RESOURCE RECOVERY FROM CO-DIGESTION OF ORGANIC WASTE WITH SURPLUS ACTIVATED SLUDGE VIA THE CARBOXYLATE PLATFORM

by

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The candidate confirms that the work submitted is his own and that appropriate credit has been given where reference has been made to the work of others.

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ABSTRACT

Waste activated sludge (WAS) is an important residue generated from Wastewater Treatment Plants (WWTPs) with a high amount of organic and inorganic resources. In view of this, WAS management systems have changed towards improving the use of waste biomass as a feedstock for bioenergy generation and nutrient recovery and reuse.

This study assessed the potential of using WAS as the main feedstock for the generation of high-value chemicals like volatile fatty acids (VFAs), via the carboxylate platform. In order to achieve that, a series of experiments were conducted with the aim to identify the main process variables controlling VFA production in batch and semi-continuous stirred tank reactors (CSTRs).

In the first stage, acidogenic fermentations were run for 21 days using iodoform as an inhibitor of methanogenic bacteria, reaching VFAs yields of 0.238 g TVFAs/g TVS_{WAS} with iodoform (CHI_3) in a ratio of 6 mg CHI_3/g VSS and an Organic Loading Rate (OLR) of 5 g TVS_{WAS}/L.

The second stage comprised the acidogenic fermentation of high pressure thermal hydrolysis (HPTH)-WAS under different pH conditions (4-1) with results of 0.415 g VFAs/g TVS at pH 9.0 and C/N=8.77, which emphasize the strong effect that pH has on VFA production and speciation and, on the inhibition of methane (CH_4) generation.

In order to improve VFAs production from HPTH-WAS, acidogenic co-fermentations at pH 9.0 were conducted using thermally pre-treated food waste and algal biomass (*Chlorella vulgaris*). Optimum results reported a yield of 0.496 g VFAs/g TVS at C/N=12.72 for fermentations using a blend of 25% HPTH-WAS/75% HPTH-Food waste and 25% HPTH-WAS/75% HPTH-*Chlorella vulgaris* with VFA yields of 0.378 g VFAs/g TVS, C/N=5.08. This suggests that HPTH pre-treatment and co-fermentation had a positive effect on the final production of VFAs despite of the C/N ratio used. Finally, experiments using semi-CSTR reactors fed with HPTH-WAS at pH 9.0 reported yields of 0.539, 0.328 and 0.364 g VFAs/g TVS for fermentors with OLRs of 0.3, 0.6 and 1.0 g TVS WAS/L·d, respectively. This suggests that increments in OLR have a null effect on VFAs production. Fermentations working with 0.3 g TVS WAS/L·d presented overall VFAs production which stoichiometrically exceeds in 31% the methane produced in AD experiments ran in this project. The OLR presented a null effect on the speciation of the VFAs as acetic acid was present in concentrations above 80% of the carboxylic acids content in all CSTR experiments. These results confirm the potential opportunities for high-value chemicals production from HPTH-WAS as part of the development of the biorefinery concept in existing WWTPs.

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Abbreviations

acetyl-CoA	Acetyl Coenzyme A
AD	Anaerobic Digestion
AF	Acidogenic Fermentation
BES	2-bromoethanesulfonic acid sodium
BMP	Biochemical Methane Potential
CH_4	Methane
CI	Confidence Intervals
C/N	Carbon-to-Nitrogen ratio
CO_2	Carbon Dioxide
CP	Carboxylate Platform
CSAF	Continuous Acidogenic Fermentation
CSTR	Continuous Stirred-Tank Reactor
d	Day
FW	Food Waste
GC-FID	Gas Chromatography–Flame Ionisation Detector
HPTH	High Process Thermal Hydrolysis
HRT	Hydraulic Retention Time
L	Litre
$L_{fermentation\ broth}$	Litre of fermentation broth
ND	Not detected
NmL	Millilitres normalised at 1 atm and 0°C
$N\text{-}NH_4$	Nitrogen Ammonia
OFMSW	Organic Fraction of the Municipal Solid Waste
OLR	Organic Loading Rate
ppm	Parts per million
SCOD	Soluble Chemical Oxygen Demand

SD	Standard Deviation
SMP	Stoichiometric methane potential
SPSS	Statistical Package for the Social Sciences
SRT	Solid Retention Time
STP	Standard Temperature and Pressure
Su-COD	Suspended Chemical Oxygen Demand
TCOD	Total Chemical Oxygen Demand
$TCOD_f$	Total Chemical Oxygen Demand final
$TCOD_i$	Total Chemical Oxygen Demand initial
TKN	Total Kjeldahl Nitrogen
TS	Total Solids
TSS	Total Suspended Solids
TVFAs	Total Volatile Fatty Acids
TVS	Total Volatile Solids
$TVS_{substrate}$	Total Volatile Solids from the substrate
TVS_{WAS}	Total Volatile Solids in WAS
VFAs	Volatile Fatty Acids
$VFAs_{SCOD}$	VFAs in terms of SCOD (mg O_2/L)
VSS	Volatile Suspended Solids
$VSS_{inoculum}$	Volatile Suspended Solids from the inoculum
WAS	Waste Activated Sludge
WWTP	Wastewater Treatment Plant
WWTW	Wastewater Treatment Works

Chapter 1. INTRODUCTION

1.1 Justification

The evolution of modern societies, population growth and changes in consumer habits and lifestyle are inextricably linked to world's demand for petroleum and its derivatives. However, the discovery of new oil and gas deposits has decreased in the past decades, which brings more uncertainty over world's fossil fuels reserves. Coupled with this lack of fossil fuel resources to meet future demand, environmental greenhouse gas emissions (e.g. CO_2, N_2O and CH_4) have raised sharply contributing to the global climate change. On the other hand, the production of wastes (e.g. Municipal Solid Waste - MSW, wastewater, sewage sludge, etc.) has also increased resulting in issues of its own.

Sustainable energy sources (e.g. solar, wind, hydro-power, biomass, etc.) are an alternative to fossil fuels to meet the increasing demand for energy, however, the main dilemma is how to make such alternatives economically feasible and environmentally sustainable in the long term.

Considering the uncertainty of oil reserves, their non-renewability and the increasing of waste production, the most promissory pathway to provide an alternative to fossil fuels and their derivates seems to be the development of organic waste-based energy and by-products. This route has the potential to convert undesirable wastes into sustainable/alternative fuels and/or chemicals with the subsequent reduction in treatment/production costs dependence on fossil fuels (Angenent et al., 2004; Agler et al., 2011; Chang et al., 2010).

A novel process to produce high-value products by fermentation is the Carboxylate Platform (CP), which can use organic wastes as feedstock and operates through inhibiting the action of methane-producing bacteria. It is aimed for recovering the short-chain carboxylic acids (e.g. acetic, propionic, butyric, etc.) to convert them into liquid biofuels (e.g. ethanol, butanol, etc.) or valuable chemicals (e.g. acetic acid, propionic acid, esters, etc.). Products from the carboxylate platform process can easily join existing market biofuels chains, unlike gas biofuels - particularly for transportation. However, further research is needed to determine the best process conditions for the potential technical and economic feasibility of the CP, to enhance the production-supply chain and to define a simplistic and cost effective process,

which meets social, economic and environmental needs in order to develop the biorefinery concept for the valorisation of organic wastes.

A promising waste for energy recovery via the carboxylate platform is the municipal waste activated sludge (WAS) (organic fraction: 60-80%), which currently is stabilised and used for methane production via Anaerobic Digestion (AD) (Holtzapple et al., 1999; Mottet et al., 2009; Rughoonundun et al., 2012).

According to DEFRA (2012a) and (EUROSTAT, 2014), the United Kingdom (UK) registered a production of 1.53-1.81 millions of tonnes of sewage sludge in 2008, 1.42 in 2010 and 1.13 in 2012; 66% of this waste was treated by AD and only 60% of the biogas was used as renewable energy (i.e. 115 megawatts, equivalent to 90% of energy produced by AD in the UK). Considering that methane generation yield from sludge can reach 9 to 16 m^3/wet ton of treated sludge (Stephen Allen, 2011), it can be envisaged that by 2020 biogas produced from sewage sludge could represent between 0.2-0.7% of the total energy consumed in the UK (Defra, 2011). Alternatively, WAS can be processed by acidogenic anaerobic fermentation in the production of VFAs as the first step of WAS stabilisation, and with this, new ways are open for resource/nutrient removal and recovery (Kim et al., 2006; Maharaj and Elefsiniotis, 2001; Wu et al., 2009; Yan et al., 2010; Yuan et al., 2009; Yuan et al., 2011; Zhang et al., 2009; Zhuo et al., 2012). In addition, VFAs can be used as feedstock for high-value chemicals and biofuels, but more research is needed to enhance process efficiency and yields to make the process profitable and environmentally sustainable. Several researchers have worked with different organic wastes such as green/lignocellulosic material, industrial wastewater, chicken manure, cattle manure, paper, sugarcane bagasse, glycerol, etc. (Bengtsson et al., 2008; Forrest et al., 2010a; Rughoonundun et al., 2010; Rughoonundun et al., 2012) but very few focus on the valorisation of municipal waste activated sludge and the co-fermentation with thermally treated organic wastes using different conditions (pH or iodoform dosis) that could achieve the inhibition of the methane generation and lead to the production/accumulation of VFAs. Regarding this, the present project aimed to determine the best process conditions to produce of volatile fatty acids (VFAs) in acidogenic fermentation of municipal waste activated sludge (WAS) and mixtures with food waste (FW) or microalgae for the development of the Carboxylate Platform (CP) concept in wastewater treatment works.

In summary, the use of WAS, as a feedstock in the production of alternative liquid fuels and high-value chemicals, can be a sustainable option to replace fossil fuels, although further research is needed to assess its technical, economic and environmental viability.

1.2 Aim and Scope

1.2.1 Aim

The aim of this research project is to determine the best process conditions for the production of volatile fatty acids (VFAs) from mixed acidogenic fermentation of the mixtures of municipal waste (surplus) activated sludge (WAS) and food waste (FW) or microalgae to provide scientific evidence towards the development of the Carboxylate Platform concept in municipal wastewater treatment works.

1.2.1.1 Objectives

- To identify the effect of iodoform on mixed acidogenic fermentations for VFAs production from thermally pre-treated municipal HPTH-WAS under batch conditions.
- To identify the effect of pH on mixed acidogenic fermentations for VFAs production from thermally pre-treated municipal HPTH-WAS under batch conditions.
- To evaluate the effect of different thermal pre-treatment conditions of food waste or microalgae on the net solubilisation of organic matter as a first step in the formation of VFAs during mixed acid fermentation from low-grade waste.
- To assess the influence of the C/N ratio on the co-fermentations of HPTH-WAS with food waste and microalgae on the production of VFAs in batch anaerobic acidogenic fermentation.
- To test different OLRs in a mixed acid fermentation carry out in a semi-continuous stirred-tank reactor (CSTR) for maximising VFAs production by fermenting thermally pre-treated organic feedstock.
- To carry out mass balances of the mixed acidogenic fermentation reactors for the understanding of the carboxylate platform process operated with different conditions for its implementation in sewage treatment works.

1.2.2 Scope

This project studied the potential production of short chain fatty acids as acetic, propionic, butyric and valeric acids from mixed acid fermentation of thermally treated waste activated sludge on its own and mixed with pre-treated/untreated food waste or microalgae, in order to present an alternative route by producing valuable chemicals instead of biomethane and also minimizing the investment net in the treatment of waste activated sludge dealing with the challenges in the UK wastewater treatment (water scarcity, water quality, climate change, population growth, rising customer expectations, rising environmental standards and economy) (OFWAT, 2015; Van Dijk et al. 2013)

Chapter 2. LITERATURE REVIEW.

2.1 Energy Demand and Supply

The recent evolution of the worldwide society and the change of their consumption habits are extremely bound to fossil fuels usage, specifically crude oil and its derivatives. This has induced to an increase on the usage of liquid/gas fuels for transportation and the production of petrochemicals due to a rapid and sustained growth of world population in the past five decades. Owing to this issue there is a big political and social concern about the security of energy since the fossil fuels are a non-renewable resource. Coupled to this limitation of resources, the net emissions of greenhouse gases such as carbon dioxide, methane and nitrous oxide, have been raised due to the effect on the climate change (Agler et al., 2011; Chang et al., 2010; Cherubini, 2010; Fernando et al., 2006; Solomon, 2007). In addition, the production of organic wastes (municipal solid wastes, wastewater, sewage sludge, agricultural and industrial biowastes) has also increased producing other problems worldwide (Golub et al., 2013; Marshall et al., 2013).

Considering all this current problems, the society has begun to look for new and sustainable energy sources as an alternative to fossil fuels in order to supply the increasing demand of energy (Fernando et al., 2006; Marshall et al., 2013). Nowadays, there are some well-known options of renewable energy sources including solar, wind, hydro-power and biomass, but the main dilemma is how to make these green energies economically feasible. The advantages of these renewable energies are the reduction of net greenhouse gasses emissions and the valorisation of the biomass by turning waste into a substrate (Chang et al., 2010; Cherubini, 2010). Recently there has been a shift to view biomass as a feedstock which could be converted into compounds that can be useful as fuels or chemicals; this energy is known as bioenergy and is defined as the production of any form of renewable energy from biological sources (Agler et al., 2011; Angenent et al., 2004; Kamm and Kamm, 2004; Karp and Shield, 2008).

Concurring with this, two promissory pathways to substitute crude oil and sub products seems to be the biomass-to-energy route, which has a high potential due to the large amount of raw materials available; and the waste-to-energy route, due to the increase production of waste and the potential

reduction of the cost in treatment, especially of organic material (Angenent et al., 2004; Chang et al., 2010; Cherubini, 2010).

2.2 Sustainable Energy Sources

Sustainable energy technologies are necessary for the potential problems on global energy security and at the same time, achieve low or zero footprint. Currently, there are many technologies for the energetic sustainability such as hydro energy, wave energy, solar energy, wind energy, geothermal energy, hydrogen production, fuel cells, biomass energy among others (Dinçer et al., 2014). Energy production based on biomass conversion technologies has become more popular in recent years as it implies the recovery of solar energy and carbon dioxide in the atmosphere via the photosynthesis according to the unbalanced formula below (McKendry, 2002):

$$CO_2+2H_2O+\text{light energy}\rightarrow(CH_2O)+H_2O+O_2 \quad \text{.....Equation 2.1}$$

The main advantages of the energy based on biomass are the reduction of the wastes, the increase on the carbon cycle efficiency and the production of energy and valuable chemicals (Bastidas-Oyanedel et al., 2015).

2.3 Bioenergy And Biofuels

The economy of biomass fuels is based in the utilisation of many different types of raw material: animal wastes, starch, sugarcane, paper, wood, agricultural crops, organic wastes, industrial wastes, algae and seaweed, sewage sludge, etc., being most of these options greener alternatives to fossil fuels (Cherubini, 2010; IEA, 2009; Fernando et al., 2006).

By definition, biofuels are gaseous or liquid products from biomass processing that can be used for either transport or heating purposes (Dufey, 2006; Mabee et al., 2004). The generation of biofuels can be assorted according to the biomass used and their competition with food production (Chang et al., 2010). There are three recognized types of biofuels; first, second and third generation (Figure 2.1):

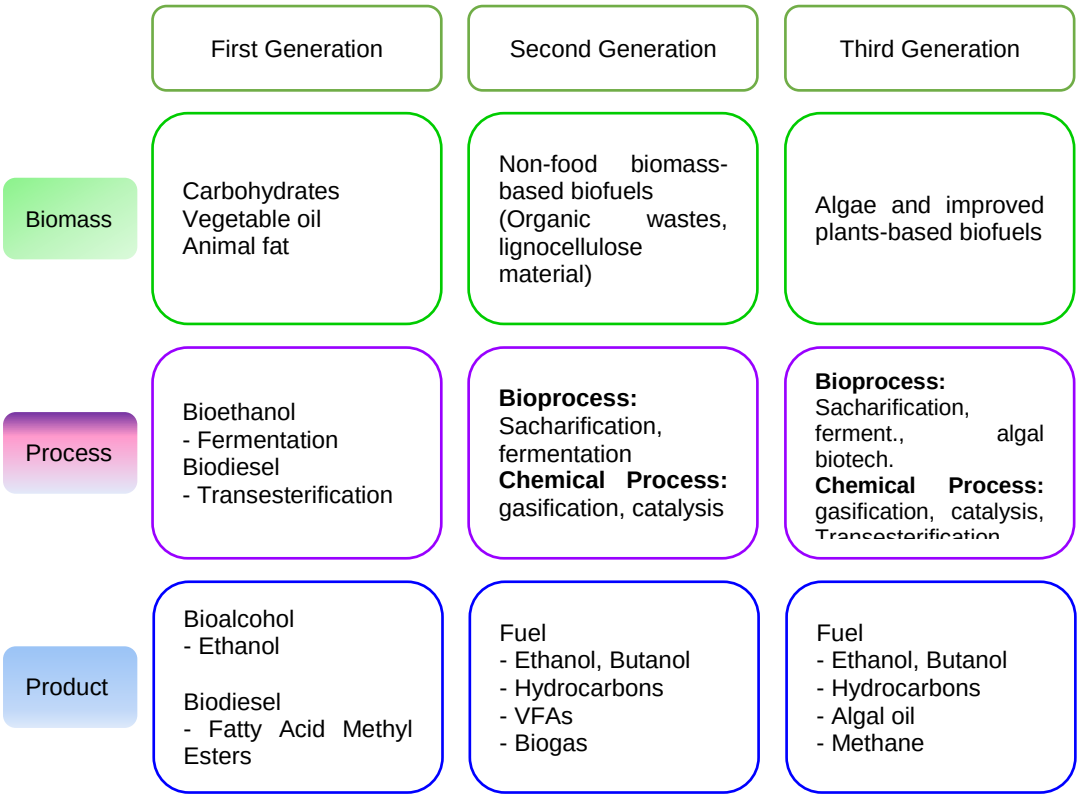

Figure 2.1. Generation of biofuels. (Adapted from Chang et al. (2010); Cherubini (2010); Fernando et al. (2006)).

2.3.1 First Generation Biofuels (Energy from biomass)

The first generation biofuels use grains (i.e., wheat, corn, triticale and rye), sweet potato, cassava, sugarcane and sugar beet, as main feedstocks, which are rich in sugar and starch although there are other feedstock less used as vegetable oil (e.g., sunflower, oil palm and soybean) and animal fat. The production of biofuels from raw materials with high content of simple carbohydrates (i.e., sugar and starch) is extensively spread because the use of traditional, simple and inexpensive technologies and processes as fermentation and sacharification, which represents their main advantage (Chang et al., 2010; Chen et al., 2012). However these varieties of biofuels have some drawbacks like competition with food production, giving agricultural products away from the human food chain; the usage of large extensions of fertile land granted to biofuel production instead of agriculture and farming, which can increase the price of food and feedstock and in the long term, deterioration of productive land (Chen et al., 2012; Cherubini, 2010).

2.3.2 Second Generation Biofuels (Energy from organic waste)

In comparison with the first generation biofuels, the second generation biofuels provide a greener and more sustainable alternative to fossil fuels because their production is based on non-crop feedstocks. There is a wide range of materials used for this purpose from lignocellulose biomass (i.e., grass, wood, chaff, reed, paper, cellulosic municipal solid waste and crop residues) to industrial and municipal organic wastes (e.g., municipal solid wastes, wastewater, manure, and industrial biosludge) (Chang et al., 2010; Cherubini, 2010; Mabee et al., 2004).

The production of second generation biofuels employs a wide variety of feedstocks with different chemical composition and hence, there is a need for diverse routes aimed at converting raw materials in the specific final products (i.e., gasification, torrefaction, hydrothermal processes, enzymatic oxidation, pyrolysis, etc.). Thus, the characteristics of the final products will vary depending on feedstock and the transformation route applied. Utilizing wastes biomass as feedstock in biofuels production has a clear advantage in waste management because residues are considered as a resource for generation of products instead of a waste which needs to be treated; consequently, biofuels can be produced in a sustainable way and thus maximise the social, economic and environmental benefits (Angenent et al., 2004; Cherubini, 2010; Fernando et al., 2006).

2.3.3 Third Generation Biofuels (Algae based biofuels)

The third generation of biofuels focused in using solar energy to produce big quantities of algae (non-lignin content) and genetically modified plants, which are considered feedstock with low growth requirements and high productivity and availability, to produce biofuels by chemical (gasification – the Fischer-Tropsch process, trans-esterification, etc.) or biological processes (fermentation, etc.) (Beer et al., 2009; Chang et al., 2010).

It is been stated that microalgae biomass could be used for the extraction of biochemicals and biofuels by several methods, such as, direct combustion, pyrolysis, gasification, liquefaction, hydrogen production by biochemical processes, fuel cells, fermentation to bioethanol, transesterification to biodiesel and anaerobic digestion. The latest option presents few advantages, for instance, the usage of the wet microalgae directly from the cultivation reactor and its potential for the utilisation of a high percentage of the organic biomass inside the reactor for energy production (Milledge and Heaven, 2014). Since biomass represents a sustainable way to produce

energy, the industry and research sectors have developed the biorefinery concept as an alternative to fuel/chemical production from crude oil (Fernando et al., 2006).

2.4 The Biorefinery Concept

According to IEA (2009) "*Biorefinery is the sustainable processing of biomass into a spectrum of marketable products (food, feed, materials, chemical) and energy (fuels, power heat)"* (Figure 2.2). Thus, the biorefineries should manage different technologies and process to separate a wide range of biomass resources and maximize the transformation of them into valuable products while minimizing waste streams, in order to replace the current petroleum refineries (Sokhansanj et al., 2003). With the purpose of substituting the petroleum refinery and their non-renewable products, biorefinery tends to be an equivalent complete process in which exist several unit operations and routes to obtain different high-value products (chemicals and fuels) from inexpensive materials, enhancing the profitability, accomplish with the global energy demand, providing sustainable fuels and reaching a complete utilization of the biomass. The main aim of the biorefinery is focused in the production of transportation fuels; being these biofuels able to be mixed with gasoline, diesel or natural gas to couple with the existing energy infrastructure and also being competitive with the current prices of fossil fuels (Cherubini, 2010; Fernando et al., 2006; IEA, 2009).

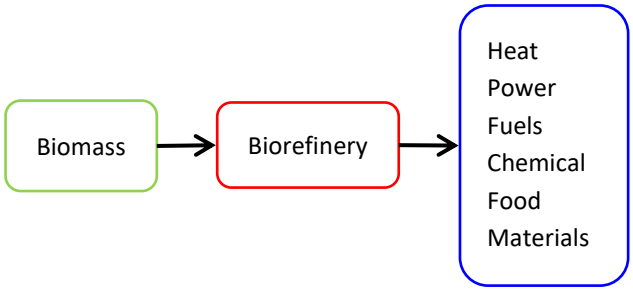

Figure 2.2. Biorefinery concept and the transformation of biomass. Adapted from IEA (2009).

Despite of all this evident advantages of biorefineries, there still are some problems to solve in order to make the process economically viable, for example physical and chemical heterogeneous composition of biomass, unevenness on amount of biomass due to geographical and seasonal conditions, competition from fertile land, etc. (Sokhansanj et al., 2003). Due

to all of this differences and difficulties, there were developed a classification of biorefineries which can be mainly divided according to the biomass used.

2.4.1 Classification Of Biorefineries

Owing to the wide variety of feedstocks and their heterogeneous composition it has been a development of different systems to convert biomass into valuable products. Currently there is no an absolute classification of biorefineries because it can be also based on the feedstock, the actual transformation process (platforms) and the final products (Figure 2.3) (IEA, 2009).

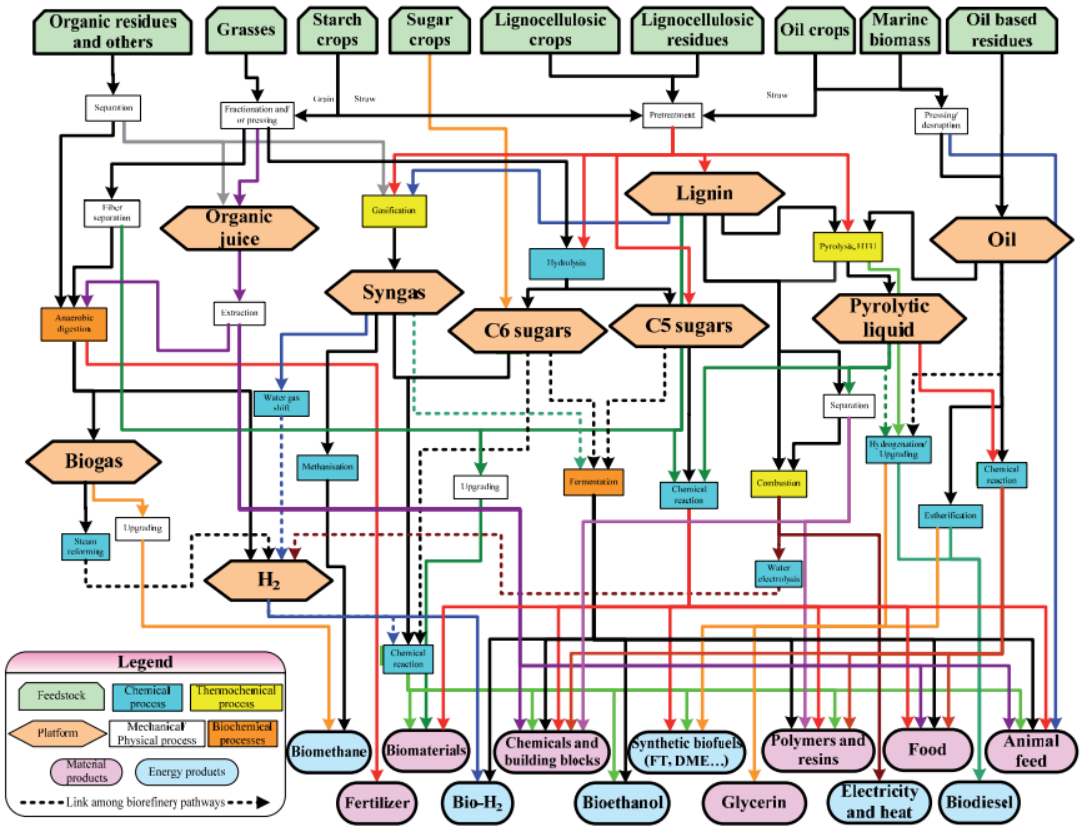

Figure 2.3. Summary of the classification of biorefineries (IEA, 2009).

