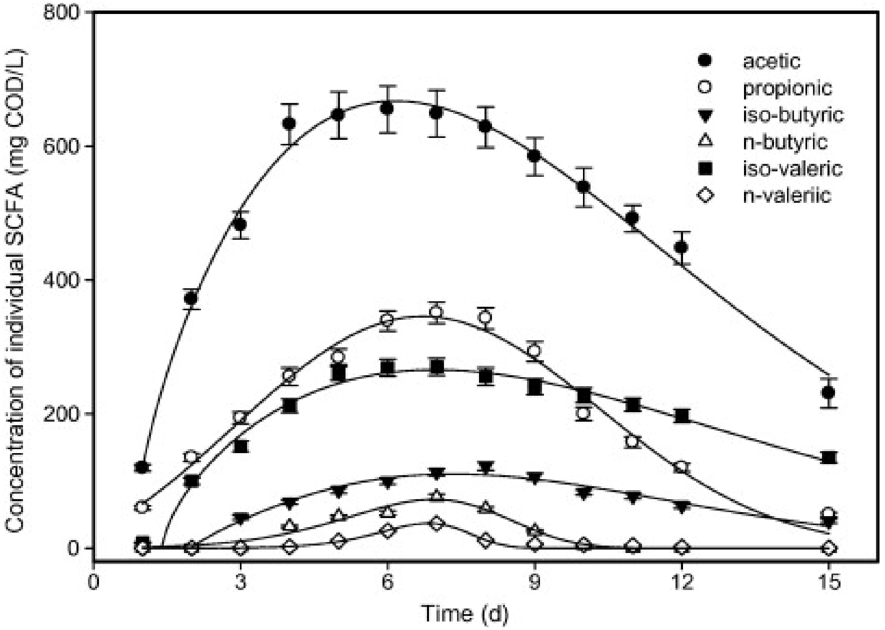


Fig. 30. Distribution of the VFA during the acidogenic fermentation for the ME + SDS system (Luo et al., 2011)

FNA + APG:

Alkylpolyglucose (APG) is a comprehensive new type of non-ionic surfactant, that is non-toxic and has a high surface activity. Also, it is rapidly biodegradable and easy to use. Moreover, it is relatively low cost and effective compared with other biological surfactants. It has been reported that APG promotes disintegration, hydrolysis, and acidification while inhibits the methanogenic step enhancing the production of VFA (Xu et al., 2016, Zhao et al., 2015c)

Liu et al. 2018 combined FNA pretreatment with the addition of APG. The concentration added of APG was 0.2 g/g TS, that is the optimal APG dosage for SCFAs production found by Zhao et al. (2015c). The results of their experiments are shown in Figure 31.

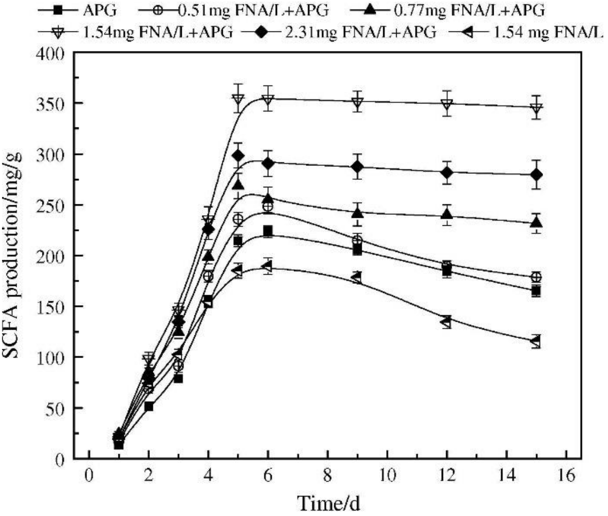


Figure 31- Effect of FNA concentration on the production of VFA from WAS acidogenic fermentation in the presence of APG. (Liu et al., 2018)

As we can see in Figure 31, the maximum yield of VFA was 354.6 mg/g at 1,54 mg/L FNA and APG presence, which was great higher than the yield of FNA or APG alone. The combination of FNA and APG pretreatments also shortened the fermentation time to reach the maximal VFA production to 5 d, whereas FNA alone or APG alone had a fermentation time of 6 d. It was shown by a mechanism study that the FNA pretreatment combined with the presence of APG promoted the sludge disintegration, hydrolysis and acidification while it inhibited the methanogenesis (see Table 12, where the enzymes activity are reported).

Regarding the VFAs distribution, in Table XX are summarized each VFA percentage for all the different pretreatments. As stated in this Table, in all the tests acetic acid was the main VFA followed by propionic, iso-butyric, n-butyric, n-valeric and iso-valeric acids.

Table 11. VFAs distribution for each different dosage of FNA and with or without APG. (Liu et al., 2018)

Individual SCFA/%	**APG**	**0.51 mg/L FNA + APG**	**0.77 mg/L FNA + APG**	**1.54 mg/L FNA + APG**	**2.31 mg/L FNA + APG**	**1.54 mg/L FNA**
Acetic acid	35.6 ± 1.2	34.9 ± 1.5	36.9 ± 1.6	38.9 ± 1.7	36.2 ± 1.3	41.5 ± 2.1
Propionic acid	23.6 ± 1.1	26.5 ± 1.1	24.5 ± 1.1	28.9 ± 1.2	21.6 ± 1.1	27.1 ± 1.6
n-butyric acid	12.6 ± 1.4	12.3 ± 0.7	10.3 ± 0.6	10.3 ± 0.6	11.3 ± 0.6	9.2 ± 0.8
Isobutyric acid	13.5 ± 1.0	11.6 ± 0.6	12.3 ± 0.9	10.3 ± 0.5	12.3 ± 0.7	8.6 ± 0.4
n-valeric acid	9.5 ± 0.6	7.9 ± 0.5	9.5 ± 0.8	7.6 ± 0.4	10.5 ± 0.6	7.5 ± 0.5
Isovaleric acid	5.2 ± 0.3	6.8 ± 0.5	6.5 ± 0.4	4.0 ± 0.2	8.1 ± 0.4	6.1 ± 0.4

Table 12. Effect of FNA combined with APG on the key enzyme activities in sludge anaerobic fermentation.

	Protease	α-glucosidase	AK	BK	F420
FNA/%	100 ± 5.6	100 ± 4.5	100 ± 5.6	100 ± 4.7	100 ± 4.2
APG/%	98 ± 4.2	102 ± 5.2	95 ± 4.6	106 ± 6.1	112 ± 5.2
FNA + APG/%	121 ± 6.2	126 ± 6.3	134 ± 5.8	112 ± 5.2	85 ± 3.2

Sulfite pretreatment + pH 9.5:

Sulfite pretreatment is another chemical pretreatment that consist in maintaining the WAS to dosage from 0.2 to 0.48 g S/L of sulfite at pH 5.0- 7.0 for 12 -36 h (Zan et al., 2016). It is a promising pretreatment since it is eco-friendly because sulfite can be recovered from the sulfite-laden wastes.

Zan et al. (2016) found that this pretreatment facilitated the release of organic substrates by 2- 5 times when compared to WAS without pretreatment. These same authors, in a posterior study (Zan et al.,2019), verified that the hydrolysis rates and biochemical methane potential (BMP) of WAS were significantly improved when it was pretreated with 0.8 g S/L of sulfite at pH 6.0 for a duration of 24 h.

Liu et al. (2020) studied the sulfite as a pretreatment for the alkali fermentation (pH 9,5) of WAS. The pretreatment consisted in achieving the desired sulfite concentration on the reactor (100, 300, 500, and 800 mg S/L), and then maintain the pH at 6.0 for 1 d (Zan et al., 2016; Zan et al., 2019). The results are shown in Fig. 32.

These authors reported that the optimal VFA production were 324.8 ± 9.5 mg COD/g VSS at 0.5 g S/L pretreatment combined with 4 d of alkaline (pH 9.5) fermentation, that was 16.2-, 2.0- and 2.9-fold of that in the blank, sole pH 9.5 and sole sulfite pretreatment systems, respectively. Also, the total time of this combined pretreatment was only 5 d, while it was of 9 d in the blank and 7 d in the sole pH 9.5 systems. This was thanks to the abundance of bacteria related to hydrolysis, acidification, and especially acetogenesis (including Proteiniclasticum sp., Alkaliphilus sp., Romboutsia sp., Tissierella sp., etc.) that were improved in the integration system. Moreover, the sulfite pretreatment performed well the disintegration of EPS and cell envelope of sludge flocs. Regarding the distribution of VFA, as the concentration of sulfite increases the acetic acid composition increases and the propionic acid composition decreases. This was because of the residual $SO_3{}^{-2}$ that facilitated the conversion of propionate and butyrate acid to acetate during alkaline fermentation.