Because of the extensive types of materials and their composition, every waste could require a different application of transformation process. In view of this, it can be envisaged three biorefineries approaches: 1) the low flexibility biorefinery, where the substrates and products are fixed, for example, fermentation of sugarcane for the production of ethanol and, 2) the medium flexibility biorefinery, which employs few materials for the production of different products depending on the demand. Finally, a third classification

of the biorefinery is the most flexible one, where a wide range of organic substrates such as wastes from agriculture, cellulose material, grass, green plants, the organic fraction of municipal solid wastes, used cooking oil, manure, residues from fruit and vegetables industries, sewage sludge, etc. can be used and the products obtained can be visualized as energy products (bioethanol, biodiesel) or material products (biomaterials, polymers and resins, food, animal feed, fertilizer, etc.)(Cherubini, 2010; de Jong, 2014; Fernando et al., 2006; IEA, 2009; Kamm and Kamm, 2004).

Regarding the transformation processes, the classification of biorefinery includes four principal groups:

1) Biochemical process; which comprises fermentation, anaerobic digestion, enzymatic cleavage, etc.
2) Thermochemical process; involves technologies as gasification, combustion and pyrolysis
3) Chemical process; embraces methods as hydrolysis, hydrogenation, electrolysis, esterification, etc.
4) Mechanical (Physical); process includes unit operations as filter separation, fractionation, extraction, size reduction, etc. (IEA, 2009; Cherubini, 2010).

Taking in account that different feedstock can generate different products due to the wide diversity of technologies applied to the biomass in order to produce valuable products, a special interest is set in research and application of technological routes to engineer the most efficient, economical and profitable system to obtain bioproducts that can compete with the current products from non-renewable fossil fuels (Lee et al., 2012). For the production of these types of biofuels, several technologies have been investigated and applied in bench, pilot and full scale, in order to increase the efficiency on the system and the economic feasibility; these technologies are discussed next.

2.5 Processes for the conversion of organic wastes to energy (bioenergy platforms)

Energy recovery form biomass has been investigated by using different processes such as thermal, chemical, and biological or a combination of them (Figure 2.4). The process election for every particular case depends in

several factors, for example, technical feasibility, simplicity, economical viability, as well as political and social acceptability, in order to reach the sustainability, the energy recovery and the pollution control (Angenent et al., 2004; Chang et al., 2010; Fernando et al., 2006).

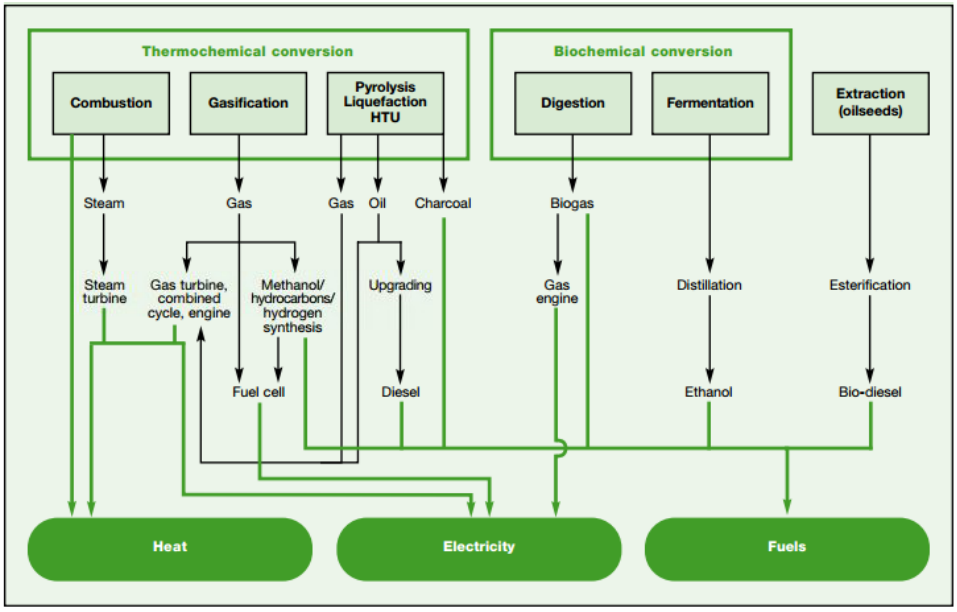

Figure 2.4. Main conversion technologies for biomass to energy (Turkenburg, 2000).

2.5.1 Thermochemical Process

Combustion processes are the most popular methods for biomass conversion and energy recuperation because of its ease of operation. The heat obtained from the combustion process can be used directly for combined heat and power via a heat exchanger. The conventional treatment process includes four standard unit operations, drying, pyrolysis, combustion of volatile material and combustion of the residual char (Bastiaans and van Oijen, 2014; Chang et al., 2010; Fernando et al., 2006).

Typically, after removing moisture from the biomass, thermal decomposition in anaerobic conditions with temperatures above 200ºC, named as pyrolysis, occurs for the production of tars (complex hydrocarbon compounds), liquids and gaseous products (carbon monoxide, carbon dioxide, methane, among others). Tars are furtherly heated up to temperatures above 600ºC to convert it to carbon monoxide, hydrogen and chars. The chars (solid carbon and ash) are finally oxidised at high temperature in aerobic conditions for the

production of carbon dioxide and water vapour (Bastiaans and van Oijen, 2014).

On the other hand, gasification process consists on the transformation, in oxidative conditions at high temperatures which range between 700 to 1500ºC at 7 MPa, of liquid or solid biomass into a combustible gaseous product called syngas, which comprises H_2, CO, CO_2, CH_4, high molecular weight hydrocarbons, H_2O and N_2. The resulting gas can be further used for the production of heat and power (electricity) as well as biofuel and production of chemicals such as bioethanol, methanol, or biodiesel (Kersten and de Jong, 2014).

Last thermochemical process involves pyrolysis, which transforms the biomass into bio-oil which can be used furtherly to produce heat, fuels, electricity and chemicals. This technology implicates the usage of temperatures between 220 to 550ºC in the absence of oxygen at atmospheric pressure, for the generation of charcoal, pyrolysis bio-oil and gases products. Similar to the other two thermochemical processes, the final aim of the pyrolysis is the generation of heat, electricity and biofuels (Oudenhoven and Kersten, 2014).

2.5.2 Biochemical Processes

2.5.2.1 Fermentation

Fermentation of biomass has been perceived as type of first or second generation bioenergy and involves the oxidation of carbohydrates for the generation of ethanol and carbon dioxide according to the equation below (Cuellar and Straathof, 2014):

$$C_6H_{12}O_6 + 2ADP \rightarrow 2CH_3CH_2OH + 2CO_2 + 2ATP \quad \text{.....Equation 2.2}$$

Ethanol is produced currently from petrochemical resources and from renewable sources such as sucrose from sugarcane, starch or lignocellulosic sugars. The main advantages of the production of ethanol from biomass could be the potential quick integration to the current fuels system due to its common usage as a feedstock in other processes. Some important factors to be considered for the production of bioethanol are the price of the feedstock, stability of the system, competition of land when producing organic material for food versus biofuel, etcetera (Angenent et al., 2004; Cuellar and Straathof, 2014).

2.5.2.2 Anaerobic Digestion

The anaerobic digestion refers to the decomposition of the organic matter into gasification and mineralisation. AD is a complex process which involves several stages and biochemical reactions and changes performed by several types of bacteria which works in different conditions, such as, facultative anaerobes and anoxic microorganism for the final production of biogas composed of CH_4 and CO_2 (Khan et al., 2016; Kondusamy and Kalamdhad, 2014; Taricska et al., 2007).

Among the biochemical technologies currently applied at industrial scale, the anaerobic digestion is probably the most popular worldwide due to its low cost, high organic removal, stabilisation of the organic and inorganic waste, low energy requirement, production of biomethane, and simplicity (Angenent et al., 2004; Appels et al., 2008; Dinsdale et al., 2000; Kleerebezem and van Loosdrecht, 2007).

2.5.2.2.1 Biochemistry of the Process

Anaerobic digestion is developed in 4 main stages:

1. Hydrolysis of polymers: in this stage the complex organic material (lignocellulose, starch, proteins, complex carbohydrates, etc.), is converted by a mixed culture bacteria ecosystem and enzymes to soluble and simple compounds as glucose, pentose, aminoacids, etc. It is considered the limiting step as it tends to occur at slow rates (Zhang et al., 2015)
2. Fermentation/acidogenesis: during this phase, the simple compounds from hydrolysis are converted to volatile fatty acids, hydrogen and CO_2.

$C_6H_{12}O_6 + 2H_2O \longrightarrow 2CH_3COOH + 4H_2 + 2CO_2$ ..(Facultative anaerobes) …..……..Equation 2.3

$C_6H_{12}O_6 \longrightarrow CH_3CH_2CH_2COOH + 4H_2 + 2CO_2$ ….(Strict anaerobes)……. .....Equation 2.4

3. Acetogenesis: Conversion of volatile fatty acids to acetate and hydrogen.

4. Formation of methane (methanogenesis): Anaerobic microorganisms classified as archaea transform acetate and hydrogen into methane and carbon dioxide, which are the final products of the process (Figure 2.5) (Hu and Chen, 2007; Metcalf et al., 2010; Nath and Das, 2004; Singhania et al., 2013).

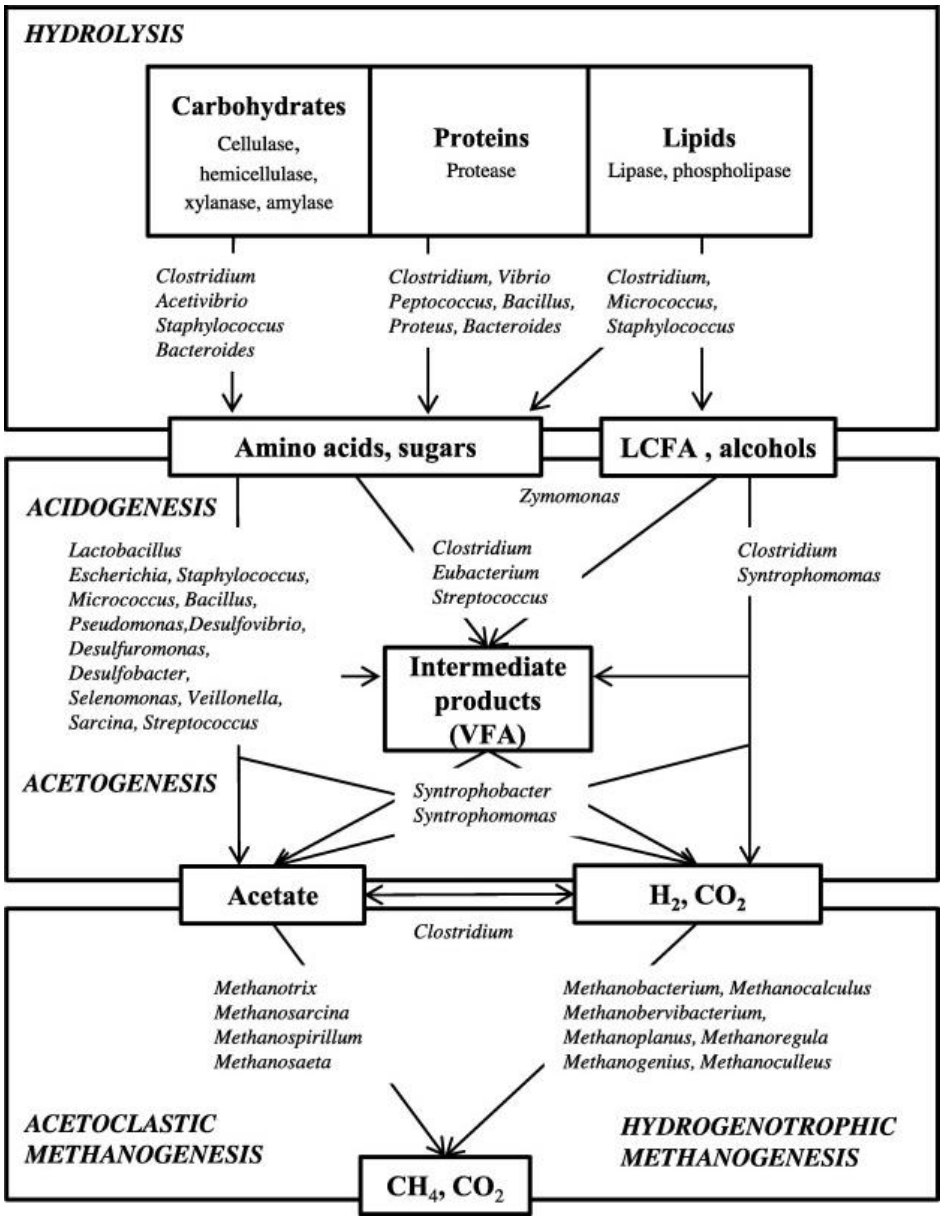

Figure 2.5. Stages of Anaerobic Digestion. Adapted from Metcalf et al. (2010); Singhania et al. (2013); and Gonzalez-Fernandez et al. (2015).

Feedstocks used for anaerobic digestion comprise wastes such as woody biomass and forest residue, agricultural residues, municipal solid waste,

waste activated sludge, organic industrial wastes, beverage industries, etc (Levy et al., 1981).

However, the anaerobic fermentation presents few weaknesses to take in consideration, for example, the presence of recalcitrant compounds in the substrate, too low or high carbon-to-nitrogen ratio (20<C/N>30), toxicity caused by the production of ammonia by the decomposition of proteins and aminoacids, the production of a low cost gaseous product, low/high pH and/or low concentration of substrate (Strathern et al., 1982; Ward et al., 2014).

2.6 The Carboxylate Platform

Taking into consideration the anaerobic fermentation process, it could be envisioned a fourth platform, the Carboxylate Platform or volatile fatty acid platform, which operates the acidogenic anaerobic fermentation in AD through inhibition of the methanogenic phase and recovering volatile fatty acids (VFAs), which are envisaged as building blocks for the production of biofuels or biochemicals (Agler et al., 2011; Angenent and Wrenn, 2008; Kleerebezem and van Loosdrecht, 2007; Marshall et al., 2013).

Acidogenic anaerobic fermentation using a mixed culture provides several advantages as:

5. Inexpensive fermentation reactors can be used.
6. A wide variety of raw materials can be employed (sewage sludge, agricultural residues, manure, organic fraction in municipal solid wastes, food industry wastes, etc.)
7. Pure culture, addition of antibiotics and broth sterilization to prevent contamination are not required.
8. External enzyme inoculation is no required.
9. Mixed cultures have the capability to transform all biomass components (starch, cellulose, proteins, fats, sugars), except lignin, since microorganisms can manage different metabolic pathways.
10. Microbial community is flexible and can adapt to changes in feeding material, slight pH changes and toxins.
11. Volatile fatty acids are main products, which are a single class of compounds.
12. Reduction of waste mass before anaerobic fermentation.

13. Mixed culture fermentation can operate in a continuous system instead of batch process (Angenent and Wrenn, 2008; Datta, 1981; Granda et al., 2009; Marshall et al., 2013; Yuan et al., 2011).

2.6.1 Biochemistry of the process

The carboxylate platform comprises several metabolic changes which are part of the tricarboxylic acid cycle, where the key compounds are the pyruvate, lactate and oxaloacetate as the main intermediates of the metabolism (Figure 2.6).

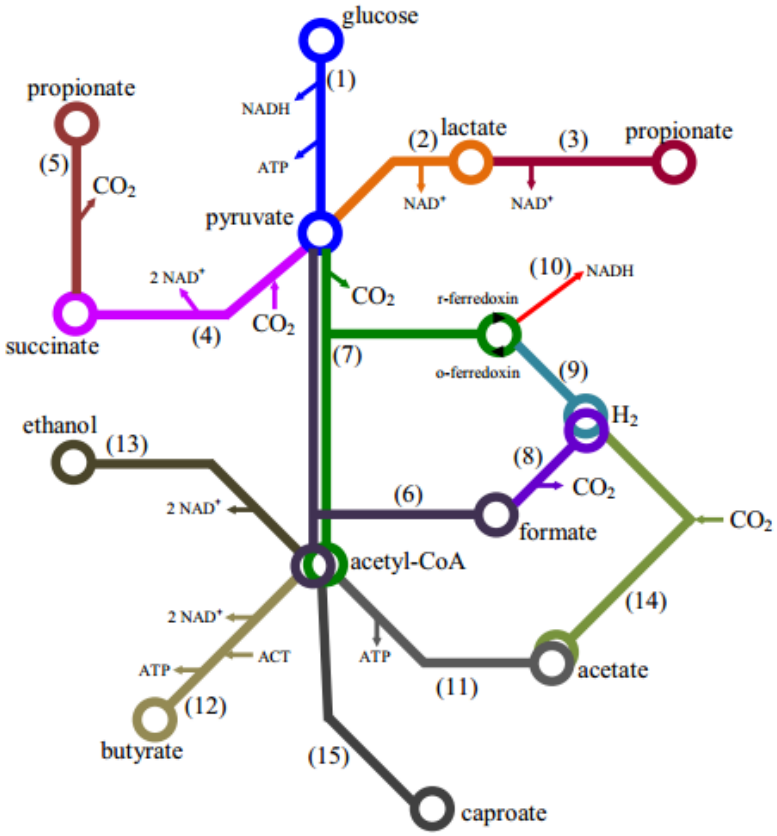

Figure 2.6. Metabolic pathways via the carboxylate platform (Bastidas-Oyanedel et al., 2015).

The glucose suffers a lysis and is converted to pyruvate (1) and 1 mol of ATP. Pyruvate is then transformed into (3, 5) propionate via the succinate pathway (4) or by the lactic acid fermentation (2) (Schiel-Bengelsdorf and Dürre, 2012; Prabhu et al., 2012). In case the pyruvate is not converted into propionate, it might be transformed into Acetyl-CoA which is a complex enzyme-substrate that could have experiment several changes. For example, the direct conversion of Acetyl-CoA to formate (6), acetate (11),

ethanol (13) and caproate (15) performed by different strains of bacteria and conditions, such as the reaction (11) where acetic acid is synthetized by the phosphotransacetylase and the acetate kinase (Thauer et al., 1977b). Acetic acid can be also produced via homoacetogenesis (14) which uses the hydrogen and CO_2 in the system. Butyric acid formation (12) is via the condensation of acetyl-CoA with acetic acid with the involvement of the butyril-CoA dehydrogenase (McInerney and Bryant, 1981; Ntaikou et al., 2010). Other transformations include the decomposition of formate to H_2 and CO_2 (8) via the formate-hydrogen lyase and the pyruvate dehydrogenase pathway (7) which produces ferredoxin as in intermediate for the production of H_2 (Bastidas-Oyanedel et al., 2015). Thus, metabolic routes should be taken into consideration when running experiments from the production of VFAs as certain conditions such as type of inoculum, OLR, HRT, pH, co-digestion, C/N ratio, temperature, etc., could lead to inhibition or spontaneity of specific reactions and then, different profiles of VFAs could be produced (Zhang et al., 2005; Zhang et al., 2009; Zhang et al., 2015).

2.6.2 Operational conditions

The goals of acidogenic fermentation are: 1) high product concentration; 2) minimum methane production; 3) reasonable residence time and 4) high rates of biomass conversion (Holtzapple et al., 1999; Singhania et al., 2013). Currently there are some studies employing acidogenic fermentation of different types of feedstock to produce VFAs (Table 2.1).

For the purposes of obtaining high VFAs yields or to guide the fermentation towards of specific specie of carboxylic acid from the acidogenic fermentation, numerous approaches have been taken, for example, the usage of different types of bioreactors, the co-digestion of different substrates to achieve an optimum C/N ratio, inhibition of the methanogenic phase by additives, tests with different HRT or OLR, and/or the pre-treatment of the biomass (Zeikus, 1980).

One of the most popular practices among anaerobic digestion and fermentation investigations and works is the co-digestion of different organic substrates as it grants two main advantages, firstly, the mixture could perform better because presents a C/N ratio close to optimal and secondly, the reduction on the need of artificial nutrients (Smith and Holtzapple, 2011). According to Georgacakis et al. (1982) the optimal C/N ratio for the production of biogas is from 20/1 to 30/1. If conditions are lower than 10/1, digesters can experience inhibition by the ammonia released, whereas at ratios higher than 30/1 the low bicarbonate alkalinity could cause a failure.

It has been stated that the hydrolysis is the limiting-step on the AD process, which advices the need of pre-treatment of the organic substrate to be used. Among the different pre-treatment processes already tested, alkaline treatment with lime or NaOH at high temperatures and pressure has been proven to be one of the most used (López Torres and Espinosa Lloréns, 2008; Mottet et al., 2009; Rughoonundun et al., 2010; Rughoonundun et al., 2012). However, other pre-treatment process have been tested and include the use of chemical additives (Ji et al., 2010), biological processes (Fdez.-Güelfo et al., 2011; Kim et al., 2006; Pham et al., 2013), acid hydrolysis (Hu and Chen, 2007), and ultrasonic hydrolysis (Yan et al., 2010; Zhuo et al., 2012), which are aimed at breaking down complex compounds as proteins, fats and some carbohydrates, in order to improve its digestibility and consequently, to obtain better fermentation kinetics and product yields.

Instead of a conventional anaerobic process, the pre-treated biomass can be used as feedstock for the mixed acidogenic fermentation for the production of VFAs (acetic, propionic, lactic, butyric, valeric, and caproic acids), which is usually carried out by a dark fermentation microbiome (unknown mixed microbial community). The production and accumulation of biochemicals joined to the avoidance of the methanogenesis step, represent the main differences between the acidogenic fermentation and the conventional anaerobic digestion, in which main products are CH_4 and CO_2 (Holtzapple et al., 1999; Singhania et al., 2013).

Once the fermentation is running, the operation conditions of the process play a key role on the maximisation of production. Among those factors is the pH, which could lead to a higher production of medium-chain carboxylic acids and solvents (propionic and butyric acids, ethanol, propanol) when working under acidic conditions (4-7); whereas alkaline conditions (9-12) could guide the fermentation towards the production of shorter-chain carboxylates (acetate) (Chen et al., 2007; Fang and Liu, 2002; Yang et al., 2014; Zhang et al., 2015). The temperature is also an important factor to consider when working with anaerobic acidogenic fermentation as temperatures below 35°C could cause a decrease on the kinetics of the hydrolytic process and high temperatures could provoke a quick adaptation of the inoculum and hence, causing a shorter lag-phase for the production of VFAs and/or biogas (Bastidas-Oyanedel et al., 2015; Zhang et al., 2015). Others vital aspects are the mixing, solids retention time, bioreactor configuration and additives (Ntaikou et al., 2010).

Table 2.1. Batch studies for volatile fatty acids production.

Reference	Parameter												
	Feedstock	Inoculum	Feedstock pre-treatment	Fermentation temperature (°C)	Substrate's concentration (g/L)	Iodoform (mg/L)	Total VFAs produced (g/L)	Productivity (g total acid/L_{liq}/day)	Yield (g acid/g TVS fed)	Acetic acid (wt%)	Propionic acid (wt%)	Butyric acid (wt%)	HRT
Golub et al. (2013)	Paper	Soil	No treatment	55	8.97 (as TVS)	1.6	2.31	--	0.04	72	1	26	30
Datta (1981)	Corn stover	Cow manure/SS	Lime/ Na_2CO_3	25	--	Low T	--	--	0.55	--	--	--	12
Pham et al. (2012)	Macroalgae	Sewage Sludge	0.5 N NaOH	35	50	30	15.2	-	0.30 – 0.41	52	36	11	5
Pham et al. (2013)	Macroalgae	Sewage Sludge	Biologic	35	40	30	15.6			53	27	15	5
			0.5 N NaOH				12.2			59	23		
Forrest et al. (2010b)	Glycerol	Marine sediment	--	55	80	2	24.0	0.75	0.29	61.6	1.8	36.5	30
Rughoonundun et al. (2010)	WAS	Marine sediment	$Ca(OH)_2$/100ºC	55	50	0.016	10.72	0.34	0.34	65.9	8.76	12.8	28
Forrest et al. (2010a)	Water hyacinths	Marine sediment	$Ca(OH)_2$/100ºC	40	100	1.6	19.93	--	0.30	73.81	14.48	9.90	30
Ross and Holtzapple (2001)	80:20 MSW:SS	Rumen fluid	$Ca(OH)_2$/121ºC	40	88	0	30	-	0.219	-	-	-	12
	Cattle manure	Rumen fluid	$Ca(OH)_2$/121ºC		105	2	20		0.158				5
Smith and Holtzapple (2011)	Paper	Marine sediment	--	40	93	3-1.6	30.02	0.84	0.239	-	-	-	32
Lee et al. (2014)	Macroalgae	SS	--	37	92	-	29.17		0.35	40.4	18.3	26.0	60
Rughoonundun et al. (2012)	70:30 Sugarcane bagasse: SS	SS	Lime	55	50	0.016	15.1		0.36	79	2	17	30

2.6.3 VFAs as Building Blocks for a Biorefinery

Although carboxylic acids are valuable products on their own right, they are mostly considered as substrates to obtain higher-value products as ethanol or esters by biochemical, chemical or thermochemical process. Figure 2.7 presents some routes to convert VFAs into higher-value products (Granda et al., 2009; Holtzapple and Granda, 2009).