Liu et al. (2020) reported that the cost of this integration system was lower than sole alkali fermentation in terms of cost the cost of the chemicals for the VFA produced. In fact, the cost of the sulfite pretreatment is 70 .3 USD/t TS (Liu et al., 2020) that is cheap compared to others.

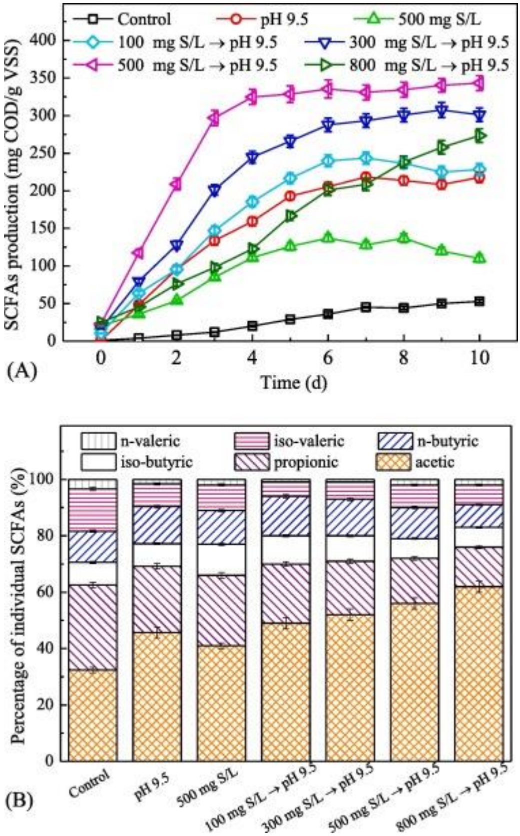


Figure 32. Total VFA production (A) and distribution (B) during acidogenic fermentation of WAS with different pretreatments (A) (Liu et al., 2020)

ULTRASONIC PRETREATMENT:

The ultrasonic pretreatment of WAS consist in maintaining WAS at a frequency around 20-30 kHz and a short time (around 10 - 60 min) (Yan et al., 2010; Liu et al., 2009).

In Yan et al. (2010) this pretreatment was performed at a frequency of 20 kHz and a time of 10 min. The results of their study are represented in Fig. 3XX. In this Figure, the reactor of blank test one corresponds to ultrasonic pretreatment (1.0 kW/L) without pH adjustment and blank test two correspond to the reactor with no ultrasonic pretreatment and pH adjusted to 10.0.

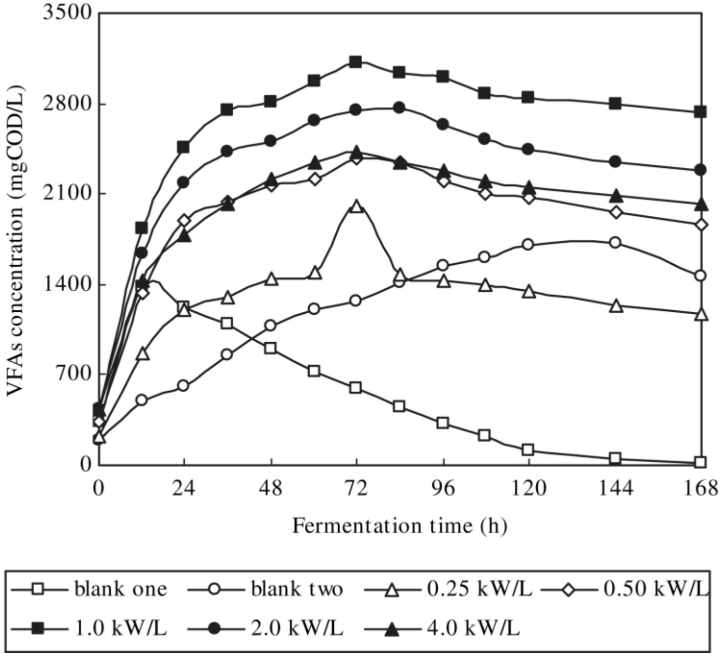


Fig. 33- Effect of ultrasonic energy density on VFA production. From Yan et al. (2010)

As it is shown in Fig. 33, in all the reactors (except the blank one and two), the production of VFA increased between 0 and 72 h and decreased in the range of 72-168 h. This might be attributed to the fact that the increase of fermentative soluble protein and carbohydrate apparently slowed down when the fermentation time exceeded the 72 h. The maximal VFA production was at ultrasonic energy density of 1.0 kW/L with a VFA production of 3109.8 mg COD/L. When ultrasonic energy density increased further than 1.0 kW/L, the VFA production decreased due to the fact that most microbial cells were destroyed. Also, the activity of key hydrolytic and acidogeneic enzimes were optimized at ultrasonic energy density of 1.0 kW/L.