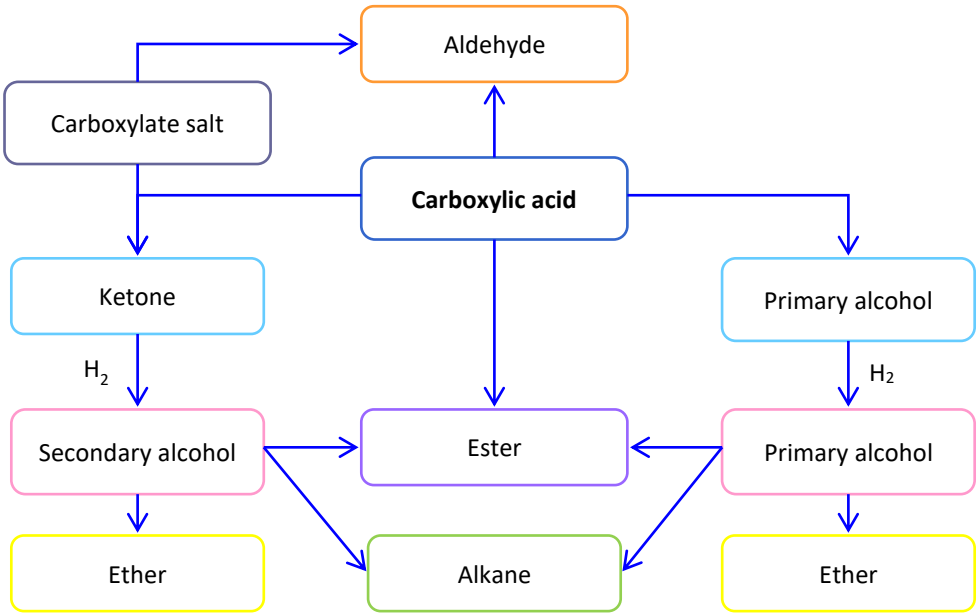

Figure 2.7. Routes to transform carboxylic acids to chemical products and biofuels. Adapted from Agler et al. (2011) and Granda et al. (2009).

Acetic acid could be easily converted via a secondary fermentation with the promotion of the reduction to butyric acid, ethanol, propanal, butanol or n-hexanol, and via a chemical post-processing to ketones, aldehydes, esters, alcohol or alkanes, which could have a higher market price and could be easier to commercialize (Agler et al., 2011).

These processes are biological reduction of carboxylates to the corresponding alcohols; biological elongation of short-chain carboxylates to longer chain products; and bioelectrochemical systems (BESs), in which biological reactions are coupled to reactions at solid electrodes to produce electric power or valuable chemicals. Regardless of the conversion method, further processing of acetate relies on being able to separate it from the

undefined mixed culture broth, because consolidated bioprocesses in which the primary and secondary fermentation reactions occur in the same reactor are often precluded by incompatible optimal conditions. One of the main barriers for large-scale liquid fuel and chemical production with the carboxylate platform is limitations with its separation. The other barrier is that hydrogenotrophic methanogenesis must be ceased.

Despite all this promising view of carboxylate platform, there still are some technical bottle-necks to be overcome which are listed below:

- Proficient pre-treatment: enhancing the digestibility, efficient removal of lignin.
- Improve the final concentration, productivity, yield and inhibition of methanogenic microorganisms.
- Reduce the separation and purification cost.
- Enhance the energy level of products.

Considering carboxylate platform advantages and disadvantages and results from other studies (Table 2.1), this process is an auguring route to produce valuable liquid biofuels from different type of wastes with high organic content and relatively easy degradable compounds, as organic fraction of municipal solid wastes (OFMSW), WAS, food waste, green biomass, algae, high organic load effluents, etc., as a previous energy recovery before the production of methane by anaerobic digestion due to methane is a gaseous fuel and therefore it is more complicated to deliver, store and use it as transportation fuel; proposing thereby, the wastes treatment as an integrated bioprocess to produce valuable products (Chang et al., 2010; Liu et al., 2013b).

2.7 Wastewater, Waste Activated Sludge and Food Waste the UK

In this section, current technologies for the treatment of wastewater, waste activated sludge and food waste in the UK are explained.

2.7.1 Wastewater and Waste Activated Sludge Production in the UK

With the increase of population, the pressure on the water sector has increased due to the fact that water resource all over the world is limited (DEFRA, 2016a; WATER-UK, 2006). Wastewater treatment works serve

around 96% of population in the UK, meaning that more than 16 billion litres of wastewater is collected daily and transferred into one of 9000 wastewater treatment plants by 624,200 km of sewer (DEFRA, 2012b; WATER-UK, 2013). Over 1 million tonnes of sludge as dry solids are generated during wastewater treatment process every year in the UK about 80% of the sludge is used for agriculture, almost 20% of sludge is incinerated and the little remaining part is sent to landfill (WATER-UK, 2013; WATER-UK, 2006).

2.7.1.1 Treatment of Wastewater in the UK

Wastewater treatment works in the UK are based on well-established bacterial process including four main treatment stages which is called Activated Sludge Process (Figure 2.8).

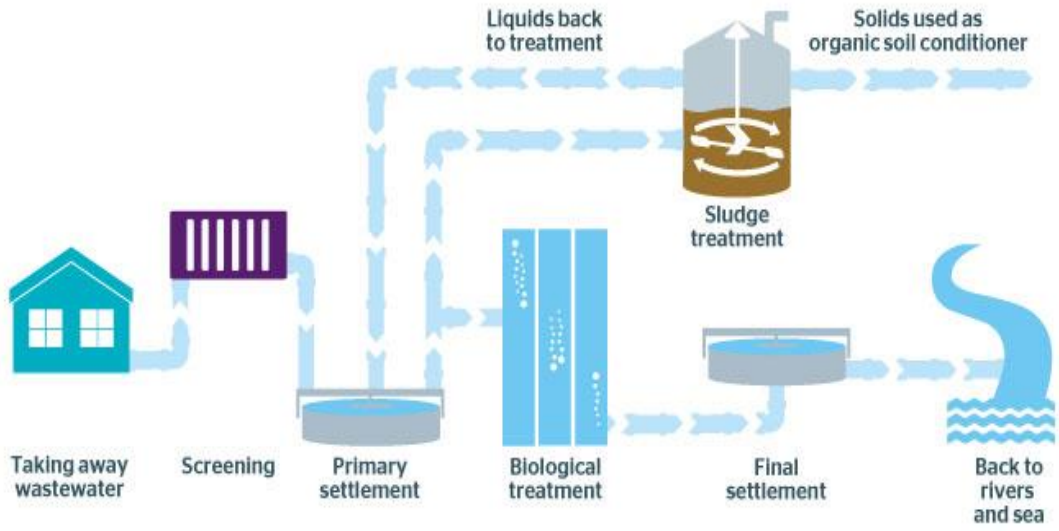

Figure 2.8. Municipal Wastewater Treatment Works scheme in the UK (SouthernWater, 2010)

The first step of wastewater treatment work is preliminary treatment that wastewater stream passes through the screens to remove large solid. As the second one, primary treatment, primary settlement tanks are used to settle larger organic matters. The third stage involves aeration tanks and final settlement tanks aiming to break down organic materials by bacteria and to settle bacteria, respectively. This process is called as secondary treatment. The fourth one is called as tertiary treatments which could be applied when they require to remove different contaminants.

2.7.1.2 Treatment of Waste Activated Sludge

Sludge collected from primary and final settlement are named raw sludge and waste activated sludge, respectively (Metcalf&Eddy, 2003). Anaerobic digestion, dewatering of sludge and incineration are common processes to treat sludge in the UK. Anaerobic digestion which is not a new method, has been used over 100 years. Organic materials are broken down under anaerobic conditions by microorganisms and converted into biogas as methane (CH_4) and carbon dioxide (CO_2) gases in anaerobic digester. This process takes between 15 – 28 days at 35°C. In addition, digestate is produced and used as nitrogen rich fertilizer (DEFRA, 2011a; Camargo-Valero et al., 2015). Dewatering process is applied to thicken sludge by gravity or mechanical separator as centrifuge, screw press or belt press. Up to 25% of dry solids could be obtained by dewatering process (Bamelis et al., 2015; WATER-UK, 2006). Incineration process is generally preferred to generate electricity via the heat produced turns into water stream and stream drives turbines (WATER-UK, 2006; Camargo-Valero et al., 2015)

2.7.2 Carbon Footprint of Wastewater and Waste Activated Sludge

Wastewater treatment works are one of the biggest source to emit greenhouse gases (GHG) as carbon dioxide according to equation 2.1 (Chai et al., 2015; Metcalf&Eddy, 2003). Carbon dioxide is produced by hydrolytic bacteria under aerobic conditions in activated sludge process (Kampschreur et al., 2009). Activated sludge process releases 88 kg of CO_2 per million litre treated water (Environmental Agency, 2009).

$$3C_6H_{12}O_6\ (\text{organic matter}) + O_2 + 2NH_3 \rightarrow 2C_5H_7NO_2\ (\text{new cells}) + 8CO_2 + 14H_2O \quad \text{.....Equation 2.5}$$

Methane produced from anaerobic digester is also considered as another main resource of the emission of greenhouse gases. There are 4 main stages including hydrolysis of polymers, fermentation, acidogenesis and methanogenesis (formation of methane) to convert organic materials into methane in anaerobic digester (Kumaran et al., 2016).

Anaerobic digester releases 18 kg methane per tonne of sludge in the case of that wastewater treatment works do not include Combined Heat and Power process (CHP). When methane is sent to CHP, the emission of methane gas should be minimum level. In addition, CO_2 is emitted from AD which is smaller amount compared to the amount of methane produced. The

emission of CO_2 from AD is calculated to be 0.549 kg CO_2 per tonne of sludge treated with CHP while the amount of CO_2 emission is estimated to be 25.4 kg CO_2 without CHP (EnvironmentalAgency, 2009) .

2.7.3 Wastewater and Energy

Electricity is created from wastewater treatment plants. Primary sludge and waste activated sludge are collected into anaerobic digester. Biogas containing 60 – 65% methane and 35 – 40 % carbon dioxide is produced by breaking down organic materials under anaerobic conditions by microorganisms in anaerobic digester. Biogas is sent to combined heat and power system to generate electricity by stream turbines (WATER-UK, 2006; Camargo-Valero et al., 2015).

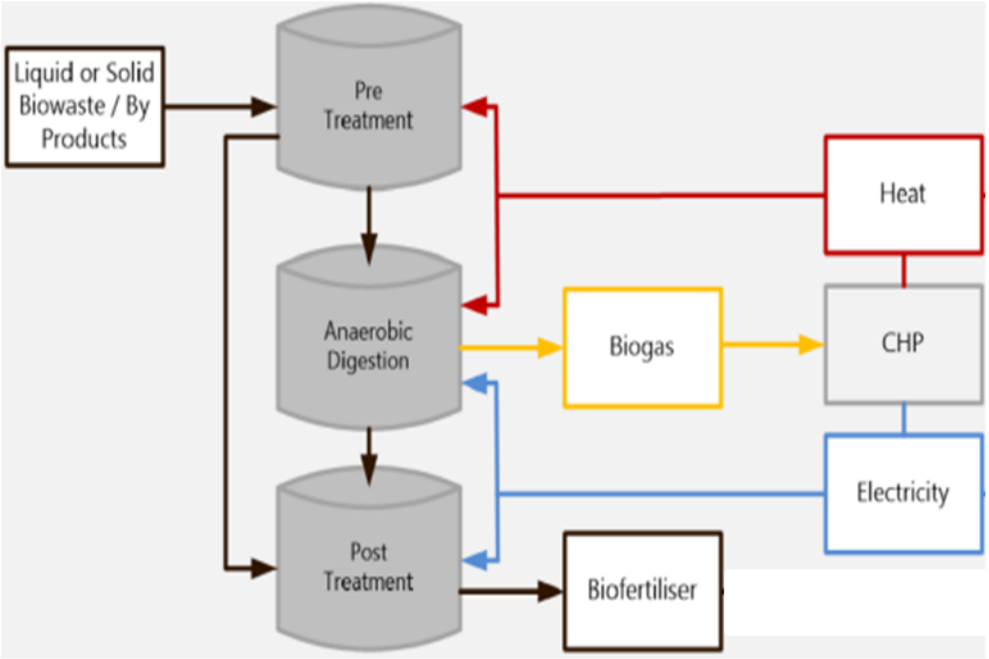

Figure 2.9. Process scheme to produce energy from waste activated sludge. (DEFRA, 2012b).

Waste combustion (including sludge) and biogas production were achieved to meet 10.8% and 4.2% of UK renewable energy in 2015 and 493 gigawatt hours energy generated from water industry in 2015 – 2016, of which 6,4% of the total energy is consumed for water and wastewater treatment. It is aimed to increase the amount of electricity produced from renewable energy sources up to 20% until 2020 (Parliamentary Office of Science and Technology, 2007).

2.8 Production and Treatment of Food Waste in the UK

The amount of food waste in the UK has presented a dramatic increase. Figures are about 7.3 million tonnes in 2015 while up to 7 million tonnes of food thrown away in 2012. Regarding the worth of food, around £13bn was wasted from households each year. Food waste is valuable product as it can be used as fertilizer in the case of is treated by compost. In addition, methane can be produced when it is used in anaerobic digester as feedstock.

2.8.1 Food Waste and Energy

The process with using food waste in anaerobic digester has the same principle as to use waste activated sludge. Food waste is broken down by bacteria in the absence of oxygen and methane is generated in anaerobic digester; thereafter, methane is transferred to CHP to produce electricity (Figure 2.10).

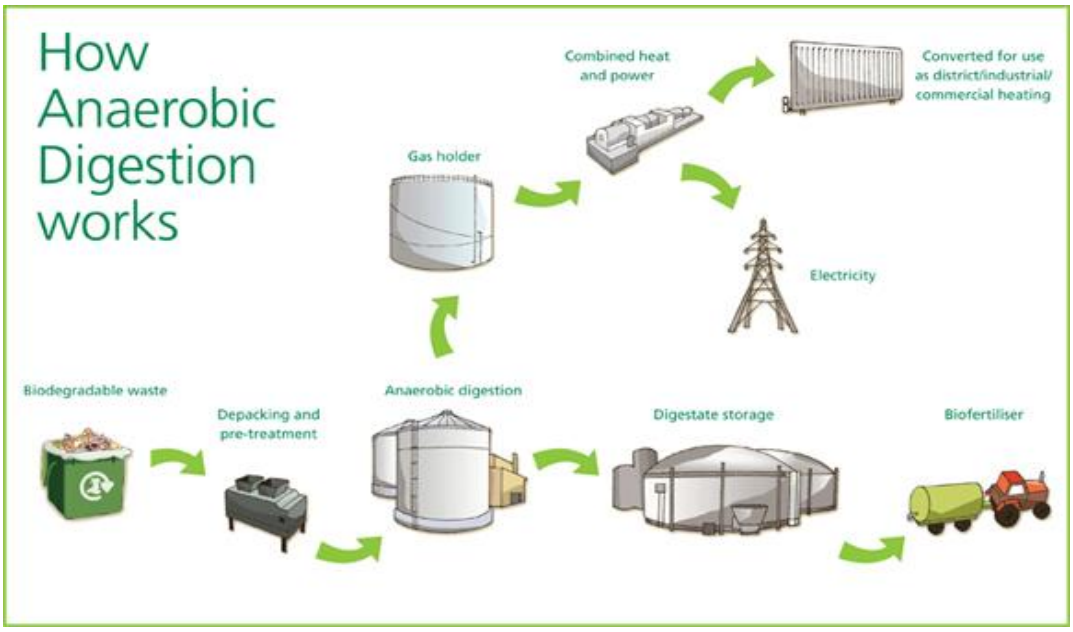

Figure 2.10. Process flow diagram for anaerobic digestion with food waste (WRAP, 2013).

The use of food waste in anaerobic digestion rather than dispose to landfill has contributed to decrease the emission of methane from landfill. Moreover, the accumulation of fat, oil and grease in the pipes of sewage network and wastewater treatment works could be prevented. The benefit of co-digesting waste activated sludge and food waste is to enhance the amount of energy

generated from wastewater treatment works at least three times and to increase the quality of digestate for the use of fertilizer (WRAP, 2013).

2.9 Promising Biomass For Biorefineries

2.9.1 Waste Activated Sludge

With regard to production of liquid biofuels by mixed acidogenic fermentation in the United Kingdom (UK), Waste Activated Sludge (WAS); considered as the residue produced after the wastewater treatment which contains a mixture of organic, inorganic and biological compounds (Liu et al., 2013b; Wu et al., 2009), is a promising feedstock which currently is stabilized by anaerobic digestion. Biogas production from WAS treatment by anaerobic digestion is a favourable renewable energy source because the increasing of this waste with a high organic fraction (60-80%), the recent government investments in the this sector and to diminish the cost of appropriate treatment (DEFRA, 2012a; Liu et al., 2012a). In 2008, the UK registered a production of 1.6 million of tonnes of sewage sludge, 66% of this waste was treated by anaerobic digestion and only the 60% of biogas was used as renewable energy which represents 115 megawatts (90% of energy produced by anaerobic digestion in the UK). Also Stephen Allen (2011) reports that methane generation yield by WAS treatment can reach from 9 to 16 m^3/wet ton. It can be envisaged that for 2020 biogas produced by AD of WAS will be between 0.2-0.7% of the energy consumed in the UK (DEFRA, 2011b; Rughoonundun et al.; 2010, Rulkens, 2007). WAS can be process by acidogenic anaerobic fermentation for production of VFAs as the first step of WAS stabilization, and with this, new ways are open for nutrient removal (Kim et al., 2006; Maharaj and Elefsiniotis, 2001; Wu et al., 2009; Yan et al., 2010; Yuan et al., 2009; Yuan et al., 2011; Zhang et al., 2009; Zhuo et al., 2012) or for using them as feedstock of other products (Bengtsson et al., 2008; Forrest et al., 2010a; Huang et al., 2002; Rughoonundun et al., 2010; Rughoonundun et al., 2012) although more research is needed to enhance the efficiency and yield to know the viability of producing VFAs from acid fermentation of WAS mixtures with other organic compounds.

Considering the characteristics and amount of sewage sludge generated annually in the UK, it can be envisaged the carboxylate platform process as an alternative route for WAS treatment and energy recovery due to WAS represents a favourable feedstock for mentioned purpose.

Between the pre-treatments reported for WAS (alkaline, acid, enzymatic, etc.), high pressure thermal hydrolysis (HPTH) process has been suggested as the most convenient pre-treatment due to it can obtain high degree of hydrolysis of carbohydrates, proteins and lipids and bacterial cell wall biomass, energy efficiency, higher digestion yield and increase biogas production. HPTH pre-treated WAS (165°C, 10 bar, 30 min) has been used amply as feedstock for methane production with good yields and productivity in many countries, included the UK, USA, Norway, Germany, Portugal, etcetera (Abu-Orf et al., 2011; Kepp et al., 2000; Panter, 2001; Ross et al., 2010).

2.9.2 Food Waste

Food waste is a worldwide social and environmental issue, reaching an approximate total volume of about 1.3 billion tonnes per year (FAO, 2011; Gustafsson et al. 2011; Lipinski et al. 2013). The amount and composition of food waste in the UK has been studied by the organisation Waste and Resources Action Programme (WRAP), reporting estimations of 7.3 million tonnes of food and drink waste post farmgate in the 2015, 60% of which can be avoided and represents a retail value of about £13 billion (House of Commons, 2017; Quested and Parry, 2015).

The main implications of the waste of food is the cost on the production (fertilizers, water, energy) and the further disposal and treatment by local authorities, which at the same time contributes to the increase on the greenhouse emissions (Quested and Parry, 2015).

In terms of the management of food waste in the UK, the main process used is the anaerobic digestion, being reported that in 2014 the AD projects under development for the treatment of food waste had a capacity around 5.7 million tonnes per annum (UK Green Investment Bank, 2015). In view of this, food waste is a promising substrate for the co-digestion with WAS as it could contain a high amount of carbohydrates (easy biodegradable material), increasing the C/N ratio and hence, promoting a healthier and quicker microbial degradation for efficiency purposes. Other benefits of the treatment and valorisation of food waste via the anaerobic digestion process in WWTP are the usage of existing facilities, the low investment on the management and treatment and the food waste treatment (Iacovidou et al., 2012)

Depending on the composition of food waste, some pre-treatment could be needed such as mechanical and physical for the reduction of particle size to more aggressive processes such as chemical and thermal treatment (acidic

or alkaline pH, microwave, HPTH, autoclaving, dry thermal, etc.) for the increase on the solubilisation of complex compounds such as lignin-based residues and fats (Qiao, 2011; Yin 2014). High process thermal hydrolysis has proven to be a robust process on the treatment of WAS in WWTP for the solubilisation and disruption of fats and cell walls and considering that FW could be a co-substrate for the resource recovery with HPTH-WAS, this method is visualized to be able to be used for both feedstocks with the further AD or acidogenic fermentation process.

2.9.3 Microalgae

Other potential raw material to produce liquid fuels via carboxylate platform is microalgae due to its high digestibility, low lignin content, high productivity and accessibility, effective solar energy use and not competition with arable lands; and food waste due to its high amount due to increasing of population and urbanization, high percentage in municipal solid wastes (50 – 70%) and high content of volatile organic compounds (Chang et al., 2010; Zhang et al., 2005; Ward, 2014). Although WAS, algae and food waste are promissory feedstock, more research needs to be done to develop the highest potential as raw material. One disadvantage of these raw materials is the lack of knowledge of pre-treatment process to reach high hydrolysis grade of solids for quick conversion to VFAs.

The main advantage of using microalgae as feedstock for AD and the production of biomethane, is the usage of the whole cell and all the organic material instead of only the macromolecules (carbohydrates, proteins, lipids) for the production of biodiesel or bioethanol (Mendez et al., 2013). Microalgae is visualised as a capable feedstock for the carboxylate platform and the production of VFAs because is a substrate rich in proteins, which are the main substrates for the generation of long-chain VFAs such as butyrate and valerate (Gonzalez-Fernandez et al., 2015; Nagase and Matsuo, 1982)

Nevertheless, microalgae has been pointed as a very difficult substrate for the digestion of methanogenic bacteria due to it is protected by a semi-rigid structure, its cell wall which is mainly composed by compounds such as algaenans and sporopollenin (Burczyk and Dworzanski, 1988). In order to hydrolyse the microalgae and release the intracellular organic material before the AD process, several pre-treatments have been employed such as thermal, chemical, physical or a combination of these, being the thermal-chemical the one that has presented the highest solubilisation, reaching solubilisation 7 to 11-fold higher than the initial soluble matter content

(González-Fernández et al., 2012; Gonzalez-Fernandez et al., 2015; Mendez et al., 2013).

In summary, the use of biomass as feedstock to obtain energy can be a sustainable and alternative option to reduce the consumption of fossil fuels. Carboxylate platform is a promissory technology that could be attached to the current anaerobic digestion system to produce renewable energy, apart from CH_4, and at the same time, to reduce the accumulation of greenhouse gases into the atmosphere, by making CO_2 a renewable source; although further research is needed to enhance the productivity, economic feasibility and process simplicity to accomplish with social, political and environmental outlooks. Owing to this, the current document presents a proposal to valorise and use WAS via acidogenic fermentation to produce liquid chemicals and/or biofuels via carboxylate platform instead of biomethane.

Although there is a clear understanding of the biochemistry and the effect of some operational conditions on the AD and the carboxylate platform, there is a lack of information that can provide strong evidence of the feasibility of the CP process for the development of the biorefinery concept in the WWTPs in the UK. The research gaps investigated in this project were:

1. The effect of the inhibition of the methanogenic bacteria with iodoform for the accumulation of VFAs on the treatment of HPTH-WAS.
2. To impact of the pH on the generation and speciation of carboxylates and biosolvents (acetone, alcohols) on the acidogenic fermentation of HPTH-WAS.
3. The influence of different thermal pre-treatment conditions of food waste or microalgae on the net solubilisation of organic material for the usage in a mixed acidogenic fermentation.
4. The effect of the the co-fermentation of HPTH-WAS with food waste or microalgae (*Chlorella vulgaris)* and different C/N ratios for the production of high-valuable chemicals (VFAs) from HPTH-WAS.
5. The impact of the organic loading rate in a mixed acid fermentation carry out in a semi-continuous stirred-tank reactor (CSTR) using thermally pre-treated organic feedstock.
6. To understand the effect of different operational conditions on the mixed acidogenic fermentation system by the calculation of mass balances.

Chapter 3. EXPERIMENTAL METHODOLOGY

To achieve the objectives in this project, a four stages methodology is proposed which comprises the pre-treatment and characterisation of organic feedstocks, its further anaerobic acidogenic fermentation of mixtures of WAS and different organic wastes by varying processes conditions (pH and/or methanogenic inhibitor dosage), a semi Continuous Stirred-Tank Reactor (CSTR) using the best conditions found in previous experiments and finally, a mass and energy balance of the CSTR process.

3.1 Methodology stages

Information about the stages for the acidogenic fermentation for the production of short-chain volatile fatty acids production is provided below:

1. Hydrothermal pre-treatment of organic feedstock (waste activated sludge, food waste and microalgae)
    - WAS used in this project was obtained directly from a wastewater treatment plant, where it is pre-treated under High Process Thermal Hydrolysis (HPTH) conditions (165°C, 6 bar and 30 min) (Kepp et al., 2000; Panter, 2001).
    - For food waste and microalgae (*Chlorella spp.*), two different pre-treatments are proposed; a lab HPTH, (165°C, 6 bar and 30 min) and autoclave process (120°C, 1.5 bar, 30 min) to increase hydrolysis and digestibility of feedstocks and at the same time, to find the best mixtures that can produce the highest concentration of VFAs (Dong et al., 2010b; Kuo and Cheng, 2007; Liu et al., 2012b; Zhou et al., 2014). Food waste was taken from the Refectory at the University of Leeds, whereas freeze dried microalgae (*Chlorella vulgaris*) was obtained from Synergy Natural Ltd (Prymont, Australia).
2. Batch mixed acid fermentation of WAS, WAS/FW, WAS/Microalgae.
    - To carry out batch anaerobic fermentations of WAS and its mixtures with treated or untreated food waste/microalgae to find the best conditions to reach the highest concentration of VFAs and to determine how different conditions can influence the final product composition as is shown below:

- Determination of the best methanogenic inhibitor ratio (mg iodoform/g VSS inoculum) when using iodoform as inhibitor of the methane bacteria producers in acidogenic fermentation of WAS.
- To assess the effect of pH in the production of VFAs in mixed acid fermentation of WAS.
- To test the influence of two different factors in a mixed factorial design;
    1. Food waste/microalgae pre-treatment: raw; autoclaved; HTP
    2. Blend ratios WAS plus FW/Microalgae: 75/25; 50/50 and 25/75

3. Mixed acid fermentation of organic feedstock in semi continuous stirred-tank reactor.

    - To carry out anaerobic fermentations in semi continuous stirred reactors with the mixture of organic waste that presented the best performance in VFAs production to examine the effect of different organic loading rates (0.3, 0.6 and 1 g TVS/L_{Liq}•d) to know the influence of solid loading rate in the final product yields and composition (Chinellato et al., 2013; Fiore et al., 2016; Gruhn et al., 2016; Karthikeyan et al., 2016; Pokój et al., 2015).

Further detailed information of each stage is giving in sections 4.2 to 4.3.

3.2 Characterisation and pre-treatment of waste activated sludge, food waste and microalgae.

3.2.1 Feedstocks characterisation.

HPTH pre-treated waste activated sludge from ESHOLT wastewater treatment plant (Yorkshire Water, Esholt Hall Estate, BD17 7QX, Lower Esholt, Shipley, Bradford) and raw and laboratory hydrothermal pre-treated food waste and microalgae (*Chlorella vulgaris*) were used as feedstock for anaerobic acidogenic fermentation to determine the effect of the pre-treatments on hydrolysis and organic matter solubilisation, and its consequent production of VFAs. Wastes characterisation was conducted to evaluate pH, total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD) total solids (TS), total volatile solids (TVS), ammonia, volatile fatty acids (VFAs) and elemental analysis.

3.2.2 Feedstock pre-treatment.

3.2.2.1 Waste activated Sludge

A limiting factor to achieve high yields and productivity of target compounds in acid fermentations is the hydrolysis of organic compounds in the substrate (carbohydrates, proteins and lipids) and bacterial cell wall biomass, and its consequent solubility for microorganism digestion (Fiore et al., 2016, Ucisik and Henze, 2008).. WAS was collected from Esholt wastewater treatment plant where is pre-treated under industrial HPTH conditions (165°C, 6 bar and 30 min) (Kepp et al., 2000; Panter, 2001). Inoculum was collected at Esholt WwTW for all experiments and was maintained in the lab under anaerobic mesophilic conditions, feeding it every 15 days and adapted according to the operational conditions in every batch of experiments (different pH or substrate).

3.2.2.2 Food Waste

Forty kilograms of food waste sample was taken from the refectory at the University of Leeds in one week during in July 2014, then, mixed with water in a proportion 7 FW:3 tap water (w:w), blended in a lab blender, sieved to a particle size up to 1 mm and finally mixed and homogenized in order to maintain the same characteristics in all the sample and, at the same time, to promote a quick and easy attack of the hydrolityc bacteria in the acidogenic fermentation system. As mentioned in the literature review, an integrated pre-treatment process is adviced because of its simplicity and energy efficiency. Regarding this, it is desirable that all the waste entering the WWTP is mixed and treated in the existing HPTH system as WAS is currently being treated. Thermal pre-treatment of organic feedstocks is proposed, to increase the overall process efficiency, to reduce liquid and solid retention times in the fermentor and to enhance the production of VFAs. About 10 kg of food waste were taken to run a pre-treatment using 2 different thermal processes (5 kg each): 120°C, 1.5 bar and 30 min (standard autoclaving); and High Pressure Thermal Hydrolysis (HPTH) (165°C, 6 bar and 30 min), in a lab reactor (Figure 3.1) in order to find the pre-treatment that achieve the highest organic matter solubilisation (Dong et al., 2010b; Kuo and Cheng, 2007; Liu et al., 2012b). After treatment, all food wastes were characterized to determine the effect of the pre-treatment in the solubilisation of organic compounds and then, preserved at -18°C.

3.2.2.3 Microalgae

As well as food waste, freeze dried microalgae (*Chlorella vulgaris*), obtained from Synergy Natural Ltd (Prymont, Australia) was treated using same thermal pre-treatments as food waste; a conventional autoclaving and a HPTH as described in 3.2.2.2, followed by characterisation and storage at -18°C.

Food waste and microalgae were used as an alternative feedstock that can provide more organic content to add to an integrated system for the production of fuels/chemicals in a wastewater treatment plant for the valorisation of different types of organic wastes.

Figure 3.1. Reactor used for high pressure thermal hydrolysis of food waste and microalgae.

3.2.3 Methods.

TCOD, SCOD, VSS, TVS, VFAs, pH analysis were run according to Eaton et al. (2005). Soluble COD was considered as the organic matter in the liquid that pass by 1.2 µm glass fibre filter (GF/C Whatman) (Morgan-Sagastume et al., 2011; Park and Lee, 2005; Saby et al., 2002; Shehu et al., 2012; Trussell et al., 2006).

Gas chromatography with a flame ionization detector (GC-FID) was used to determine VFAs production using the modified methodology from Eaton et al. (2005) and Smith and Holtzapple (2011). Samples were acidified to pH 2 with H_3PO_4 (85%) to assure VFAs are protonated and then was centrifuged

(14,000 rpm) to separate solids. The supernatant was filtered through a disposable in-line filter (0.2 µm pore size) and placed in a 1.5 mL glass vial for GC-FID analysis. Chromatographic analysis conditions were: Agilent Technologies® chromatograph, helium as carrier gas, inlet temperature 200°C, split 5:1, column gas flow 3 mL/min, column Supelco Carboxen® 1010 PLOT, Oven program: hold a 35°C for 7 min, ramp of temperature of 24°C per minute until to 225°C and hold for 5 min, detector at 230°C).

For biogas analysis (H_2, CH_4, N_2, O_2, and CO_2), a gas chromatograph equipped with a thermal conductivity detector was employed (GC-TCD) (Agilent Technologies® 7890A GC system). 1.0 mL biogas sample was injected manually in split-splitless 5:1, mode in a Supelco Carboxen® 1010 PLOT column, 30 m, 0.53 mm I.D., inlet temperature 200°C, column gas flow 0.7 mL/min; oven program: hold a 35°C for 7 min, ramp of temperature of 24°C per minute until to 225°C and hold for 5 min, detector at 230°C). Argon was used as carrier gas at a flow rate of 3 mL/min, and the run time was 20 min (Eaton et al., 2005).

All feedstocks were analysed to determine its elemental composition (C, H, N, S) using a CE Instruments Flash EA1112 Series elemental analyser). About 3 mg of sample was introduced into aluminium tin which was then placed in a furnace for combustion at 1100°C for 50 s. The products of combustion (CO_2, H_2O, NO_x and SO_x gases) were carried through the system by the He carrier to be quantified. Adjustments for blank, standards and weights were applied to the final integrated signal; results are reported in percentage of C, H, N and S (Arnaiz et al., 2006; Callaghan et al., 1999; Eaton et al., 2005; Ji et al., 2010; Mottet et al., 2009; Wang et al., 2009).

3.3 Mixed acid fermentation of mixtures of waste activated sludge and food waste.

Batch acid fermentations were run to determine the best operational conditions for the mix acid fermentation and to obtain high yields of liquid biochemicals and VFAs.

3.3.1 Effect of iodoform as inhibitor of methanogenic bacteria for VFAs production in mixed acid fermentation of waste activated sludge.

3.3.1.1 Effect of alcoholic solution of iodoform in mixed acid fermentation of waste activated sludge.

Two batches of acid fermentation were run to find out the effect of the addition of iodoform diluted in pure ethanol (batch 1) as an inhibitor; and to determine the best ratio of iodoform (CHI_3) with regards to the amount of VSS from the inoculum (relation: mg CHI_3/g $VSS_{Inoculum}$) (batch 2) to get a correct inhibition of methane production and to enhance the accumulation of VFAs generated during the acidogenesis/acetogenesis.

The first batch of acid fermentations was carried out in a 1 L bioreactor (0.8 L working volume) in mesophilic conditions (37°C) and stirring in an incubator shaker at 140 rpm with an organic loading of 8 g TVS/ L in a ratio of 1 g TVS of feedstock/ g $VSS_{inoculum}$ to ensure high microorganism activity and low risk of inhibition (Mottet et al., 2009; Nachiappan et al., 2011; Valo et al., 2004). Due to the characteristics of the feedstocks, which contain vitamins and nutrients needed by the microbial consortia, it was not considered the addition of nutrient media. Iodoform (20 g/L of iodoform diluted in pure ethanol) was used as a methane inhibitor (Smith and Holtzapple, 2011; Nachiappan et al., 2011; Fu and Holtzapple, 2010a; Domke et al., 2004) and was added at the beginning of each test and every other day afterwards, adding each time 320µL de ethylic solution which is equivalent to a concentration of 10 mg iodoform/$L_{fermentation\ broth}$ (Adewale, 2015) without having reporting any specific dosage with regards to TVS or VSS. Calcium carbonate (1.0 g/g of substrate) was added at the beginning of the fermentation to keep neutral pH conditions (6.5-7.5) (Pham et al., 2012). Gaseous nitrogen was bubbled through the broth for two minutes to remove oxygen in the broth and in the headspace on day 0 and each day after the iodoform solution was added. Fermentors were sealed with rubber stoppers with 2 tubes to collect gas and liquid samples. Fermentations were run for a period of four weeks in batch mode, and samples were taken on days 0, 2, 5, 7, 9, 14, 19, 23 and 28. Ten millilitres of liquid sample were taken from the fermentor every other day to conduct characterisation. Gases generation were measured by gas displacement to quantify the amount of gases produced.

Although iodoform has been applied widely as methanogenic inhibitor for the production of VFAs at different dosages and/or different periodicities (at the

beginning of the experiment, every other day and, sometimes or without specific information) (Boonsawang and Harnnarong, 2006; Chan and Holtzapple, 2003; Domke et al., 2004; Forrest et al., 2010b; Fu and Holtzapple, 2010b; Holtzapple et al., 1999; Nachiappan et al., 2011; Pham et al., 2012; Ross, 1998; Rughoonundun et al., 2012; Smith and Holtzapple, 2011; Thanakoses et al., 2003a); all of these studies use iodoform dissolved in ethanol which increases the operational costs and also do not report a ratio which relates the amount of iodoform and volatiles suspended solids as a dosage ratio, as VSS content because it is considered as an indirect measure of the amount of bacteria in the broth.

3.3.1.2 Determination of solid iodoform dosage as methanogenic inhibition in mixed acid fermentation of waste activated sludge.

For the second batch of experiments, iodoform dosage was established taking into consideration only the amount of VSS in the inoculum used as this value indirectly represents the content of microorganism capable to degrade organic matter. The concentration of VSS from the substrates was not taken into consideration when calculating the iodoform dosage as the feedstock has been treated thermally and the content of microorganism was considered negligible. Only studies from Suresh et al. (2013) reported a ratio of 530 mg CHI_3/g VSS of inoculum (other authors do not report iodoform dosage ratio based on the VSS from the inoculum). Thus, in this research it was decided to try low concentrations of solid iodoform with regards to the dosage previously provided (530 mg CHI_3/g VSS) in order to decrease the amount of iodoform and hence, the investment on the methanogenic bacteria inhibition. The concentrations tested were 0, 3, 6, 9 and 15 mg CHI_3/g $VSS_{Inoculum}$ (0, 0.56, 1.13, 1.70 and 2.83%) to be added only at the beginning of the fermentation process. The set for the second batch is explained below:

Three different types of reactors were carried out as follows:

1. Five mixed acid fermentations with five different inhibition ratios were tested with the conditions described as follows: 0, 3, 6, 9 and 15 mg CHI_3/g $VSS_{Inoculum}$, in 1 L reactors, 0.8 L working volume, using 5 g TVS of WAS as substrate and 5 g VSS of inoculum (inoculation 50:50). Iodoform was added once, at the beginning of the fermentation, in solid form. To maintain neutral pH (7-8), $CaCO_3$ (1 g $CaCO_3$/g $TVS_{susbtrate}$) was added at the beginning of the experiment. Mesophilic conditions (37°C) and no agitation were used. Fermentations were ran in duplicate for 21 days, taking samples at

day 0, 2, 5, 7, 10, 14, 17 and 21 (to maintain optimal conditions through the entire fermentation, the final volume taken as sample was less than 10% of the initial working volume). Parameters analysed were SCOD, TCOD, VSS, TVS, VFAs, pH, alkalinity every sampling day and gas composition on days 7, 14 and 21 by CG-TCD.

2. One reactor in duplicate was run to simulate bio-methane potential (BMP) conditions (anaerobic system, 1 L volume reactors, 0.8 L working volume, no inhibitor, pH not controlled, no addition of $CaCO_3$, 37°C and no agitation) to investigate the potential production of methane with the waste activated sludge and to compare it with the acidogenic fermentations.
3. Control fermentors with no substrate added and containing 5 g VSS of digested sludge was carried out using the same conditions than mixed acid fermentation and/or BMP reactors to determine the effect of the inhibitor in the inoculum during anaerobic fermentation (Figure 3.2).

A compilation and key for the second batch of experiments is presented in Table 3.1. Experiments were divided in two groups due to the amount of analysis to be run.

Table 3.1. Experimental design for second batch of fermentations.

Fermentor	Inoculum (g VSS/L)	WAS Substrate (g) TVS/L)	Inhibition ratio (mg CHI_3/mg VSS)	Group
Control 1	5	0	0	**1**
Control 2	5	0	0	
Control 3	5	0	3	
Control 4	5	0	6	**2**
Control 5	5	0	9	
Control 6	5	0	15	
BMP 1	5	5	0	**1**
Acid Ferm 0 (AF0)	5	5	0	
Acid Ferm 3 (AF3)	5	5	3	
Acid Ferm 6 (AF6)	5	5	6	**2**
Acid Ferm 9 (AF9)	5	5	9	
Acid Ferm 15 (AF15)	5	5	15	

Figure 3.2. Reactors used in the mixed anaerobic fermentation of WAS and different organic substrates.

3.3.2 Effect of pH as inhibitor of methanogenic bacteria in mixed acid fermentation of waste activated sludge.

Similarly to the determination of iodoform dosage, six different pH levels in acidogenic fermentations process were tested to discover the influence of pH on the production/accumulation of biochemicals and VFAs and also, the inhibition effect on the methane production during the acidogenesis/acetogenesis process (Chen et al., 2007; Gottschal and Morris, 1981; Zhang et al., 2009; Zhang et al., 2015).

Six different pH levels (4, 5, 6, 8, 9 and 10) and their control fermentors were tested: acidic pH to promote alcohol-acetone production, and basic pH to act as a buffer to neutralize the VFAs produced during the acidogenesis and avoid the methane production (Chen et al., 2007; Horiuchi et al., 2002; Li et al., 2010; Liu et al., 2012a; Zhang et al., 2015), using the same conditions as the experiments in section 3.3.1.2: 1 L reactor (0.8 L working volume), 37°C, 10 g TVS/L in a ratio of 1 g TVS of feedstock/ g $VSS_{inoculum}$, no fermentation media; no iodoform or $CaCO_3$ addition as a buffer. To set and control pH, NaOH 2N or HCl 1N were used to adjust pH at the beginning of the process and when the target value was higher or lower in 0.15 units by measuring it with a pH meter, under nitrogen flux to ensure anoxic conditions. Fermentors were sealed with rubber stoppers with 2 tubes to collect gas and liquid samples. Fermentations were run for 3 weeks in batch mode and samples were taken on days 0, 2, 5, 7, 10, 14, 17 and 21. Gas analysis were run at days 7, 14 and 21. Gas results from these experiments are important to help to define if fermentations using different pH can show an inhibitory effect on methanogenic bacteria in order to increase biochemicals production.

Inoculum was adapted for 14 days previous the anaerobic fermentations by adjusting pH to every specific level and re-adjusting when necessary, on days 2, 5, 7, 10 and 14.

3.3.3 Factorial design experiments to assess the effect of FW pre-treatment and ratios of mixtures of WAS/FW.

Bearing in mind the factors that affect acidogenic fermentations (pH, inhibitor, HRT, organic loading rate (OLR), agitation, feedstock blend and pre-treatments), two more factors were tested in this project, feedstock pre-treatment and blend ratios WAS/FW; to find out their effect on the anaerobic fermentation, especially on the production of VFAs, changes on the VFAs profile and/or hydraulic retention time. For this purpose, a mixed factorial design with two 3-level factors (feedstock pre-treatments and mixtures WAS/FW) using the best conditions for VFAs production, with duplicates and controls. Experiments were carried out operating with the same conditions described in section 3.3.1.2. Part of the factorial design can be seen in Table 3.2. As the aim of this section was to test the effect of the fermentation of HPTH-WAS from a municipal wastewater treatment plant with food waste or microalgae, tests were conducted to reach ratios of WAS:FW/Microalgae of 3:1 (75%/25%), 1:1 (50%/50%) or 1:3 (25%/75%).

Table 3.2. Factorial design for the acidogenic fermentations of mixtures WAS/Raw FW.

Factor 1. pH	**Factor 2. Feedstock pre-treatment**	**Factor 3. Mixture WAS/FW**
Best operational conditions (21 days at pH 9)	Raw Food Waste	75/25
		50/50
		25/75
	Autoclaved Food Waste	75/25
		50/50
		25/75
	THP food waste	75/25
		50/50
		25/75

3.3.4 Mixed acid fermentation of mixtures of waste activated sludge and food waste in semi continuous stirred-tank reactor.

After detailed statistical analysis and consideration of the results obtained from the batch mixed acid fermentation with regards to VFAs yields,

additional costs (pH buffers, pre-treatments, methanogenic bacteria inhibitor) and shortest HRT; the best fermentation conditions were chosen, being a fermentation at pH 9 with a HRT of 14 days with WAS as feedstock. With this information, a semi-continuous stirred reactor was set up on AMPTS II (Bioprocess Control Sweden AB) (Figure 3.3), employing the acidogenic fermentation conditions previously described (0.5 L, 0.45 L working volume, 5 g/L TVS of WAS, 5 g VSS of inoculum, pH 9). During the first 14 days, acid fermentation was run in batch, after that, HPTH pre-treated waste activated sludge was fed to the reactors at different TVS loading rates (0.3, 0.6 and 1 g/L·d, fed three times a week) to understand the effect of different OLR on the behaviour of the acidogenic process and on the production of VFAs (Chinellato et al., 2013; Fiore et al., 2016; Gruhn et al., 2016; Karthikeyan et al., 2016; Pokój et al., 2015).

Figure 3.3. Fermentations ran in semi-continuous stirred reactors using a AMPTS II equipment (Bioprocess Control Sweden AB).

The semi-continuous acid fermentation was maintained for 56 days to reach steady state conditions (Lee et al., 2014; Lin et al., 2011). During semi-continuous culture, OLR levels will be maintained in mesophilic conditions at 120 rpm, for 1 min every 10 min, in order to examine acid production, yield and biochemical methane potential in the system (Athanasoulia et al., 2014; Lee et al., 2011; Sosnowski et al., 2003). Samples were taken at day 0, 2, 5, 7, 10 and 14 and then, three times a week, to analyse variations on SCOD, TCOD, TVS and VFAs. Biogas from the fermentors passed through a solution of NaOH 10N to clean the gas products and remove the CO_2 and hence, it was assumed that only CH_4 was quantified by the AMPTS II gas

counter. Other gaseous products such as H_2, NH_3 and H_2S were considered negligible.

Chapter 4. EFFECT OF IODOFORM FOR VFAs PRODUCTION IN MIXED ACIDOGENIC FERMENTATION OF WASTE ACTIVATED SLUDGE.

4.1 Effect of alcoholic solution of iodoform in mixed acidogenic fermentation of waste activated sludge

As a first step, acidogenic fermentation (also named as dark fermentation) using iodoform (CHI_3), diluted in pure ethanol with a concentration of 20 g CHI_3/L to inhibit the production of methane, as firstly reported by Holtzapple et al. (1999) and Holtzapple and Granda (2009), was tested using WAS as main feedstock. Acidogenic fermentors were carried out in triplicate in order to assess the potential production of VFAs from WAS. The fermentors were set-up with the conditions described in the methodology for the first batch of experiments. Control reactors were also carried out to track the behaviour of the inoculum.

The characterisation of WAS, treated by HPTH, was run before carrying out the acidogenic fermentation experiments; the results are presented in Table 4.1. The results of the elemental analysis of HPTH-WAS were nitrogen 4.44%; carbon 39.10%, hydrogen 5.24%, sulphur 0.58% and oxygen 22.04% (Oxygen content was calculated as follow: Oxygen= 100% TS - % CHNS- % ashes). The empirical formula of WAS is $C_{10.3}H_{16.5}NO_{4.3}$ (Rittmann and McCarty, 2001).

Table 4.1. Waste activated sludge characterisation.

Parameter	WAS	Inoculum
TCOD (g/L)	98.97±1.54	46.55±0.0
SCOD (g/L)	24.23±0.22	3.63±0.10
TS (g/L)	80.98±0.55	48.78±0.78
TVS (% of TS)	72.1	62.1
TSS (g/L)	68.19±0.64	46.48±0.29
VSS (% of TSS)	69.0	61.4
Alkalinity (g/L)	6.58±0.09	7.54±0.15
N-NH_4 (g/L)	1.61±0.02	2.24±0.01
TKN (g/L)	2.61±0.25	4.23±0.00
pH	7.39	7.9
SCOD/TCOD	0.24	0.07
Ash content (% of TS)	28.6	--

±: Figures are presented with one standard deviation

In summary, HPTH-WAS in this study was found to have high concentrations of COD, SCOD and solids, which are similar to the results in studies from Morgan-Sagastume et al. (2011) for high-pressure thermal hydrolysis (165°C, 6 bar, 30 min) which reported values for three different wastewater treatment plants in Denmark and Australia; giving ranges from 78-83 g/L TS and 49-59 g/L TVS, 84-97 g/L TCOD, and 31-34 g/L SCOD. Additionally, TKN results can give an estimate of the protein in the WAS, considering that protein contains 16.5% (w/w) N (Raunkjær et al., 1994) showing values of about 6.06 g protein/L WAS (10.38% of TVS). The carbon-to-nitrogen ratio was determined to be 8.8:1 according to the results from the elemental analysis which was smaller than the ratios reported as optimum by Shanmugam and Horan (2009) for the co-digestion of WAS which is in the range 17 to 21; being 20-30 the most common for the production of CH_4 (Smith and Holtzapple, 2011).

4.1.1 Effect of iodoform ethylic solution on chemical oxygen demand

One fermentor was carried out in triplicate and results of TCOD are shown in Figure 4.1 where WAS 1, WAS2 and WAS3 represents each fermentor in the experiment. Is clear that total COD presented a growth from the beginning of the fermentation process until the last day as seen in, which taking into account that the system remained closed and was not fed with organic feedstocks, the additional COD (about 6 g/L), must have come from external sources.

Among the fermentations carried out, it is noticeable that none of the reactors presented a steady concentration on COD, which gives evidence that such increment must come from external factors/feedstocks. At the same time, the concentration of SCOD increased along the acidogenic fermentation experiment, which suggests the augment of soluble compounds either from the hydrolysis of organic substrate by microbial activity or by the addition of soluble compounds into the reactor. The highest SCOD concentrations reached a value of 12.48 g/L on day 21 which represents a 77.2% of the TCOD inside the reactor and denotes an increase of 181% with regards to the initial SCOD value. Other studies working with anaerobic acidogenic fermentation for the production of VFAs focus only on TVS reduction and VFAs production and do not report VSS values in order to inspect the evolution of biomass or gas analysis to ensure there is no

production of methane or COD values to examine the concentration of organic content and its change along the experiment.

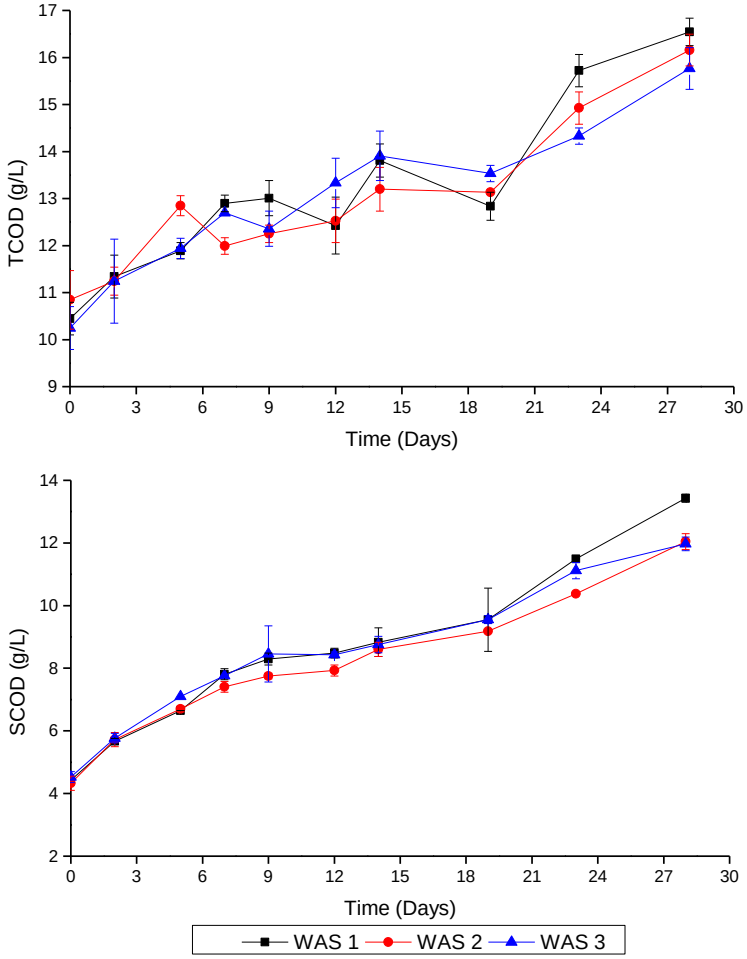

Figure 4.1. Total and soluble COD results from acidogenic anaerobic fermentation of HPTH-WAS in batch culture with iodoform.

(Key WAS1: fermentor with HPTH-WAS number 1, WAS2: fermentor with HPTH-WAS number 2, WAS3: fermentor with HPTH-WAS number 3).