It can also be seen in Fig. 33 that the maximal VFA production in blank test one took place at the short fermentation time of 12 h (1373.2 mg COD/L). This might be since the VFA were mainly produced from the soluble organic matters hydrolyzed by ultrasonic treatment and only a small amount of particulate of the substrate were hydrolyzed during the fermentation. Also, it was due to the activity of the methanogenic bacteria that were not inhibited in the working pH range. In blank test two, the fermentation time was 144 h (6 days) with a maximal concentration of VFA 1713.3 mg COD/L. So, these data suggest that the combination of ultrasonic pretreatment and the alkaline fermentation enhances the VFA production and reduces the fermentation time for the maximal VFA production.

Regarding the distribution of VFA, as we can see in Fig. 34 the ultrasonic energy density increased from 0 to 4.0 kW/L, the acetic acid percentage decreased from 52.3 to 44.9%, and the iso-valeric and propionic percentages both increased from 14.9 and 11.4% to 21.5 and 15.2%, respectively.

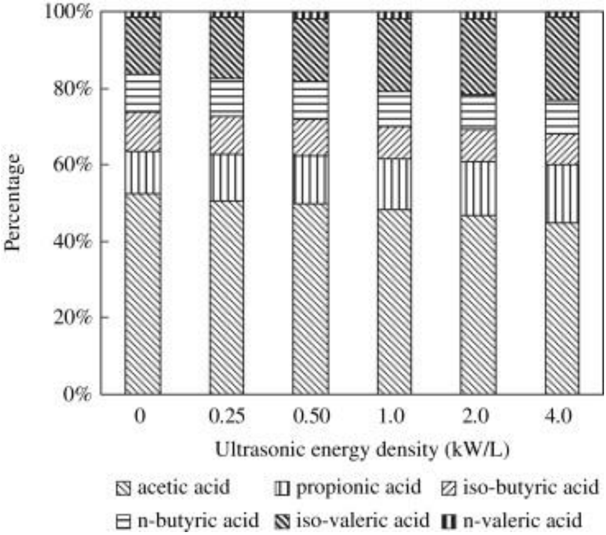


Fig 34 -Distribution of VFAs at different ultrasonic energy densities with fermentation time of 72 h. (Yan et al., 2010)

APPENDIX 2: Polyhydroxyalkanoates (PHAs)

From a biological point of view, polyhydroxyalkanoates (PHA) are reserve materials under unbalanced growth conditions produced by various prokaryotic microorganisms (Mannina et al 2020, Koller 2021). Indeed, PHAs provide microbial cells a competitive advantage to withstand harsh environmental conditions, such as lack of nutrients or environmental stress. In fact, this is used to select PHA-producing biomass as will be shown. From a technological point of view, PHAs are materials with physicochemical properties like plastics produced by the petrochemical industry and are expected to gradually replace polyolefins derived from crude oil as they have the advantage of being fully biodegradable, biocompatible and produced from renewable carbon sources. The most common PHAs are poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate), i.e. P(3HB) and P(3HB-co-HV), respectively. The properties of these biopolyesters, such as degradability, composting performance, thermal melting and decomposition behavior, crystallinity, strength or elasticity, are predefined during their biosynthesis in living cells.

The current price of these PHAs depends on the monomer composition and is generally higher for copolymers. Their price ranges from 2.2 to 5.0 € kg^{-1}, which is significantly lower than the 10-12 €/kg^{-1} price range they commanded last decade (Gholami et al., 2016). Currently, PHA is marketed and used in packaging, food services, in the agri-food industry and in biomedicine (a field in which it has experienced the highest growth in the last 5 years). However, according to the RES URBIS project, discussed below, PHA generated from organic waste by CMM is expected to be used for other applications, such as secondary packaging or contaminated soil remediation, among others (Dosta and Mata-Álvarez, 2017).

Current efforts to make PHA production efficient and sustainable in terms of economic and environmental impact are mainly related to the applied microbial production strains, types of advanced bioreactors, continuous or batch culture processes, sustainable and efficient techniques for product recovery and, in particular, the choice of raw materials. Only recently, also the scientific disciplines of genetic engineering, metabolomics, bioinformatics and nanotechnology consolidate their role in the field of PHA research (Koller, 2021).