4.1.2 Effect of iodoform ethylic solution on solids and gases content

Total solids (TS) and total volatile solids (TVS) were examined in order to determine its progress during the experiment; the switch of complex and non-volatile compounds (at 105°C) to volatile compounds at 550°C and, the loss of TS due to mineralisation of the feedstock (conversion to gases as CO_2, CH_4 or H_2).

TS and TVS in the fermentors decreased along the time, which implies that solids are being lost to generate a product no longer inside the fermentor, which are gases such as CO_2, CH_4 and/or H_2. The decay of TS in WAS fermentors showed a reduction of 26.7% (Figure 4.2).

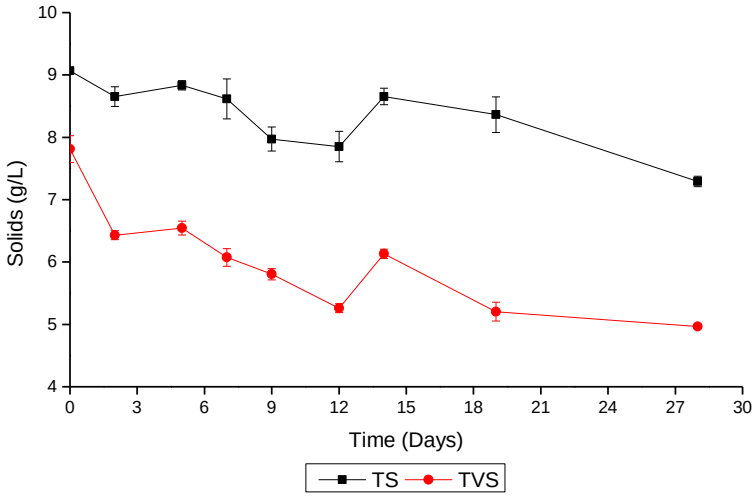

Figure 4.2. Average values of TS and TVS results from the acidogenic fermentation of HPTH-WAS (fermentors 1 to 3) in batch culture with iodoform.

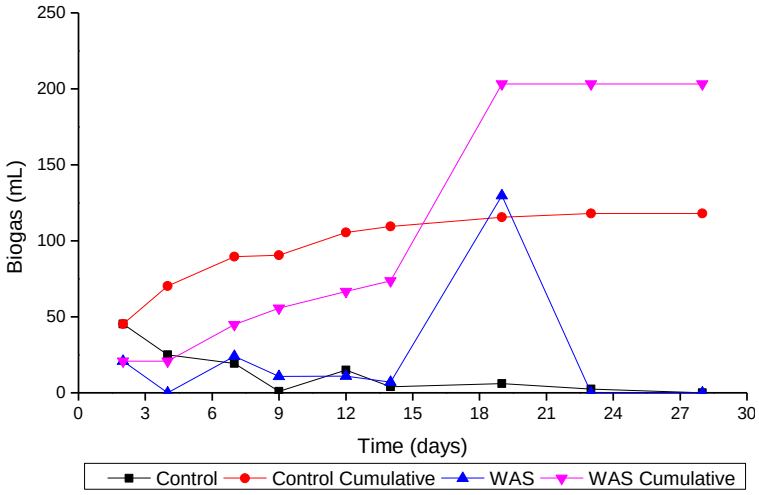

Figure 4.3. Gas production from the acidogenic fermentation of HPTH-WAS in batch culture with iodoform.

The concentration of TVS showed a reduction of 36.3% in WAS fermentors. When comparing the results of TS and TVS with the production of gas,

measured by displacement, from the fermentors (Figure 4.3), it is clear that some fermentable solids were converted to gases. Even when the production of gases was erratic during the complete experiment, the average cumulative production of gases showed that the WAS fermentors presented a generation of gas 1.7 times higher than the control fermentors. Also WAS fermentors exhibited their maximum gas production at day 19, of 203 cm^3.

It is clear that even with the addition of the inhibitor to the fermentors, biogas was produced in the fermentors which could diminish the final concentration of VFAs in the broth.

4.1.3 Effect of iodoform ethylic solution on VFAs profiles and concentration

VFAs generation was analysed by GC-FID in order to track the production rate and the final concentration in the fermentors; the results are presented in Figure 4.4.

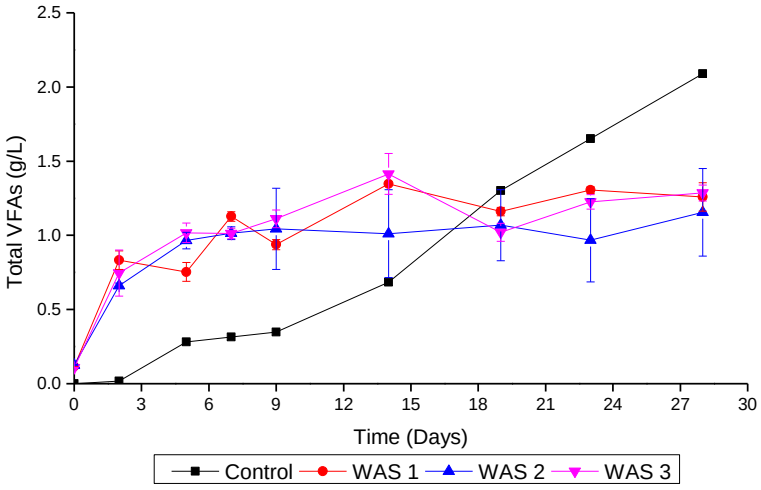

Figure 4.4. VFAs production results from acidogenic anaerobic fermentation of HPTH-WAS in batch culture with iodoform.

(Key: Control: fermentor blank with only inoculum, WAS1: fermentor with HPTH-WAS number 1, WAS2: fermentor with HPTH-WAS number 2, WAS3: fermentor with HPTH-WAS number 3).

VFAs production from batch anaerobic fermentation showed different behaviours between the control fermentor and WAS fermentors. Control fermentors showed an average production rate of 0.1 g VFAs/d while all WAS fermentors showed a rate of 0.04 g VFA/d (1.256 g/L). Also VFAs in WAS fermentors showed a asymptote from day 14 until the end of the

experiment with a production of no more than 1.5 g/L and a final concentration of 1.23g TVFAs /L (day 28). Due to a stable VFAs concentration was reached at day 14, the hydraulic retention time (HRT) chosen as the best, is day 14.

In contrast, after day 14, the control fermentor presented a higher production of VFAs, with a maximum average of 2.08 g VFAs/L and the final production rate reached was 0.11 g VFAs/d due to the bacteria having easy material to degrade (ethanol). Yields and productivity values from the fermentations are not trust worthy due to the addition of ethanol with the inhibitor.

During the black fermentation of WAS, some unidentified organic compounds which elute after all the short-chain fatty acids (C2-C7) were produced showing high peak areas, especially at minute 12.78 as shown in Figure 4.5. In comparison, the control fermentor did not present large peaks of unidentified compounds.

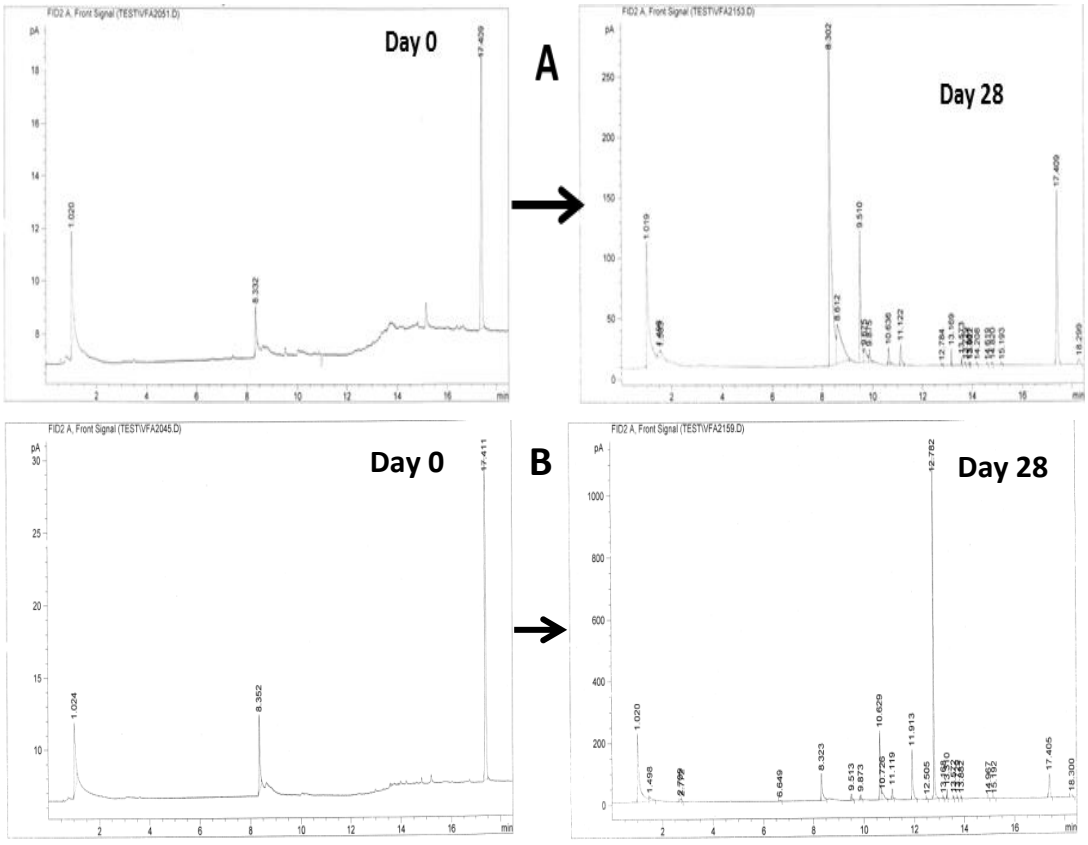

Figure 4.5. Chromatograms of day 0 and day 28 of (A) control fermentor and (B) WAS fermentors of acidogenic fermentation of HPTH-WAS in batch culture with iodoform.

Acidogenic fermentations experiments conducted by Zhang et al. (2005) using kitchen waste as main substrate with an initial organic load of 137.93 g TVS/L reached a production of 36 g VFA/L which corresponds to a yield of 0.26 g VFA/g TVS, in 5 days and a rate of 7.2 g VFA/d. Also D'Addario et al. (1993) report a production of VFAs of 15.6 g/L after 12 days (pH 5.5, substrate: organic fraction of municipal solid waste) with an initial feedstock concentration of 150 g TVS/L and a production rate of 1.3 g VFA/d with a yield of 0.14 g VFA/g TVS. Similarly, batch fermentations conducted by Babel et al. (2004), operated a pH 7 for 5 days with 50 g TVS/L of pineapple + sewage sludge mixture as initial feeding, showed a production of 21 g VFAs/L in reactors, which correspond to a yield of 0.40 g VFA/g TVS and a VFAs production rate of 4.2 g VFAs/d.

The concentration of TVFAs ranged between 2.14-2.18 g COD/L from day 14 to day 28 in reactors with HPTH-WAS and reaching its maxima of 3.91 g COD/L at day 28. Volatile organic compounds composition in the batch fermentations of WAS is shown in Figure 4.6.

Acetic acid was the main acid at day 28 in most of the fermentors (26 – 85%) which is in line with experiments conducted by Yuan et al. (2011) which worked with WAS and report results between 41-69% and Wang et al. (2014) which worked with the fermentation of kitchen wastes and descript ranges from 75 to 90% of acetic acid. The production of acetic acid is originated from the anaerobic catabolism of different substrates and routes: from ethanol via acetyl-CoA, from hexoses and pentoses via pyruvate or acetyl phosphate and/or from aminoacids such as alanine, glycine, cysteine or other carboxylic acids like lactate, citrate or fumarate via pyruvate with acetate kinase as the main enzyme (Thauer et al., 1977a). Due to the addition of ethanol to the fermentation broth, it is presumed that acetate was mainly formed following the acetyl-CoA route. Acetic acid concentration achieved the highest concentration at day 28 in WAS fermentors, with 0.59 g/L which is only 30.06% of the total concentration of volatile compounds analysed.

Propionic acid was the second most concentrated acid in the broth at day 28, reaching concentrations between 8.02–14.64% of the total VFAs concentration in the fermentation broth, which agrees with acidogenic fermentations studies conducted by Yuan et al. (2009) and Yuan et al. (2011). Propionic acid can be produced by the transformation of pyruvate to

lactate and/or succinate, which are called the acrylate and succinate routes (Bastidas-Oyanedel et al., 2015).

Finally, on the last two days of fermentation, the main compound was ethanol, showing concentrations as high as 0.83 g/L in reactor 1 at day 28, which corresponds to about 40% of the total volatile compounds analysed. This ethanol is probably coming from the inhibitor added to the fermentors.

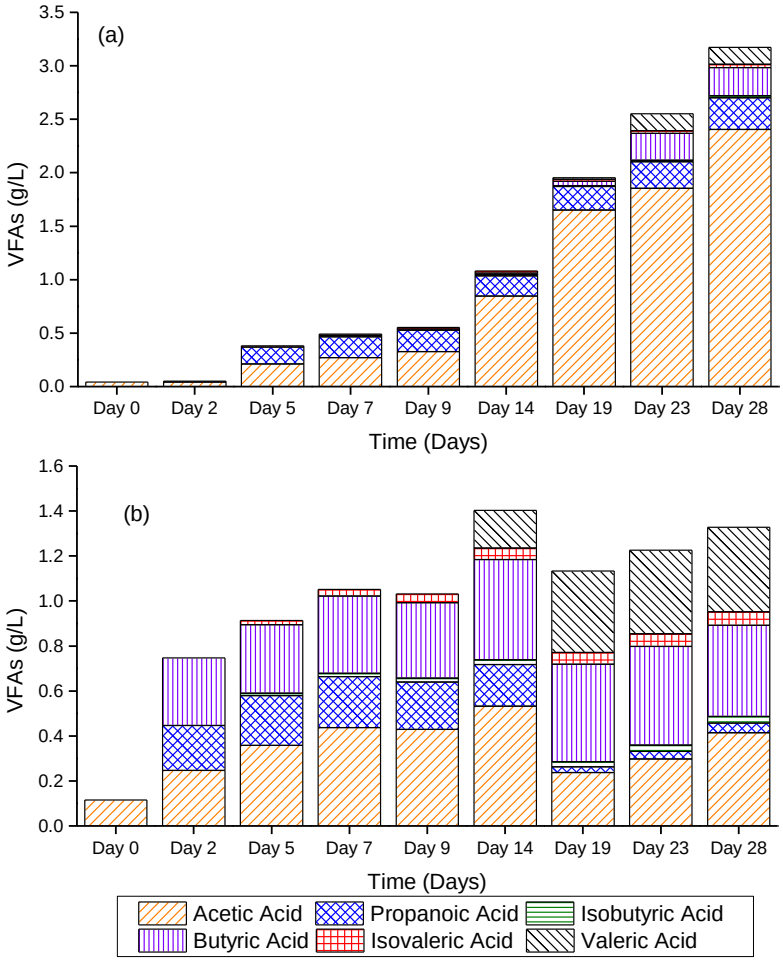

Figure 4.6. VFA profile on acidogenic fermentation of reactors Control (a) and HPTH-WAS (b) in batch culture with iodoform.

Comparing other carboxylate platform published works (Chan et al., 2011; Domke et al., 2004; Forrest et al., 2010b; Forrest et al., 2010a; Fu and Holtzapple, 2010a; Liu et al., 2013b; Nachiappan et al., 2011; Pham et al., 2012; Pham et al., 2013; Ross and Holtzapple, 2001; Rughoonundun et al., 2010; Rughoonundun et al., 2012; Smith and Holtzapple, 2010; Suresh et al., 2013; Thanakoses et al., 2003b; Thanakoses et al., 2003a) against the

results of the first batch mixed acidogenic fermentation in this project, it is clear that using iodoform dissolved in ethanol to inhibit the production of methane, involves the addition of more organic matter coming from a pure reagent (ethanol), which contributes to a higher generation of VFAs and other by-products, better yields and productivity and misleading results that cannot be trusted fully. In view of this, mass balances for these batches were calculated (4.1.4); and a further second batch of experiments was run using CHI_3 as solid powder.

4.1.4 Mass balance of acidogenic fermentation of WAS with alcoholic solution of iodoform as inhibitor

The transformation of the feedstocks to products and the behaviour of the fermentors were analysed through a mass balance of the fermentations based on TCOD values. Mass balance was useful to explain the increase of TCOD along the fermentation and the possible TCOD loss as biogas. Results are presented below (Table 4.2):

Table 4.2. Mass Balance of reactors WAS (COD terms) of acidogenic fermentation of HPTH-WAS with iodoform.

	TCOD experimental (g/L COD)			**TCOD theoretical (g/L)**	
	TCOD (average)	SCOD (average)	SCOD/TCOD		
TCOD initial ($TCOD_i$)	10.51±0.30	4.43±0.10	0.42	$TCOD_i$	10.52
TCOD final ($TCOD_f$)	16.16±0.38	12.48±0.82	0.77	TCOD ethanol added	**5.88**
				$TCOD_f$	16.39
$TCOD_f$-$TCOD_i$	5.64				
COD loss (Theoretical-Experimental COD_f)					0.24 g/L

TCOD from experimental data and calculations from fermentor Control have a difference of 0.08 g/L which represents a decrease of 0.78%. The increase of the average TCOD in control fermentors was 4.86g/L, which represents a 42.6% with respect to the initial value. The TCOD values from the WAS fermentors also presented an increase of 53.6% in comparison to the initial TCOD value (10.51 g/L COD). According to the theoretical and experimental mass balances, it is clear that TCOD values increased due to an external factor which could be attributed to the ethanol added when the solution of iodoform was dosed (400 µL every 2 days) to the reactors. Also the

theoretical concentration of ethanol added, in terms of COD, is similar to the difference between the final TCOD and its initial value from the experimental results, in both reactors, control or WAS.

The difference among TCOD calculated and TCOD experimental evidences a slight loss of organic content from the reactor, which is possibly due to the production of gaseous compounds such as CO_2, H_2 and or CH_4. It is also important to notice the decrease of the concentration of ethanol in the fermentation broth, which suggests that some ethanol was susceptible of the conversion by the microbial consortia, showing the capability of the bacteria to use alcohol as feedstock for the production of VFAs or biogas.

The tracking of TCOD done via the experimental and theoretical mass balances provides clear evidence that the similar increases of the concentrations of COD in reactors Control and WAS, was due to the addition of the ethanol from the iodoform solution (Table 4.3). It is important to highlight the role of the hydrolytic bacteria on the COD solubilisation as the final SCOD value represents an increase of 22.8% with regards to the initial TCOD after the subtraction of the SCOD from the ethanol added.

Table 4.3. TCOD values along the fermentation in WAS reactors in acidogenic fermentation of HPTH-WAS with iodoform.

	TCOD experimental data (g/L)		**TCOD+Ethanol theoretical (g/L)**
Days	TCOD (aver)	SCOD (aver)	
0	10.51	4.43	10.52
7	12.23	6.82	12.48
14	13.64	8.73	14.44
23	14.99	11.00	15.74
28	16.16	12.48	16.39
$TCOD_{f-i}$	5.65	8.05	5.87

Bearing in mind the results exposed in this mass balance, it is reasonable to assume that the main reason of the increases on the TCOD concentration during the acidogenic fermentation process, is due to the addition ethanol added via the dosage of iodoform. As mentioned before, when researching for this project, no studies were found that reported TCOD, SCOD, VSS and/or gases composition.

4.2 Determination of the best iodoform dosage as methanogenic inhibitor in mixed acidogenic fermentation of waste activated sludge

It is important to note that there are no studies that clearly report about the total amount of inhibitor added to acidogenic fermentation experiments. Some reports state initial dosages, which are between 0.36 and 1.4 mg CHI_3/g TS substrate (Fu and Holtzapple, 2010a; Pham et al., 2012; Pham et al., 2013) and 0.0057-3.8 mg/g TVS substrate (Rughoonundun et al., 2012; Nachiappan et al., 2011) but not in a ratio involving the amount of VSS from the inoculum which has a relationship with the microorganism content in it. Another drawback from cited studies is the lack of information about the total addition of iodoform as more inhibitor is added during the experiment.

A key fact that is seen in the first set of experiments is that iodoform added dissolved in pure ethanol (20 g/L) promoted the increase of the organic matter inside the fermentors due to the ethanol added. Considering that ethanol is a product/by-product of anaerobic digestion, it is feasible that ethanol added to the reactor can be interconverted for the production of other compounds like VFAs, lactate, other alcohols, succinate and some gases (Bastidas-Oyanedel et al., 2015; Gerardi, 2003).

4.2.1 Effect of iodoform dosage on the Chemical Oxygen Demand

In view of previous results, in the second batch of experiments, iodoform dosage was based on the amount of VSS in the inoculum due to its content of microorganisms capable of degrading organic matter. WAS and other substrates do not have a high content of microorganisms due to them being treated thermally and were not considered during the calculation of the addition of inhibitor. Fermentors key are: biomethane potential (BMP; no iodoform and no pH buffer) and Acidogenic Fermentation reactors (AF0= 0 mg CHI_3/g $VSS_{Inoculum}$ and 1 g $CaCO_3$/g $TVS_{substrate}$, AF3= 3 mg CHI_3, AF6= 6 mg CHI_3, AF9= 9 mg CHI_3 and AF15= 15 mg CHI_3/g $VSS_{Inoculum}$). Control reactors were carried out for every single process condition.

TCOD concentrations in control and Acidogenic Fermentation reactors tend to decrease during the experiment which suggests the conversion of organic matter to biogas although low TCOD losses were observed. The average decrease of TCOD was about 10% for Acid Fermentation reactors and about 1% for control fermentors (Figure 4.7). In contrast with the previous set of experiments, there is not a noticeable increment on TCOD which shows that

no other organic matter was added to the reactor and the only outlet was the production of biogas.

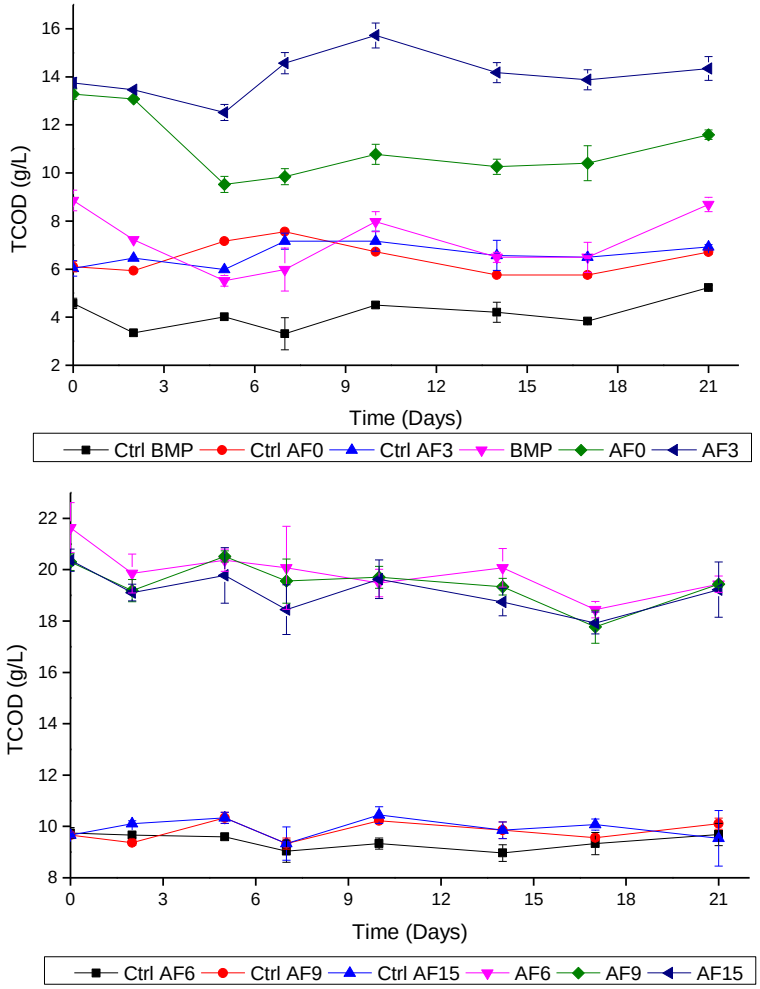

Figure 4.7. Total COD results from acidogenic anaerobic fermentation of HPTH-WAS in batch culture with iodoform.

(Key: BMP: biomethane potential test, Acidogenic Fermentation reactors (AF0= 0 mg CHI_3/g $VSS_{Inoculum}$, AF3= 3 mg CHI_3, AF6= 6 mg CHI_3, AF9= 9 mg CHI_3 and AF15= 15 mg CHI_3/g $VSS_{Inoculum}$).

With regards to SCOD values, it is noticeable that SCOD concentrations increased in all AF reactors, presenting increments of 47% (AF3) on day 10 to a final 6% increase on day 21 (Figure 4.8). Reactors AF6-AF15 presented an asymptote-like on SCOD, showing values between 35-46% from day 10 till the end of the fermentation, the maximum value was 3.63 g/L SCOD (AF15), which is 18% of the total COD inside the fermentor.