For example, continuous production processes for PHA biosynthesis look promising on lab scale when compared with batch and fed-batch cultivation setups. Thus, the next step should be the implementation of such continuously operated processes in pilot production plants. This is essential to produce high quantities of PHA of constant composition and quality on demand (Koller, 2021).

Especially, the further processing for PHA extraction still needs to be optimized as the selection of an appropriate PHA recovery method must be made on a case-by-case basis depending on the microbial production strain and the required purity and quality of the product. At present, there is no single technique that unequivocally constitutes the best solution for all types of PHA, all production strains and all fields of application of the isolated products, and the literature remains contradictory in this regard (Koller, 2021).

Finally, there are still many possibilities to develop new PHA-based composite materials with tailored properties (such as permeability, density, degradability, hydrophilicity or conductivity) by using other cheap materials to process them together with PHA and generate PHA-based blends and (nano)composites with different characteristics. These materials may be a paradigm shift in the packaging sector, especially for easily perishable products, and may even outperform current fuel-derived plastics. (Koller, 2021)

1.1. The main PHAs: polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV)

Polyhydroxybutyrate (PHB) and polyhydroxyvalerate a naturally occurring β-hydroxyacids. As for their production from VFA, PHBs are synthesized from VFA with an even number of carbons (i.e. acetic acid and butyric acid). In contrast, PHVs are synthesized from VFA with an odd number of carbons (propionic acid and valeric acid). For this reason, the distribution of VFA is important to produce the desired type of PHA. For example, if it is desired to produce more PHB, it would be useful to work at basic pH because this will produce a higher amount of acetic acid. Or, if it is desired to produce more PHV it could be used a co-substrate with a high lipid content to produce more propionic acid (Yin et al., 2016).

In addition to PHB and PHV, a copolymer of both (PHB-PHV) can be synthesized to improve the characteristics of the thermoplastic. For example, PHB can be used for bioactive and biodegradable composite implants that will guide tissue growth and be replaced eventually by newly formed tissue, due to its ability to degrade and resorb in the human body environment.

But, since its usefulness is limited by its brittleness, the addition of polyhydroxyvalerate (PHV) to the PHB polymer chains can improve the ductility and processability of the polymer. For this reason, polyhydroxybutyrate-co-hydroxyvalerate (PHB–PHV) with varying molar ratios of HV has been the material of extensive, on-going research for biomedical applications (Chen et al., 2002)

In fact, many researchers around the world are currently working on how to overcome the drawbacks of this co-polymer (such as lack of mechanical strength, water sorption and diffusion, electrical and/or thermal properties, etc.) because it is a promising material to substitute the petroleum-derived polymers (Rivera-Briso et al., 2018).

1.2. The PHA production process using mixed microbial cultures

The production of PHA with pure cultures has been studied but has very high costs. For this reason, there is an increasing tendency to use mixed microbial cultures (MMCs) in order to reduce costs. Indeed, pure cultures require working under aseptic conditions and, moreover, with a specific raw material (glucose, etc.). On the other hand, working with mixed cultures saves the requirement of having to maintain aseptic conditions and we cheap raw materials (as industrial and agricultural wastes) could be used. According to Reis et al. (2003), the use of MMC and waste streams can reduce the current cost of PHA production by up to 50%, although the efficiency of PHA accumulation is lower (60% PHA on a dry basis compared to 80% on a dry basis for pure crops).

The difference between pure cultures and mixed cultures is that a PHA-accumulating biomass selection stage must be added. The stages of a PHA production process with MMCs are shown in the Figure 1. In the following sections, these process stages are briefly discussed.