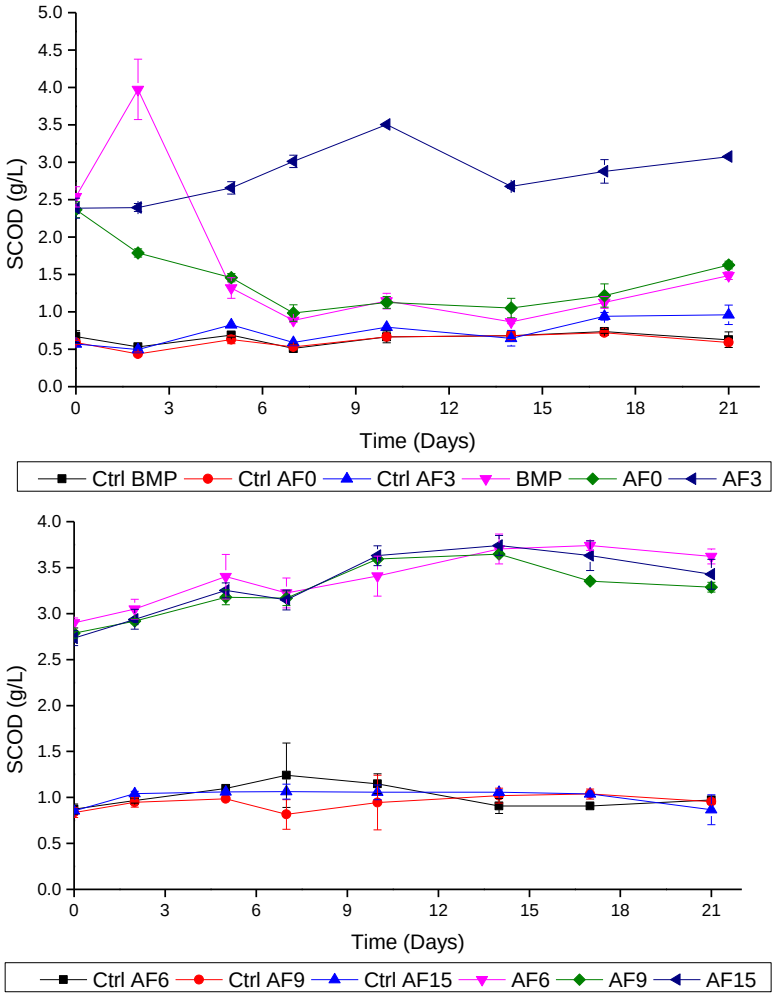

Figure 4.8. Soluble COD results from acidogenic anaerobic fermentation of HPTH-WAS in batch culture with iodoform.

(Key: BMP: biomethane potential test, Acidogenic Fermentation reactors (AF0= 0 mg CHI_3/g $VSS_{Inoculum}$, AF3= 3 mg CHI_3, AF6= 6 mg CHI_3, AF9= 9 mg CHI_3 and AF15= 15 mg CHI_3/g $VSS_{Inoculum}$).

Overall, the hydrolysis was the limiting step in these fermentations as revealed by the solubilisation degree (conversion of TCOD into SCOD in %) in fermentors AF6, AF9 and AF15, which showed a modest increase of about 4-7% from day 10 to day 21 as previously proven by Fiore et al. (2016). These results are in the same line than studies from Ucisik and Henze (2008) who report values between 1.9 and 5.6%. Fermentor AF0 did not present any sign of hydrolysis due to SCOD decrease from the beginning of the fermentation. The BMP reactor had a high SCOD value at day 2, but decreased dramatically to a value about 1 g/L COD, which implies the consumption of VFAs and the production of biogas. As the data corroborates, the effect of the iodoform in the COD content was the

accumulation of the soluble COD inside the fermentors and the low decrement on the total COD concentration, giving idea of the low conversion of the COD into biogas.

4.2.2 Effect of iodoform dosage on the solids content

In terms of TVS conversion, the results from BMP and acidogenic fermentation reactors presented a tendency to reduce its TVS content, possibly due to the conversion into biogas. Reactor AF0 showed the highest TVS mineralisation, decreasing around 27% at day 21, possibly due to the buffer addition to maintain pH 7, which promoted the production of methane. Control fermentors remained almost stable during the complete duration of the experiment with reductions of no more than 10%, which implied the low or null conversion of the VSS to biogas and also, gives good evidence of low conversion of reactants to products. When looking at VSS results, Control reactors showed similar trends to results from TVS, which means, small reductions in the region of 10%; whereas VSS from AF reactors diminished an average of 10% and only AF0 presented a 22% reduction due to the lack of inhibitor (Figure 4.9).

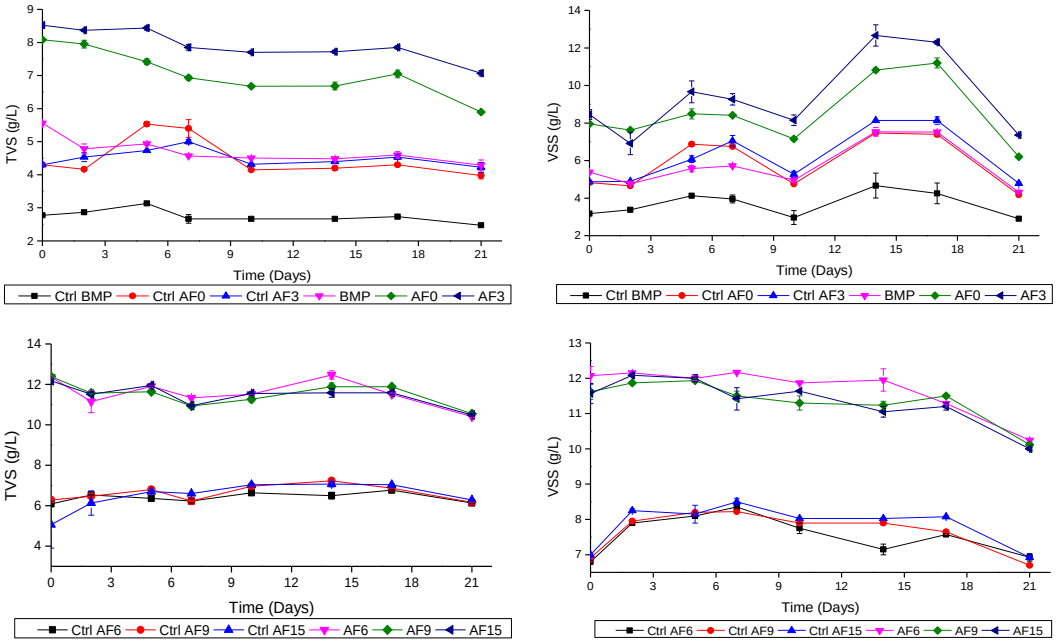

Figure 4.9. TVS and VSS results from the acidogenic fermentation of HPTH-WAS in batch culture with iodoform.

(Key: BMP: biomethane potential test, Acidogenic Fermentation reactors (AF0= 0 mg CHI_3/g $VSS_{Inoculum}$, AF3= 3 mg CHI_3, AF6= 6 mg CHI_3, AF9= 9 mg CHI_3 and AF15= 15 mg CHI_3/g $VSS_{Inoculum}$).

In general, TVS and VSS results agree with the results for TCOD, where it is evident that around of 15% of the organic matter was converted to biogas. Additionally, acidogenic fermentations showed a good performance in COD solubilisation by presenting losses lower than 10% of organic substrate as biogas, possibly due to an adequate inoculation and correct inhibitor dosage. Thus, similarly to the TCOD results, the impact of the iodoform in the TVS content was the preservation of the solids content inside the fermentor for the conversion into VFAs and the avoidance to the mineralisation of the organic matter into biogas.

4.2.3 Effect of iodoform dosage on biogas production

Biogas composition from all fermentors was analysed to understand the biological routes that bacteria followed to generate products. Reactors BMP and AF0 showed high production of methane from day 7, of about 60% (Figure 4.10) which is expected because of the lack of CHI_3 addition in those reactors. These statements are supported with the decay of TVS and SCOD on these fermentors. Reactors AF3 and AF6 (3 and 6 mg CHI_3/ g $VSS_{Inoculum}$) exhibited low production of methane on day 7 (2-30%) and then had their maximum value at day 21, with values above 40% for AF6 and 65% for AF3. Fermentors AF9 and AF15 presented the best methanogenic inhibition of all ratios with tested percentages of around 10% for the first 14 days of fermentation and then reaching values above 60% for AF9 and 10% for AF15. Hydrogen gas was detected in fermentors AF3 and AF15 in the sample for day 7, showing a 3% in the biogas mixture, which is smaller than the percentage reported by Chinellato et al. (2013) when fermenting food waste for hydrogen production (10%) after 24h, showing that hydrogen could be recovered as another product of the acidogenic fermentation. As mentioned previously, neither COD, TVS, VSS or biogas results can be compared with other studies due to the lack of data reported regarding the mentioned parameters.

It is visible the impact of the iodoform on the biogas production in the mixed acidogenic fermentation experiments as the reactors with low or nil concentration of iodoform for CH_4 inhibition (0-6 mg CHI_3/ g $VSS_{Inoculum}$) presented the highest proportion of biomethane. In contrast, higher concentrations of CHI_3 (9-15 mg CHI_3/ g $VSS_{Inoculum}$) presented much lower percentages of methane produced and most likely a high conversion of the organic material inside the fermentor into VFAs.

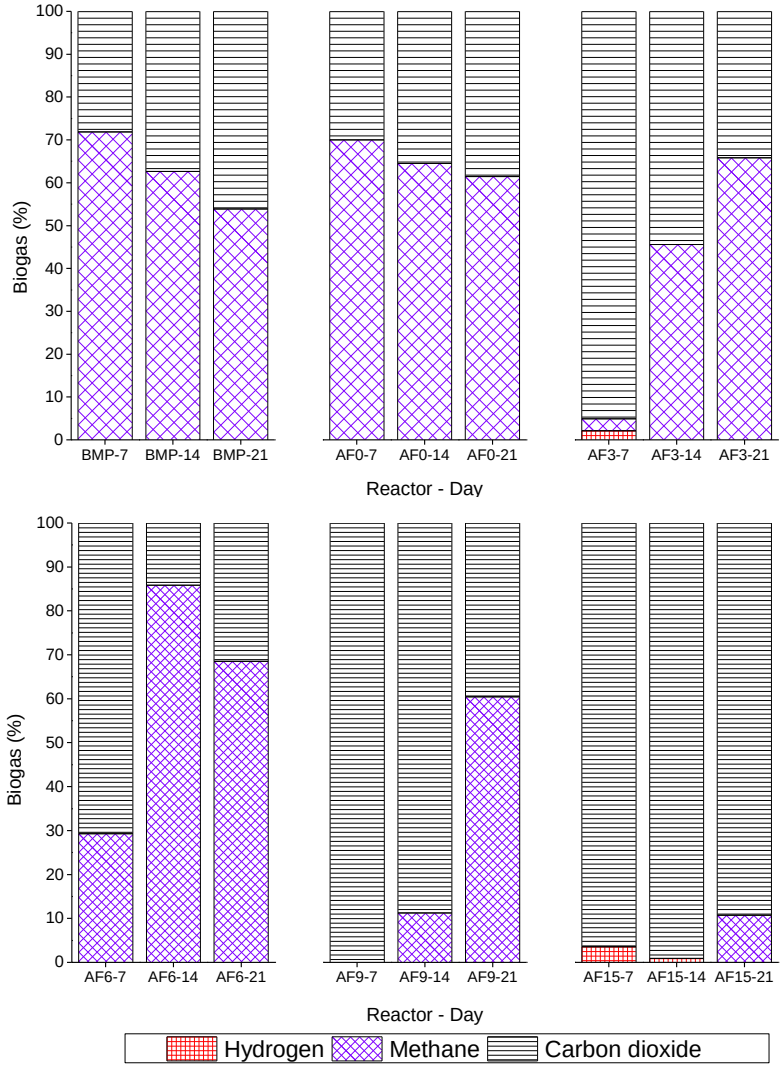

Figure 4.10. Biogas composition from the acidogenic fermentation of HPTH-WAS in batch culture with iodoform.

(Key: BMP: biomethane potential test, Acidogenic Fermentation reactors (AF0= 0 mg CHI_3/g $VSS_{Inoculum}$, AF3= 3 mg CHI_3, AF6= 6 mg CHI_3, AF9= 9 mg CHI_3 and AF15= 15 mg CHI_3/g $VSS_{Inoculum}$ on days 7, 14 and 21*).*

4.2.4 Effect of iodoform dosage on the volatile fatty acids production

A high productivity and yield of VFAs are the main aim of acidogenic fermentations to decrease cost on purification of the produced biochemicals, in view of this, VFAs analysis were carried out to determine the concentration of VFAs in the broth, its composition and the conversion of TCOD to VFAs. Figure 4.11 presents the production of total volatile fatty acids (TVFAs) in acidogenic fermentation reactors. Data reported is the

TVFAs produced in reactors, with WAS as substrate, minus the VFAs produced by the inoculum in the control reactors.

All fermentors with a dosage of iodoform (AF3-AF15) showed similar production of VFAs during the first 7 days (1.09-1.22 gTVFA/L), but after day 7, presented different behaviours, i.e. TVFAs in AF3 started to decrease (from 1.13 to 0.71 g TVFA/L on day 21) while AF6 continue increasing until day 21. On the other side, AF9 and AF15 presented an asymptote from day 10 to day 21, meanwhile TVFAs in AF6, continue increasing until day 21 with a final concentration of 1.83 g/L VFAs, when experiments were finished. It was considered that VFAs concentration presented an asymptote when the acid concentration presented a difference of no more than 5% for a period of three sampling points (Forrest et al., 2012).

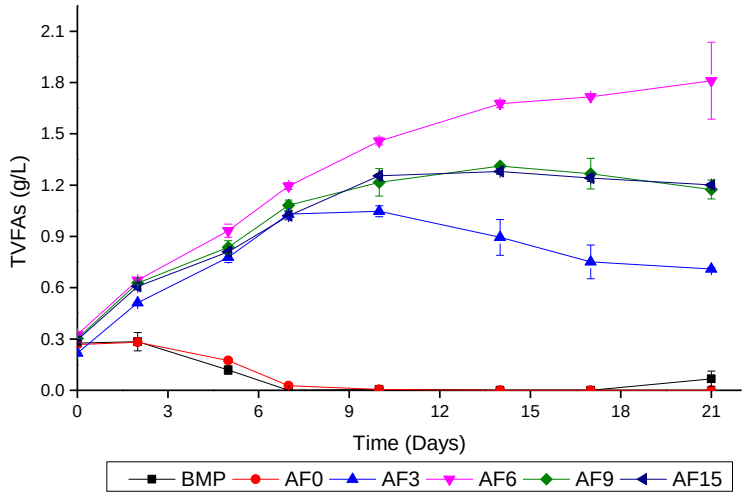

Figure 4.11. VFAs production in acidogenic fermentation of HPTH-WAS in batch culture with iodoform.

(Key: BMP: biomethane potential test, Acidogenic Fermentation reactors (AF0= 0 mg CHI_3/g $VSS_{Inoculum}$, AF3= 3 mg CHI_3, AF6= 6 mg CHI_3, AF9= 9 mg CHI_3 and AF15= 15 mg CHI_3/g $VSS_{Inoculum}$).

Despite reactors AF9 and AF15 presented the best methane inhibition in concordance to gas analysis, AF6 fermentor presented the highest TVFA concentration thus, 6 mgCHI_3/g $VSS_{Inoculum}$ might be envisaged as the best inhibition ratio to reach the highest product generation and a good methane inhibition through the 21 days of the experiment. BMP and AF0 reactors presented TVFAs lowest than 0.3 g/L from day 0 to day 2 and did not showed any increase on the concentration of VFAs after that, which means

the initial VFAs concentration came from the feedstock; and from day 5, VFAs started to decrease probably due to conversion to methane that can be corroborated with the results from biogas composition analysis (CH_4 concentration higher than 50% at day 7). This supports the net VFAs production of about 1.5 g TVFAs/L.

VFAs accounted for more than 95% of the liquid products throughout the process, which means, products like ethanol, methanol, acetone and butanol were not produced in significant percentages, hence, solventogenesis was not promoted with the conditions tested. Ethanol and other solvents were detected in very small concentrations (less than 0.06 g/L), reaching its maximum in days 5 to 7 in reactors AF3, AF6 and AF9 (0.02-0.06 g ethanol/L) which gives information that solventogenesis was not the preferable route for these experiments (Van Andel et al., 1985; Gottschal and Morris, 1981; Grupe and Gottschalk, 1992). The low production of alcohols is mainly due to the pH of the process (≥7.0) that is ideal for the production of VFAs, since it has been reported that the optimum pH for alcohol production is between 3.0 and 5.5 (Dogan et al., 2009; Grupe and Gottschalk, 1992).

VFAs yields from this batch of experiments are reported in Figure 4.12. The highest VFAs yields are from AF3 (0.208 g VFA/g TVS) at day 10 and AF6 (0.238 VFA/g TVS) at day 21, which represents an acidification of almost 25% of the initial TVS feeding. With regards to the COD, AF3 and AF6 showed yields of 0.149 and 0.148 g VFA/g COD, which represents an acid production lower than 15%. Pham et al. (2012) and Pham et al. (2013) report yields between 0.305-0.41 when working with pre-treated macroalgae with initial organic load of 40-50 g dry solids, respectively in about 5 days of fermentation and 30-70 ppm CHI_3 v/v (not specific period of time as well as total addition). Studies from Rughoonundun et al. (2010) descript yields of 0.34 g VFAs/g TVS at 28 days when working with untreated sewage sludge at 31.5 g TVS and with CHI_3 dosage of 0.016 mg/50 g TVS every 48 h (total 252 mg CHI_3). Also Rughoonundun et al. (2012) got yields of 0.36 g VFAs/g TVS with mixtures of sugarcane/WAS as substrate, in fermentations of 36 days and CHI_3 additions of 252 mg CHI_3. Liu et al. (2013b) investigations with WAS at 25 days, 20 g TS of substrate and 8 mg CHI_3/L every 48 h (total 108 mg CHI_3) inform a yield of 0.217 g VFAs/g $TVS_{substrate}$. Finally, Chan and Holtzapple (2003) report yields of 0.15 g VFAs/g $TVS_{substrate}$ in experiments with mixtures of municipal solid waste and sludge in a counter

current reactor. Other studies (Boonsawang and Harnnarong, 2006; Domke et al., 2004; Forrest et al., 2010b; Forrest et al., 2010a; Fu and Holtzapple, 2010b; Nachiappan et al., 2011; Ross and Holtzapple, 2001; Smith and Holtzapple, 2011; Thanakoses et al., 2003b; Thanakoses et al., 2003a) report yields between 0.027-0.258 g VFAs/g TVS substrate using a wide diversity of organic substrates at different fermentation times and iodoform dosages when working in a countercurrent fermentation system. All studies mentioned above, do not report the total amount of ethanol added to the fermentor when adding the CHI_3 and hence, do not take into account the quantity of the external organic substrate added to the system when calculating/reporting values for yields and productivity. These findings make difficult to stablish a solid comparison among the yields and results from this project and mentioned studies. Nevertheless, it is important to point that present fermentations showed good VFAs yields without the addition of easy degradable external substrates like ethanol. The positive and clear results from this part of the project offers a precedent on the use of CHI_3 as inhibitor in anaerobic acidogenic fermentation when added without being dissolved in pure ethanol for the production of VFAs from HPTH-WAS.

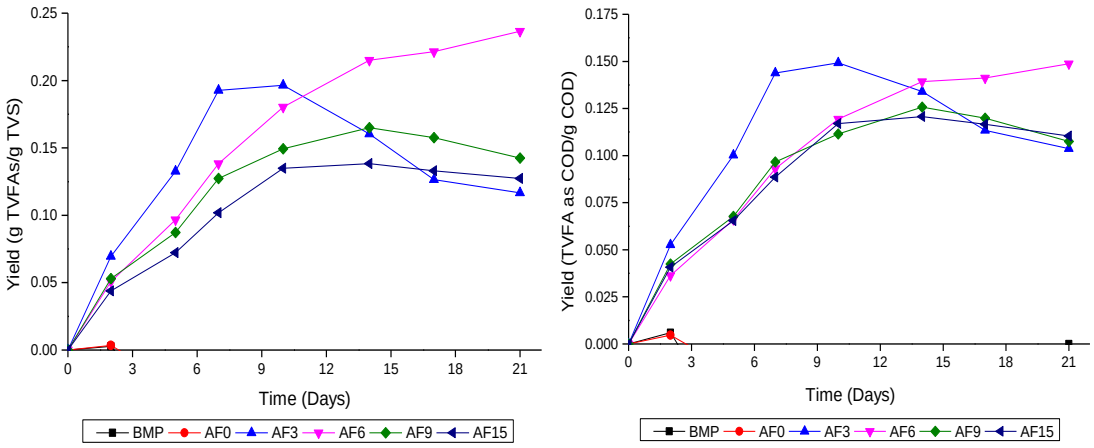

Figure 4.12. Average VFAs yields from WAS mixed acid fermentation of HPTH-WAS in batch culture with iodoform.

(Key: BMP: biomethane potential test, Acidogenic Fermentation reactors (AF0= 0 mg CHI_3/g $VSS_{Inoculum}$, AF3= 3 mg CHI_3, AF6= 6 mg CHI_3, AF9= 9 mg CHI_3 and AF15= 15 mg CHI_3/g $VSS_{Inoculum}$).

Statistical analyses were conducted in order to compare the yields reached in these series of experiments to determine the best combination of inhibition

ratio and best hydraulic retention time (HRT), which reached the highest VFAs yields.

As fermentor AF6 showed the highest yields, statistical analysis was conducted to determine whether yields for day 21 were statistically different than yields for day 14 and day 17, taking into consideration that TVFAs concentration did not change more than 7% when comparing TVFAs yields of day 14 and day 21. To accomplish this aim, paired-samples t-test was carried out in SPSS® with confidence intervals (CI) of 95% (Table 4.4). The results showed there was no statistically significant difference in VFAs yield scores from day 21 (M=0.238, SD=0.025) to day 14 (M=0.218, SD=0.002) or to day 17 (M=0.224, SD=0.000), with a $p>0.05$ (Sig. two-tailed). The eta squared statistic (0.50) indicated a large effect size which means, a large proportion of variance of the VFAs yield is explained by the fermentation time. Finally, is important to mention that day 14 could be envisaged as the best/shortest HRT in acidogenic fermentations of WAS with 6 mg CHI_3/g VSS.

Table 4.4. Paired samples t-test of yields on different days of reactor AF6 of fermentation of HPTH-WAS in batch culture with iodoform.

	Paired differences					t	df	Sig. (2-tailed)
	Mean	Std. deviation	Std. error mean	95% CI of the difference				
				Lower	Upper			
AF6D14 - AF6D21	-0.0194	0.0228	0.0131	-0.0760	0.0372	-1.47	2	0.279
AF6D17 - AF6D21	-0.0134	0.0246	0.0142	-0.0747	0.0477	-0.94	2	0.444

Additionally, an independent-sample t-test was conducted using yields values for AF3 and AF6 fermentors as they reached the highest VFAs yields at day 10 and day 21 respectively, with an $\alpha=0.05$. Yields at day 10 for AF3 fermentor were 0.208, 0.2082 and 0.2207 g VFAs/g TVS and for fermentor AF6 were 0.2128, 0.2381 and 0.2635 g VFAs/g TVS on day 21. Results can be found in Table 4.5:

Table 4.5. Independent samples t-test for fermentors AF3 and AF6 of fermentation of HPTH-WAS in batch culture with iodoform.

Yield	Levene's Test for Equality of Variances		t-test for equality of means						
	F	Sig	t	df	Sig. (2-tailed)	Mean difference	Std. Error difference	95% CI of the difference	
								Lower	Upper
Equal variances assumed	0.83	0.41	1.83	4	0.140	-0.2995	0.0163	-0.0153	0.0752

Results show a Sig. (2-tailed)>0.05 which confirm that the differences between the yields of each fermentor are not statistically significant different, hence, both conditions, 3 and 6 mg CHI_3/g VSS give the same performance and effectiveness at day 7 and 21 respectively. Considering that seven days is a shorter HRT, AF3 showed the best efficiency on producing VFAs with the lowest inhibition ratio and the shortest HRT. If the main aim on a fermentation is the correct inhibition of the system, AF6 (6 mg CHI_3/g VSS) presented the highest effectiveness because of its low production of methane.

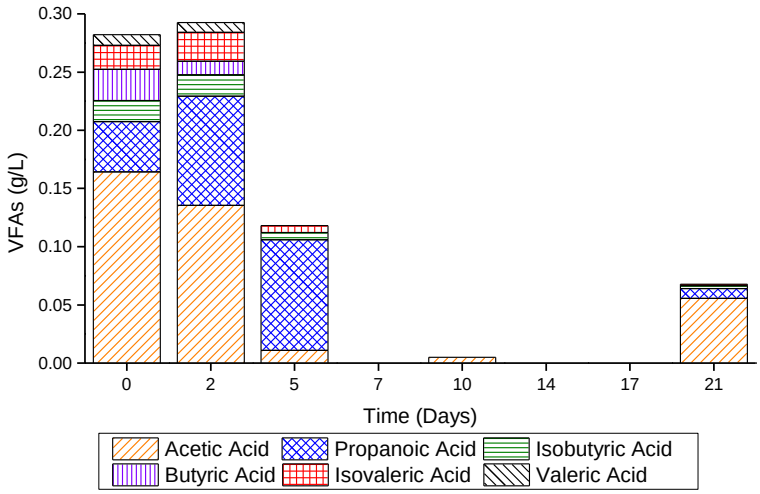

Figure 4.13. VFA profile in BMP reactor from WAS mixed acid fermentation of HPTH-WAS in batch culture with iodoform.

In concordance with Figure 4.11, BMP reactor showed a constant concentration of VFAs during the first two sampling days which showed that

VFAs in the broth came from the feedstock (WAS) as in AF fermentors, hence, VFAs were not produced but consumed to generate biogas and then decreased dramatically to values about 0.1 g/L or less during the whole fermentation which agrees with the decay of SCOD as reported previously.

Due to reactor AF3 and AF6 presented the best yields and VFAs production, a more detailed analysis of results was done (Figure 4.14).

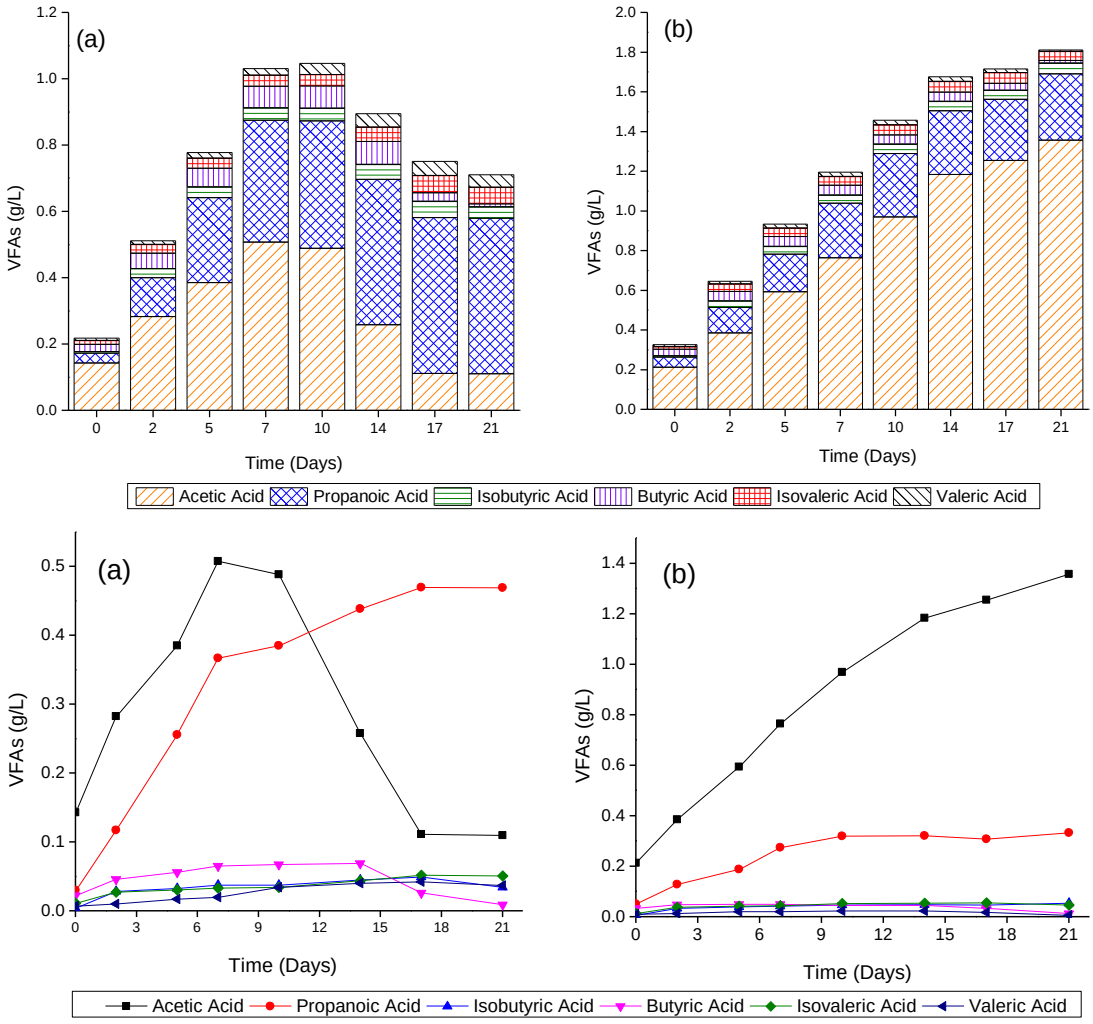

Figure 4.14. VFA profiles in reactors AF3 (a) and AF6 (b) of HPTH-WAS in batch culture with iodoform.

Volatile fatty acids concentration reached the highest value at day 10 for fermentor AF3 and then a decrease which suggests there was a conversion of VFAs probably to biogas (25-55% of the TVFAs); meanwhile AF6 presented an asymptote on VFAs concentration from day 14 till day 21

where the increment was less than 6%. Acetic acid was predominant in fermentor AF6 along the complete process, reaching the highest concentration on day 21 with 1.357 g/L with a percentage of 75%. Acetic acid concentration varies between 59% - 75% in the acid blend for AF6; its concentration is similar to percentages obtained by Forrest et al. (2010a), Golub et al. (2013), Rughoonundun et al. (2012) and Ucisik and Henze (2008) with ranges between 43-95%.

It is important to mention that reactor AF3 presented a substantial decrease of acetic acid from day 10 and increase of methane produced which is feasible due to acetic acid is the main substrate for the production of CH_4. Propionic acid was produced constantly along the experiment and no consumption or decrease is visible, presumably because it was not transformed to methane as the conversion is less favourable thermodynamically compared with butyrate or acetate (Khan et al., 2016; Yu et al., 2016). No attempts were made to identify or quantify other types of acids such as lactic, succinic and others with more than 6 carbons chain.

Propionic acid was present in AF3 and AF6 fermentors as second highest acid concentration along the whole experiment getting concentration levels between 15–22% of total acid concentration in the fermentation broth which agrees with acidogenic fermentations studies conducted by Morgan-Sagastume et al. (2011) and Pham et al. (2013) which report results from 15 to 37% when working with WAS and marine macroalgae as the main substrate, respectively.

It is visible the small or null effect of the iodoform dosage on the volatile fatty acids speciation in the mixed acidogenic fermentation as, among all the reactors, acetic acid was the main product followed by the propionic acid regardless the CHI_3 dosage applied. In terms of carboxylic acids production, the iodoform dosage showed a positive effect as its addition promoted the generation and accumulation of VFAs and the poor conversion into biomethane and the loss of VFAs.

The VFAs/SCOD relation (also called as degree of acidification) indicates the degree of acidogenesis, which is the conversion of soluble organic matter to VFAs (Bengtsson et al., 2008; Maharaj and Elefsiniotis, 2001). In order to compare SCOD with production of VFAs, VFAs concentration was converted in terms of COD by using the conversion factors given by Yuan et al. (2009): 1.07 g COD/g acetic acid, 1.51 g COD/g propionic acid, 1.82 g

COD/g butyric and isobutyric acid and 2.04 g COD/g valeric and isovaleric acid. On average, the increase on the degree of acidification was 42-46% and 56-59% for AF3 and AF6 on day 21, respectively (Figure 4.15).

The highest value of total VFAs (as g COD), 2.18 g COD, was obtained at day 21 in reactor AF6 which represents a yield of 0.34 g VFA (as COD)/g TVS, while studies conducted by Xiong et al. (2012) show a production of 5.699 g VFA (as COD)/L after 8 days fermentation when working with WAS at a TVS value of 23.78 g/L which represents a yield of 0.23 g VFA (as COD)/g TVS. Kim et al. (2006), obtained a production around of 3.5 g VFA as acetate/L (data obtained from a graph) when working with a TVS initial feeding of 8 g/L of food waste. Also Bengtsson et al. (2008) reports productions of 3.96 g VFAs (g COD/L) when using paper mill as feedstock with a yield of 0.59 g COD/g COD; and 2.27 g VFAs (g COD/L) with cheese whey as main raw material with a yield of 0.60 g COD/g COD. It was found that the percentage of acidification in this project had higher acidification when compared to other experiments working with different types of substrate although suspended TCOD did not make a significant contribution for the VFA production (3-5%). Also it is important to notice that around 10% of the TCOD was loose as biogas in fermentations AF3 and AF6 on day 21.

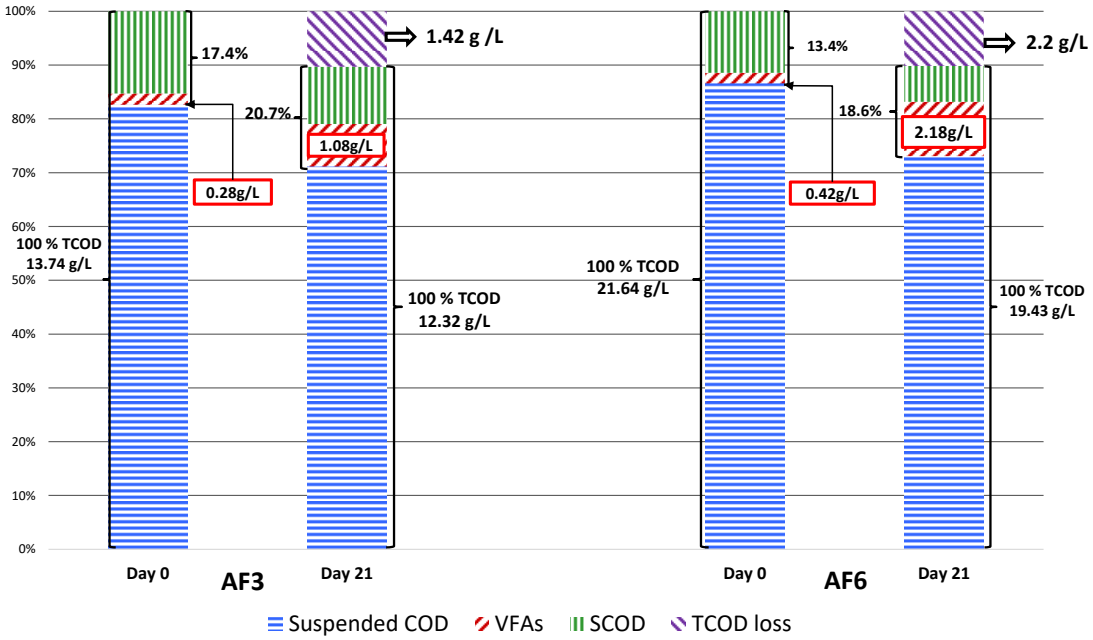

Figure 4.15. COD conversion on the acidogenic fermentation of HPTH-WAS in fermentors AF3 and AF6 in batch culture with iodoform.

4.2.5 Stoichiometric (SMP) and Biochemical methane potential (BMP) and VFAs production

With the data from the empirical formula of WAS ($C_{10.3}H_{16.5}NO_{4.3}$) and the VFAs production from fermentors AF3 and AF6, a stoichiometric (SMP) and Biochemical methane potential (BMP) were calculated in order to compare if VFAs production in the acidogenic fermentation could be competitive with the amount of methane produced in the current anaerobic digestion with methane production in a WWTP.

The stoichiometric methane potential (SMP) is used to assess the theoretical production of biogas by using the empirical formula of a particular substrate. The results of SMP are useful to estimate rapidly and to compare the methane potential yield of a determined substrate and its real BMP results tested in the laboratory (Hansen and Christensen, 2005; Shanmugam and Horan, 2009).

The calculation of SMP is done with the formula below (Symons and Buswell, 1933):

$$SMP=\frac{22.4\cdot\left(\frac{n}{2}+\frac{a}{8}-\frac{b}{4}-\frac{c}{8}\right)}{12n+a+16b+14c}=\frac{\text{STP litre } CH_4}{\text{g TVS}} \quad \text{.....Equation 4.1}$$

Where:

$$n=\frac{\%C}{12T};\quad a=\frac{\%H}{T};\quad b=\frac{\%O}{T};\quad c=\frac{\%N}{T};\quad \text{and}\quad T=\frac{\%C}{12}+\%H+\frac{\%O}{16}+\frac{\%N}{14}$$

The SMP using the empirical formula resulted in a production of 0.461 litre CH_4/g TVS of HPTH-WAS at STP conditions. As methane was not quantified, the BMP could be calculated from the VFAs produced in the reactors and taking in consideration the biochemical reactions of the VFAs according to Buswell and Mueller (1952), Heidrich et al. (2011), Liu et al., 2004, Nelson et al. (1958) and Thauer et al. (1977a) ash shown below:

$$C_nH_aO_b+\left(n+\frac{a}{4}+\frac{b}{2}\right)H_2O\rightarrow\left(\frac{n}{2}-\frac{a}{8}+\frac{b}{4}\right)CO_2+\left(\frac{n}{2}+\frac{a}{8}-\frac{b}{4}\right)CH_4 \quad \text{.....Equation 4.2}$$

Then:

CH_3COOH (acetic acid)$\rightarrow CH_4+CO_2$ ……Equation 4.3

$4CH_3CH_2COOH$ (propionic acid)$+2H_2O \rightarrow 7CH_4+5CO_2$……..Equation 4.4

$2CH_3CH_2CH_2COOH$ (butyric acid)$+2H_2O \rightarrow 5CH_4+3CO_2$……...Equation 4.5

$2CH_3CH_2CH_2CH_2COOH$ (valeric acid)$+2H_2O \rightarrow 13CH_4+7CO_2$.Equation 4.6

The empirical biochemical potential of biomethane from the reactors BMP, AF3 and AF6 were 0.220, 0.567 and 0.914 litre CH_4, respectively; whereas the amount of TVS of WAS as initial feedstock in each fermentor could give experimental yields of BMP=0.079; AF3=0.135 and AF6=0.146 litre CH_4/g TVS). Thus, experimental BMP values from acidogenic fermentation for the production of VFAs with iodoform as methanogenic inhibitor of reactors BMP, AF3 and AF6, presented percentages of 18, 30 and 33% of the theoretical methane potential respectively. Fermentations with 3 and 6 mg/L CHI_3/g VSS presented similar values; around 12% higher than the AD experiment (BMP reactor). It is worth to mention that the experimental biochemical potential from the BMP reactor reached 18% due to the initial VFAs content from the feedstock and not because of a long and sustained VFAs production inside the fermentor. In practice, the amount of biomethane recovered per gram of TVS is lower than the theoretical biomethane potential, as not all solids are biodegradable, thus, the results suggest that around 70% of the organic matter contained in WAS in this study, was not completely biodegraded in a fermentation of 21 days and possibly a longer retention time might be needed. Comparing the VFAs production and its biomethane potential from this study with biomethane potential from anaerobic digestion studies using hydrothermal pre-treated WAS as feedstock for the production of CH_4, conducted by Bougrier et al. (2007), Bougrier et al. (2008), Cano et al. (2014) and Qiao et al. (2011) (0.256-0.333 litre CH_4/g TVS), it is clear that acidogenic fermentation could reach a yield of between 43-52% of the standard biomethane process with the advantage of the unconverted material could be used subsequently for the production of biomethane in an AD system.

Finally, an economic analysis is suggested to determine the costs of running an acidogenic fermentation, its VFAs yields and its comparison with the current anaerobic digestion and methane production.

4.2.6 Mass Balance

Total COD and TVS results were used to calculate a mass balance of the fermentations with CHI_3 without the addition of ethanol to track the conversion of the feedstocks to products and also to review the performance of the fermentors when an inhibitor is added in powder (Figure 4.16).

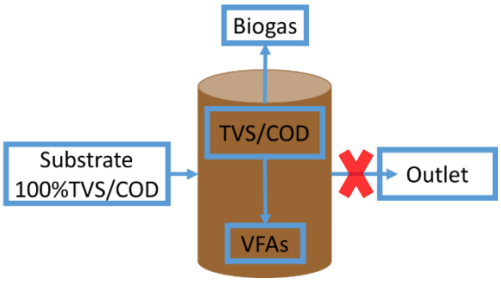

Figure 4.16. Organic matter balance of the batch fermentation of HPTH-WAS.

Mass distribution and conversion was different among the reactors due to its different operational conditions. In all reactors, TVS and COD were mainly kept inside the system (72.9-86.1% TVS, >87%COD) and low conversion to gaseous products (CH_4, CO_2) was achieved (counted as TVS or COD losses). The highest conversion of COD and TVS to biogas was achieved from reactor AF0, 14.6 COD and 37.1% TVS; probably due to the neutral pH and high alkalinity (>5 g/L $CaCO_3$) which promoted the action of methanogenic bacteria. BMP reactor reached the second highest loss of TVS (29%) (substrate mineralisation) but did not showed the highest biogas production perhaps due to low alkalinity and uncontrolled pH. Reactor AF6, AF9 and AF15 showed similar losses of TVS, between 16.2-18.8%, being AF15 (15 mg CHI_3/g VSS), the one with the lowest mineralisation.

Mass balance for control fermentors is also shown in Figure 4.17 and Figure 4.18 where is evident that changes in TCOD were lower than 1.3% for all control fermentors. Additionally, VSS losses were not higher than 12% for reactors with no inhibitor and lower than 2% for reactors with inhibitor dose. That behaviour suggests that inoculum had little or no inference on the production of gas or liquid products which agrees with results from TCOD, TVS and VFAs and also demonstrate that products were mainly generated from the substrate added and not from the consortia of bacteria in a potential decay phase.

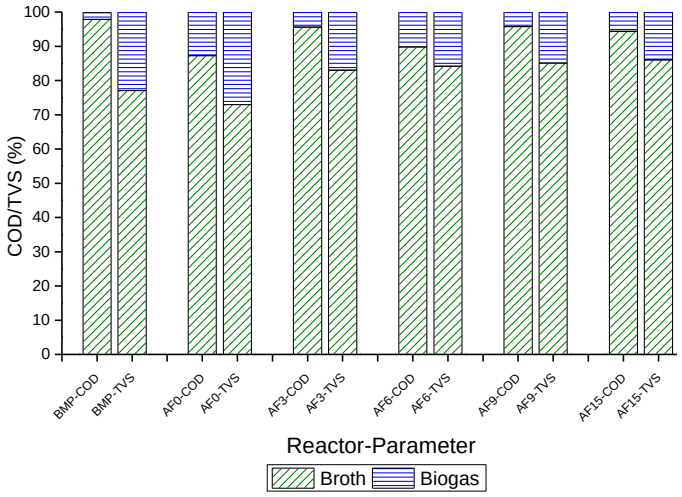

Figure 4.17. Mass balance for mixed acidogenic fermentation of HPTH-WAS as COD and TVS.

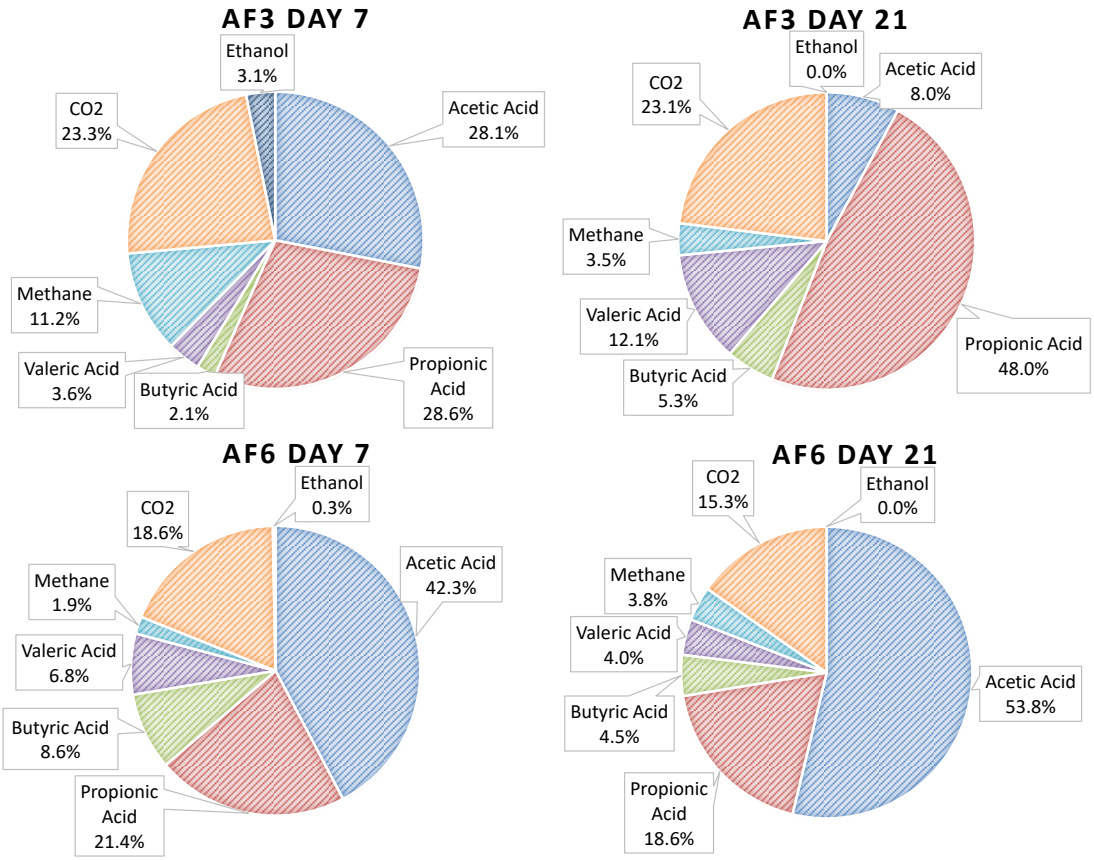

Figure 4.18. Mixed acidogenic fermentation average products expressed as mass percentage in terms of COD of AF3 and AF6.

Since the experiments were ran in batch sealed systems and biogas generation was prevented by an inhibitor, it is comprehensible that most of the organic matter remained in the batch unit. As the initial and final results of TVS and COD from control reactors were practically stable along the experiment, it is visible that the biomass (as VSS) inside the reactor remained quasi-constant and hence biomass production could be considered negligible. It is worth to mention that CO_2 was the main gas present as a product in all fermentors, with values up to 20% of the total COD (Figure 4.18).

A deeper analysis strengthened that among all the products from black fermentation, VFAs were the main compounds generated. Other products as biomass, ethanol and hydrogen were present in small percentages while formic, succinic, caproic and lactic acid were not analysed as well as pyrroles, indoles and aromatic acids which comes from the fermentation of proteins and aminoacids (Bastidas-Oyanedel et al., 2015; Morgan-Sagastume et al., 2011).

4.3 Summary

Mixed acidogenic fermentation of HPTH-WAS can produce VFAs with results as 1.83 g/L of VFAs which represents a yield of 0.238 g TVFAs/g TVS (0.34 g VFA (as COD)/g TVS) when methanogenic stage is inhibited with iodoform at a ratio of 6 mg CHI_3/g $VSS_{Inoculum}$ and low organic loading rate (5 g TVS_{WAS}/L) on day 21. However, fermentations using inhibition ratio of 3 mg CHI_3/g VSS presented a yield of 0.208 g VFA/g TVS on day 7. On average, the conversion of soluble COD to VFAs reached values of 42-46% and 56-59% for fermentations with 3 and 6 mg CHI_3/g VSS, respectively.

The results on VFAs showed an small or null effect of the iodoform dosage on the VFAs speciation in the mixed acidogenic fermentation as acetic acid was present in all reactors with contents between 50-75% and then propionic acid with contents between 15–22% of the total VFAs mixture on day 21 regardless the CHI_3 dosage applied. In contrast, the addition of iodoform showed an positive effect on the VFAs production as its generation and accumulation was promoted and the conversion into methane was poor.

Experimental BMP values from acidogenic fermentations with inhibition ratios of 3 and 6 mg CHI_3/g VSS reached percentages of 30-33% of the theoretical methane potential; suggesting that around 70% of the HPTH-WAS, was not completely biodegraded.

The clear results from this experiments offer a precedent on the use of CHI_3 as inhibitor in anaerobic acidogenic fermentation, giving as outcome, a ratio which relates the amount of inhibitor with VSS in the fermentation which is a common parameter in wastewater and WAS treatment.

In summary, the results obtained in this study of acidogenic anaerobic fermentation of HPTH-WAS to produce VFAs as an alternative of methane production, demonstrate that this could be a feasible option to produce biochemicals as a first step before existing anaerobic digestion for biogas generation and thus, reducing the wastewater treatment cost.

Chapter 5. EFFECT OF THE pH ON THE PRODUCTION OF VFAs IN MIXED ACID FERMENTATION OF WASTE ACTIVATED SLUDGE.

In acidogenic fermentation systems, some operational factors are key to improve the production of VFAs and its optimisation in laboratory experiments, pilot or full-scale; the most important parameters are: type of bioreactors, temperature, substrates and substrate pre-treatment, hydraulic retention time, organic loading rate, additives and pH (Bastidas-Oyanedel et al., 2015; Khan et al., 2016; Zhang et al., 2015). The pH plays an important role because of different values affect the production/accumulation of alcohol-acetone and elongation of VFAs carbon chain (in acidic pH) and the neutralisation of VFAs for a sustained production of VFAs (in basic pH) and also, it could act as an inhibitor of the methane production and/or the inhibition by the products (VFAs) (Chen et al., 2007; Gottschal and Morris, 1981; Zhang et al., 2009; Zhang et al., 2015). The production of VFAs and the influence of pH has been investigated in waste activated sludge (Chen et al., 2007; Liu et al., 2015; Ma et al., 2016; Wan et al., 2016; Yan et al., 2010; Yuan et al., 2006; Zhuo et al., 2012), primary sludge (Wu et al., 2009), food waste (Wang et al., 2014a; Zhang et al., 2005), glucose (Tamis et al., 2015; Temudo et al., 2007), soluble portion of WAS (Liu et al., 2012a), synthetic wastewater with gelatine as main carbon source (Yu and Fang, 2003), dairy wastewater (Yu and Fang, 2002), high-pressure thermal hydrolysis (HPTH) WAS (Morgan-Sagastume et al., 2011), among other substrates, but to the knowledge of this research, no studies have been conducted using HPTH-WAS at six different pH levels in order to determine the best operational pH and its effect on the VFAs composition and yields. For that reason, acidogenic fermentation at pH levels of 4, 5, 6, 8, 9 and 10 were investigated. Levels of pH lower than 4 or higher than 10 are unlikely to sustain appropriate microbial activity as extreme pH affects the structure of all macromolecules. While at low pH the hydrogen bonds holding together could cause the DNA break up, at basic pH lipids can be hydrolyzed. The most important macromolecule to be considered are the proteins as slight changes in the pH could modify the ionization of amino-acid functional groups and disrupt hydrogen bonding, promoting denaturation and stoping the microbial activity (Rosso et al., 1995; Russell et al., 1979).

The experiments were run using the same conditions as the experiments in section 4.2: 1 L reactor (0.8 L working volume), 37°C, 10 g TVS/L_{Liq} in a ratio of 1 g TVS of feedstock/ g $VSS_{inoculum}$, no iodoform or $CaCO_3$ addition. The pH was adjusted by opening the reactors at any sampling day the pH dropped to keep the level in a range of ±0.15 pH units, under nitrogen flux to ensure anaerobic conditions. Acidogenic fermentation at pH seven was not conducted as it was considered that AF0 in the previous chapter, was operated with the same conditions.

5.1 Effect of pH on the chemical oxygen demand

It is very important to track down the TCOD and SCOD content along acidogenic fermentation experiments as it involves the TCOD destruction and conversion to biogas, the changes on SCOD/TCOD ratio exhibiting the hydrolysis of suspended COD, and the possible conversion to products such as biochemicals and/or biogas and also, the potential effect of the conditions investigated on the COD changes.