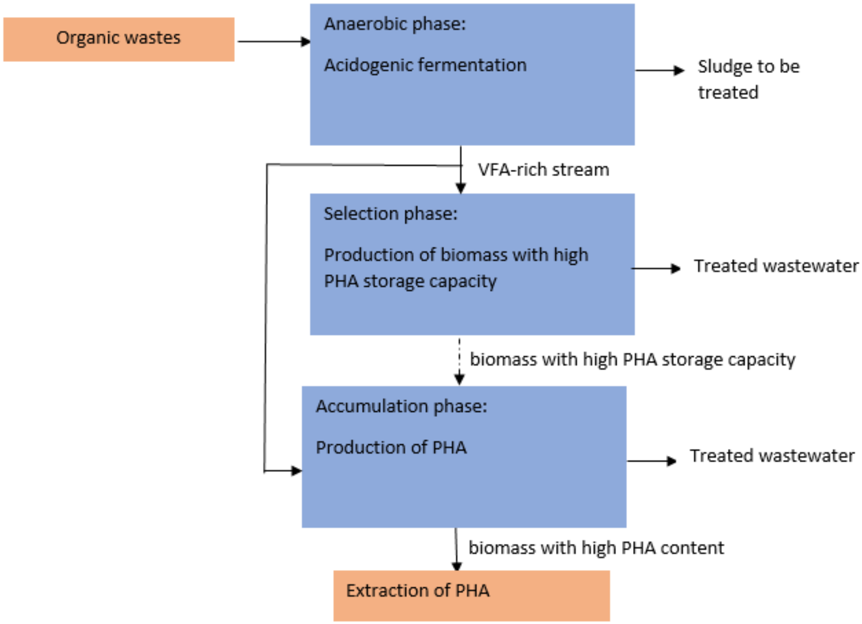


Figure 35. Block diagram of the PHA production process from organic wastes (Reis et al., 2003; Dosta et Mata-Álvarez, 2017).

Anaerobic fermentation.

Anaerobic fermentation of biodegradable organic wastes is an effective bioprocess to produce volatile fatty acids (VFA) and other low molecular weight organic compounds.

The fermenter has several operational parameters (pH, T, HRT, ORL, etc.) that are important for optimizing VFA production as studied in this project. VFA are precursors of PHA and the more VFA produced, the more PHA can be formed. For example, it is known that at mesophilic (37 °C) or thermophilic (55 °C) temperatures and without pH control, 50% of the volatile solids at the fermenter inlet can be converted to VFA (Dosta and Mata-Álvarez, 2017).

During fermentation, the pH drops due to the concentration of VFA and the concentration of ammonium (NH_4^+) increases due to ammonification processes.

Selection of PHA accumulating biomass.

Before producing PHA, biomass that can convert VFA to PHA must be selected. This is commonly done by alternating satiation stages where bacteria have readily biodegradable carbon sources, with starvation stages where bacteria lack such carbon sources. Thus, bacteria that can store PHA (PHA has the function of a storage polymer) have a competitive advantage over other bacteria in the starvation stages.

During the satiety stage, the consumption of organic carbon is due both to bacterial growth processes and to storage processes in the form of PHA. On the other hand, in the starvation stage, the bacteria that have accumulated PHA continue to carry out growth processes, while the others undergo death-regeneration processes (Henz et al., 2000).

The parameter that must be regulated to achieve a good selection of PHA-producing biomass is the duration of the satiation stage with respect to the starvation stage. It is considered that the satiation time with respect to the total aerobic reaction time of the SBR cycle should be 20% or lower (Reis et al., 2011; Tamis et al., 2014).

Although the procedure of alternating satiety stages with starvation stages is the most common, there are also others. This method is very efficient for low OLR (2 g-COD $L^{-1}d^{-1}$), but for high OLR (6 g-COD $L^{-1}d$-^{1}) other selection methods should be used. Moreover, it should be considered that it is desirable to work at high ORL to improve PHA productivity. Therefore, Oliveira et al. (2016) studied a method that separates carbon feeding from nitrogen feeding. That is, carbon is fed at the beginning of the cycle while nitrogen is fed at the end of the satiation stage. They saw that this method at high ORL is more effective than the conventional method.

Produccion of PHA.

Once the biomass enriched in PHA-accumulating bacteria has been selected, an accumulation stage should be applied to increase the proportion of PHA in this biomass. This stage is usually carried out in batch reactors in which purged biomass is fed from the PHA-accumulating biomass selection reactor and an organic carbon source is supplied under satiation conditions, in order to increase the PHA content of the biomass under aerobic conditions.

The composition of the VFA mixture in the fermentation liquid, the operating conditions (the organic loading rate applied, the working pH range, the temperature, etc.) will affect both the percentage of PHA produced and its composition.

Another key aspect in this stage is the concentration of nutrients, so that VFA consumption is promoted to accumulate bioplastics and the synthesis of new biomass is minimized (Dosta et Mata, 2017).

Extraction of the PHA.

The most common method for extracting PHA from biomass is the chloroform extraction process, as it is a simple and effective method for separating PHA granules from biomass, and their subsequent precipitation with an alcohol. By using this method, it is possible to obtain a highly purified PHA without degrading the synthesized PHA molecules. Other halogenated organic solvents can also be used or also performing cell lysis using sodium hypochlorite, although it carries the risk of partial degradation of the bioplastic and provides a PHA with lower molecular weight (Samori et al., 2015).