Figure 5.1 shows the total COD from both, control (Ctrl pH) and Acidogenic Fermentation reactors at different pHs (AFpH), where AFpH 4 is the acidogenic fermentation at pH 4, AFpH5 denotes the results from the experiments ran at pH 5 and so on until AFpH10 which are the reactors which pH was adjusted at level 10. Control and AFpH reactors showed a decrease on the TCOD content between 1.8-25.8% and 1.33-18.9% at day 21, respectively, showing the lowest decrement on AFpH 10 and the highest on AFpH 8 for the AFpH reactors and on the CtrlpH6, suggesting a high TCOD destruction and conversion to biogas at pH levels near to neutral value which agrees with the reports from Chen et al. (2007) and Gerardi (2003).

It is clear that different pH levels caused different effects on the TCOD content in the mixed acidogenic fermentation, whereas extreme pH levels (4 or 10) caused a low TCOD destruction, pH values close to seven (6-8) caused a higher TCOD mineralisation and loss, which agrees with the higher microbial activity at pH closes to neutrality (Rosso et al., 1995; Russell et al., 1979).

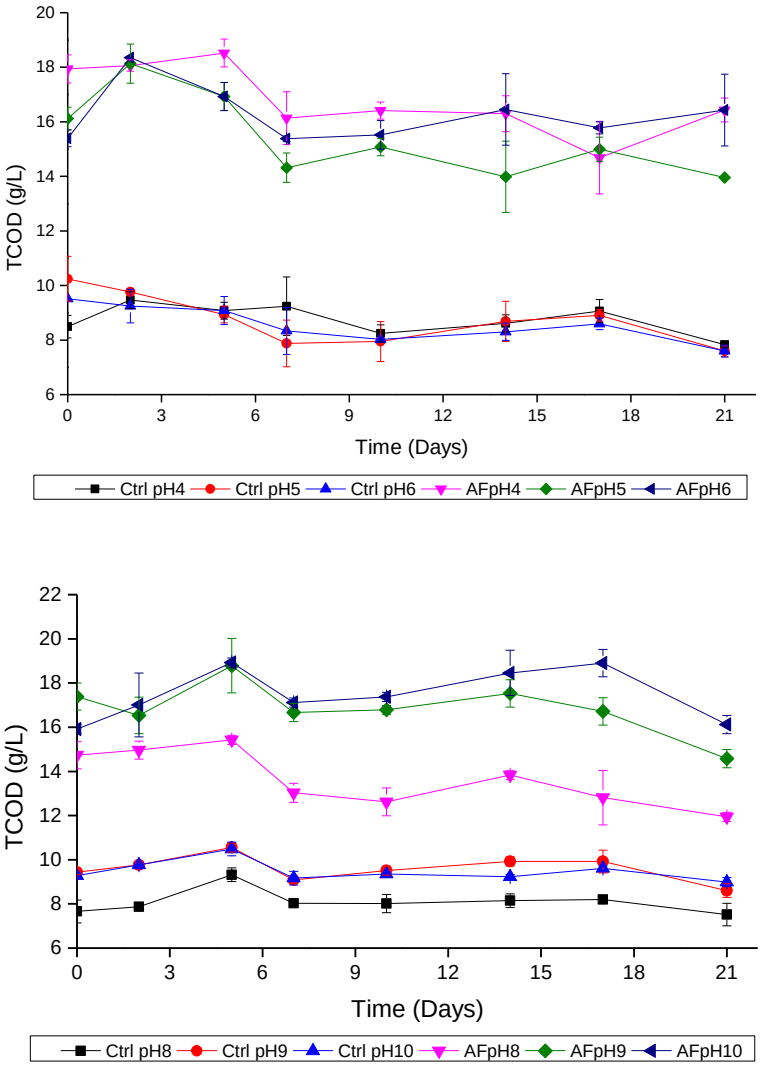

Figure 5.1. Total COD results from acidogenic anaerobic fermentation of HPTH-WAS at different pH levels.

(Key: Ctrl pH are the blanks of each fermentation with different pH levels (4-10); AFpH reactors are the acidogenic fermentation at different pHs (4-10); thus, AFpH 4 is the acidogenic fermentation at pH 4, and so on until AFpH10 which is the reactor working at pH 10).

In contrast, the SCOD results from the AFpH reactors on day 21 showed mixed results; while acidic-neutral pH fermentors (pH4-8) presented a decrease on SCOD during the time of fermentation (between 21.3-72.5%), alkaline pH levels (pH9-10) exposed an increment (2.2-11.6%) with regards to the initial SCOD content (Figure 5.2).

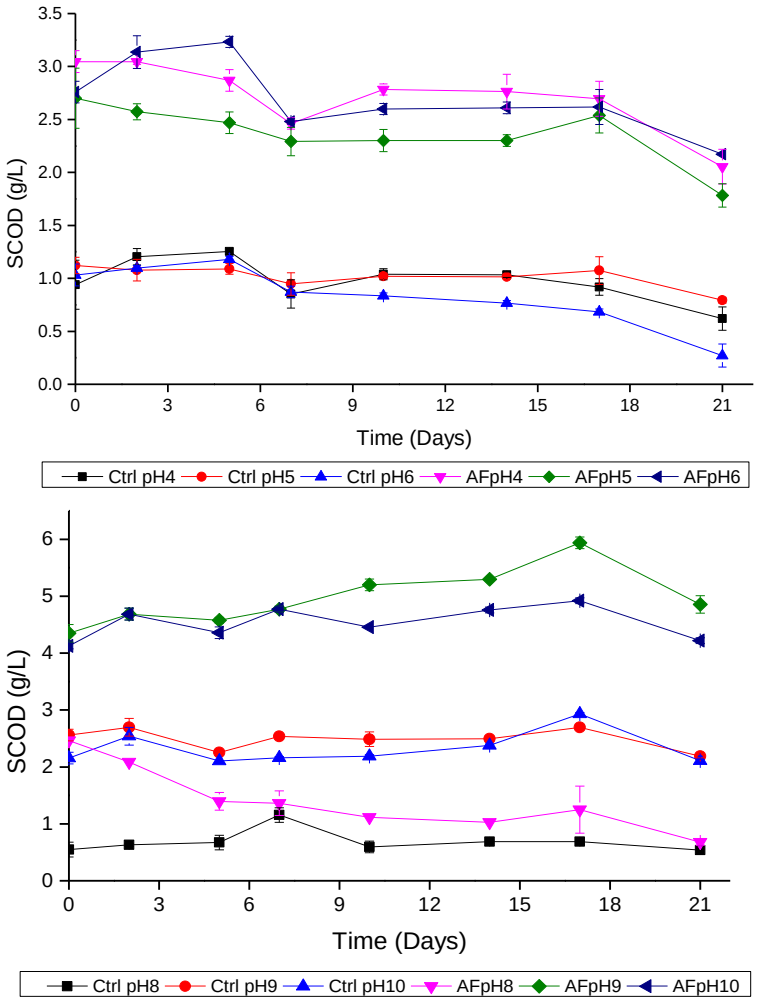

Figure 5.2. Soluble COD results from acidogenic anaerobic fermentation of HPTH-WAS at different pH levels.

(Key: Ctrl pH are the blanks of each fermentation with different pH levels (4-10); AFpH reactors are the acidogenic fermentation at different pHs (4-10); thus, AFpH 4 is the acidogenic fermentation at pH 4, and so on until AFpH10 which is the reactor working at pH 10).

There was no effect of the pH on the initial SCOD, which means, there was no hydrolysis of the COD caused by the change on the pH on day zero. When subtracting the SCOD of the control reactors of its respective mixed acidogenic fermentors (AFpH), it was seen that the resulting SCOD, which should come purely from the feedstock at different pH, did not increase and reached a value in the range of 1.57-2.10 g/L SCOD which concurs with the calculated SCOD value (~2.07 g/L) from the 5 g/L of HPTH-WAS that was added. These results exhibited that the hydrolysis reached the pre-treatment of WAS by HPTH-WAS not improved when the pH was adjusted in the

acidogenic fermentation; however, the activity of the hydrolytic bacteria in the system increased the solubilisation of the organic material.

SCOD content of AFpH9 and AFpH10 from day 0, when compared with fermentors AFpH4-8 (near neutral pH), reached an increase between 42.7-76.5% and 39.9-67.5% respectively (Table 5.1), probably because of the higher hydrolysis of TCOD from the inoculum at alkaline pH, which probably was caused by the disruption of flocs and cells, releasing and hydrolysis of proteins and other organic matter (Cysneiros et al., 2012; Kim et al., 2003b; Li et al., 2010; Ma et al., 2016; Penaud et al., 1999).

Table 5.1. Organic matter hydrolysis in acidogenic fermentations of HPTH-WAS at different pH levels.

	AFpH4		AFpH5		AFpH6		AFpH8		AFpH9		AFpH10	
Day	Su-COD	SCOD	Su-COD	SCOD	Su-COD	SCOD	Su-COD	SCOD	Su-COD	SCOD	Su-COD	SCOD
0	83.0	17.0	83.2	16.8	82.1	17.9	83.3	16.7	75.0	25.0	74.1	25.9
7	84.7	15.3	84.0	16.0	83.9	16.1	89.5	10.5	71.4	28.6	72.1	27.9
14	83.0	17.0	83.5	16.5	84.1	15.9	92.6	7.4	69.8	30.2	74.2	25.8
21	87.5	12.5	87.2	12.8	86.8	13.2	94.3	5.7	66.7	33.3	73.8	26.2

*Su-COD= Suspended COD

After day zero, SCOD in fermentor AFpH9 exposed a growth between 5-36% with respect to the initial SCOD value ending with an increase of 11.6% at day 21 and a value of 33.3% of the total COD; this further hydrolysis was caused probably due to enzymatic hydrolytic activity of the bacteria inside the fermentor.

Fermentor AFpH8 did not show evidence of COD hydrolysis but there was a decrease on SCOD on day 21 (72.5% = 1.79 g/L) which correspond with the results from TCOD loss (18.9% = 2.79 g/L) and AFpH6 presented a decrease of 34% of SCOD, which might due to the ideal pH for biogas generation is between 6.5-7.9 (Appels et al., 2008; Chen et al., 2007; Dong et al., 2010a; Liu et al., 2012a; Temudo et al., 2007; Yu and Fang, 2002; Zhang et al., 2005). The solubilisation of the TCOD in fermentor AFpH9 is clearly 5.8-fold higher than AFpH8 and 2.6-fold higher than TCOD in fermentor AFpH4.

The values of solubilisation of COD along the fermentation process in AFpH9 are similar to the values published by Ucisik and Henze (2008) who reported a degree of solubilisation of 12.1% for primary sludge in batch experiments with SRT of 5 days and no pH adjustment; and also to the results from Rajagopal and Béline (2011) who reported TCOD hydrolysis of 20% for secondary and pre-treated sludge; but smaller than the findings by Chen et al. (2007) with a 68.3% of hydrolysis after 20 days at pH 11 when working with untreated secondary sludge.

Also, Wu et al. (2009) reported a SCOD increase in fermentations at pH levels 9 and 10 from day one with a maxima on day 5 at pH 9 (105%) and pH 10 (107%) using sewage sludge from primary sedimentation tank. Chen et al. (2007) reported an increase of around 6.5 times the solubilisation of COD at pH 11 on day 8, when compared with a blank with no pH adjustment and using WAS from a secondary sedimentation tank. Also, experiments ran by Yuan et al. (2006) exhibited a growth on COD hydrolysis from pH 8 to pH 11 on fermentations of WAS from a secondary sedimentation tank, reaching a solubilisation 4 times higher at pH 11 than a neutral pH. Ma et al. (2016) reported hydrolysis of 54.3% after 10 days of fermentation of dewatered sludge at pH 10. Studies done by Zhang et al. (2005) in kitchen wastes which states a COD solubilisation of about 82%.

In terms of SCOD values, pH presented different types of effect, levels of pH from 4 to 8 presented negative effect on the SCOD accumulation in the system as SCOD was consumed during the process which indicates the possible conversion of the soluble organic matter into gaseous products. In contrast, experiments working with alkaline pH (9 and 10) presented a sustained increase on the SCOD value which denotes the low methanogenic activity and the continuous activity of the hydrolytic and/or acidogenic/acetogenic bacteria, corroborating the positive effect of the alkaline pH on the acidogenic fermentation process. These findings are in agreement with the understanding of the anaerobic microbial activity, where systems with pH close to 7 tends to the mineralisation of the organic material (Rosso et al., 1995; Russell et al., 1979).

Taking these results into consideration, it is clear that either, pH9 or 10, could improve the hydrolysis of WAS to about 9% of the initial TCOD content with similar results than other studies previously published, consequently, pH 9 was chosen as the optimum because its high performance on enzymatic hydrolysis at the lowest NaOH addition.

5.2 Effect of pH on the solids content

Figure 5.3 depicts the TVS results of the reactors at different pH. It is observed that reactors with highly acidic pH (4-5) and pH 10, showed a very low decrease (7%) on volatile solid content in the broth, which suggests a very poor conversion into biogas probably because pH was not the optimal for methanogenic or hydrogenic bacteria. On the other hand, AFpH8 and AFpH9 presented a reduction of TVS which imply a loss on organic matter, mainly as biogas.

Fermentor AFpH8 presented the TVS highest mineralisation with a TVS reduction of 16.6%, most probably due to the pH is near to neutral, which as has being previously pointed, is near the optimum pH for the production of biogas (Appels et al., 2008).

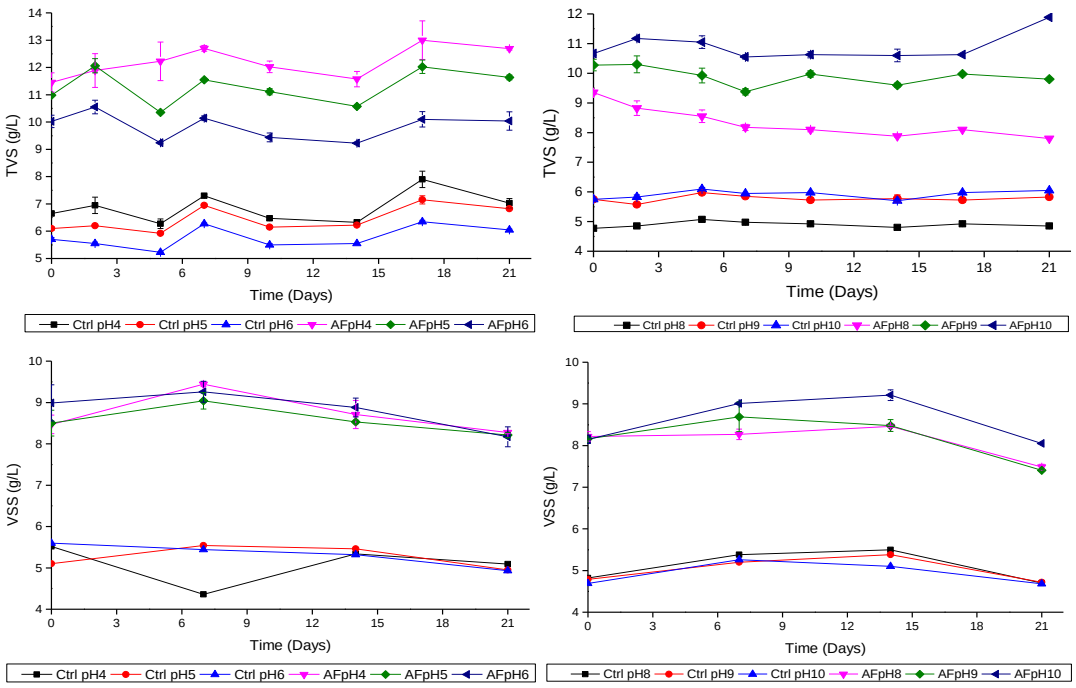

Figure 5.3. TVS and VSS results from the acidogenic fermentation of HPTH-WAS at different pH levels.

(Key: Ctrl pH are the blanks of each fermentation with different pH levels (4-10); AFpH reactors are the acidogenic fermentation at different pHs (4-10); thus, AFpH 4 is the acidogenic fermentation at pH 4, and so on until AFpH10 which is the reactor working at pH 10).

With regards to TSS results, reductions were observed, between 4.3 to 7.6%, especially on alkaline pH fermentors (pH8-10), probably because of solubilisation of solids due to either, the pH and/or the enzymatic activity. These findings concur with studies conducted by Morgan-Sagastume et al. (2011) which reached a 20% of TSS solubilisation on untreated secondary sludge. Also, it was found that AFpH6, AFpH8 and AFpH9 showed the largest VSS destruction, reaching its maxima of 9.2% at pH 9 which concurs with the reduction on TCOD due to organic matter mineralisation. This kind of behaviour has been reported in studies from Cokgor et al. (2009) and Wu et al. (2009), who account TVS drops from 25 to 52% after 20 days of fermentation. It is important to mention that hydraulic retention times had a very small impact on VSS destruction on acidic pH fermentors and at pH 10, and a higher effect on fermentors at pH close to neutrality which concurs with research carried out by Xiong et al. (2012).

Finally, TVS and VSS concentration in all control fermentors remained stable along the duration of the experiment with diminutions of no more than 10% which involves a low conversion of the organic matter into biogas.

Overall, it can be concluded that high acidic and high alkaline pH fermentors did show a very low TVS and VSS loss and mineralisation, demonstrating that bacteria that produce biogas were highly inhibited by the effect of pH, while fermentors at pH closer to 7 (6-8) were the most dynamic on the mineralisation of solids in the broth due to its conversion to biogas.

5.3 Effect of pH on biogas products distribution

Biogas analyses were conducted in all acidogenic fermentors with different pH on days 7, 14 and 21 in order to know the behaviour of biogas producer bacteria (not identified gases are not reported on the final composition in Figure 5.4). Fermentors with acidic pH (AFpH4-AFpH6) displayed a clear high production of CO_2 and a low production of other common anaerobic fermentation gases such as methane. Carbon dioxide represented the most substantial gas on any sampling days with concentrations above 90% of the composition of the biogas in the fermentors, especially on day 21 at pH 4 and pH5.

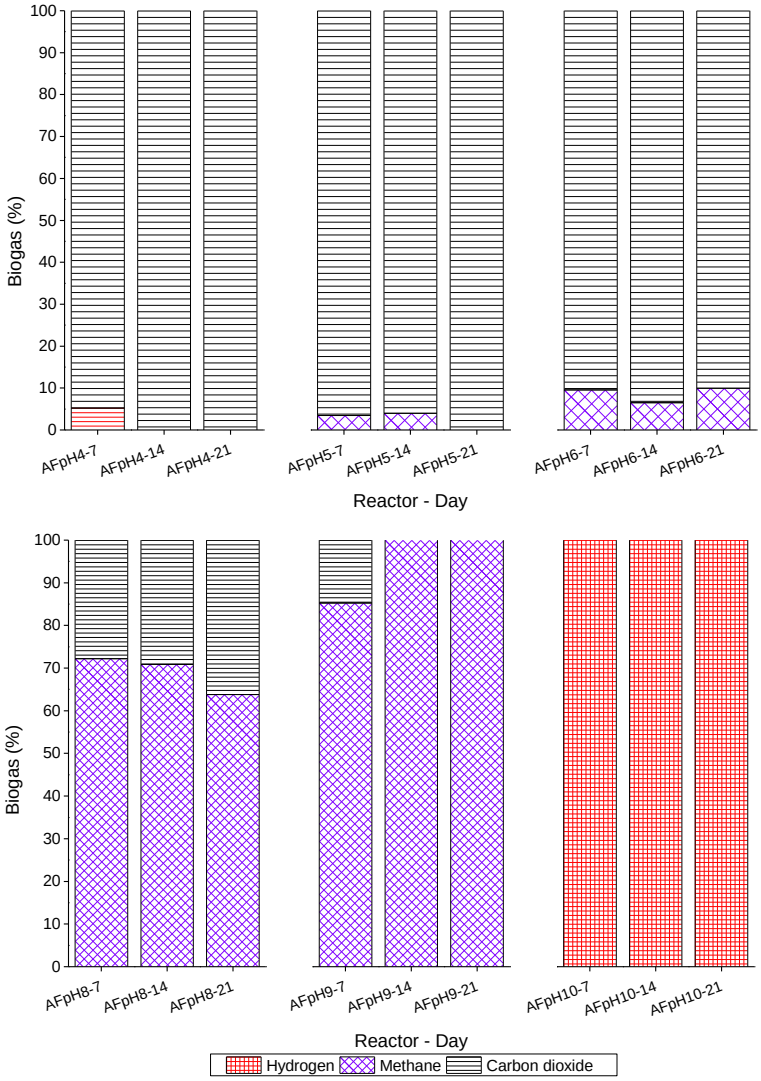

Figure 5.4. Biogas composition from the acidogenic fermentation of HPTH-WAS at different pH levels.

(Key: Ctrl pH are the blanks of each fermentation with different pH levels (4-10); AFpH reactors are the acidogenic fermentation at different pHs (4-10); thus, AFpH 4 is the acidogenic fermentation at pH 4, and so on until AFpH10 which is the reactor working at pH 10).

These outcomes agree with findings from Liu et al. (2012a) who found out that acidogenic fermentations with sludge supernatant in batch reactors at pH 3, produced no methane, small quantities of hydrogen (≤15%) and a high concentration of CO_2 (≥85%). Conclusions from Horiuchi et al. (2002) and Liu et al. (2012a), who carried out acidogenic fermentations at pH 5 using glucose and pre-treated sludge supernantant respectively, were high concentrations of CO_2 (>66%) and low methane concentration (<25%). Tamis et al. (2015) conducted experiments in anaerobic sequencing batch

reactors at pH 4.5, 5 and 5.5 with glucose as feedstock, finding that among the gaseous products, CO_2 presented the highest concentration, H_2 as the second highest while CH_4 production was not detected. Also, Li et al. (2010) reported no production of methane and about 40% of hydrogen gas on batch fermentations of kitchen waste at pH 5.1 and 5.8 in 15 days. A continuous upflow anaerobic reactor using gelatin-rich wastewater and dairy wastewater as feedstock at different pH, ran by Yu and Fang (2003) and Yu and Fang (2002), presented a biogas composition of around 30% of carbon dioxide and 56% of hydrogen without the presence of methane, at pH 4; furthermore, methane percentage in the headspace increased proportionally with the increase of pH, showing its maxima at pH 7. Chinellato et al. (2013) carried out a two-phase semi-continuous anaerobic experiment with food waste, producing hydrogen gas in concentrations higher than 40% when maintaining pH below 5.8. Finally, Cagnetta et al. (2016), Chen et al. (2007) and Yuan et al. (2006) clearly stated that at pH lower than 5, methane generation was low or nil when working with different types of sludge (CO_2 and H_2 analysis are not reported). All mentioned studies agreed with the results from fermentors AFpH4 and AFpH5 in this project.

Li et al. (2010) found that at pH levels of 6.5, 7.2 and 7.5 in batch fermentations of kitchen waste, could produce methane with a proportion of above 65% of the total biogas content at 11 days of the process. Similarly, Chinellato et al. (2013) registered CH_4 productions above 61% of the total biogas when digesting food waste and maintaining pH between 7.55 and 7.80. These findings are also supported by studies done by Chen et al. (2007), Liu et al. (2012a) and Yuan et al. (2006) with experimental conditions previously mentioned. These investigations concur with the outcomes from fermentor AFpH8 which presented ratios of methane above 60% in the biogas inside the fermentor. This conduct can be explained bearing in mind that the optimum range of pH for CH_4 generation, is between 6 and 8 (Appels et al., 2008).

Although AFpH9 fermentor was under severe alkaline conditions, its biogas composition was mainly prevailed by methane gas on any sampling day, which was even higher than in reactor AFpH8, suggesting poor pH adjustment and not clear identification of other gas products like H_2S, leading to a high biogas production, because of the consumption of some of the VFAs produced in the system. Also, it is important to clarify that percentage of CH_4 is higher than 75% possibly because of a high

concentration of unidentified gases in the gas analysis. These findings are opposite to the results reported by Chen et al. (2007), Liu et al. (2012a) and Yuan et al. (2006) who reported low production of methane (~15%) and high hydrogen (~30%) and carbon dioxide (~55%) concentration in the biogas composition at pH 9.

Then AFpH10, presented an interesting biogas composition, with a 100% of hydrogen at any sampling day, which can be caused due to the high solubilisation of proteins and carbohydrates and the stable hydrogen producing bacteria consortia at pH 10, low or null consumption of hydrogen by hydrogen-oxidising methanogens and low conversion into other gas products as shown in the equations below (Chynoweth, 1996; Wan et al., 2016; Wolfe, 1971; Zehnder and Brock, 1979). These results demonstrate that hydrogen could be recovered as another product of the acidogenic fermentation.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad \text{.....Equation 5.1}$$

$$HCO_3^- + 4H_2 + H^+ \rightarrow CH_4 + 3H_2O \quad \text{.....Equation 5.2}$$

These discoveries correspond with studies carried out by Liu et al. (2012a), Wan et al. (2016), Yuan et al. (2006) and Zhao et al. (2010) when using WAS as feedstock at different pH for the production of VFAs and/or biohydrogen.

Biogas production, especially biomethane, in AFpH8 and AFpH9 concur with the VSS destruction and TCOD reduction on these reactors, probably because of organic matter mineralisation. Biohydrogen production is an important outcome to consider as it can be visualised as a recoverable by-product of the acidogenic fermentation and at the same time, to avoid the conversion into methane by reacting with the CO_2 in the system.

Overall, the biogas analysis results provides comprehensible information about the effect of the pH on the biogas composition, with a positive impact on the methanogenic bacteria inhibition at pH 4, 5 and 6 which is corroborated by the poor conversion of the organic material to methane. On the other side, pH 8 and 9 presented a higher conversion of the organic material into methane which provides evidence of the poor effect of the pH on the inhibition of the methane generation bacteria and the mineralisation of the soluble organic content in the reactor. An special occurrence is the

impact of the pH on the acidogenic fermentation at pH 10 as there was a clear production of biogas but tended to the generation of biohydrogen instead of products such as methane and/or carbon dioxide.

5.4 Effect of the pH on the production, accumulation and composition of volatile fatty acids

5.4.1 Production and accumulation of VFAs

The carboxylate platform has been envisaged as a potential route for the conversion of many organic materials to uniform and simple products such as VFAs which then can be separated and converted into end-products with higher value (Li and Yu, 2011). The anaerobic acidogenic fermentation for the production of VFAs can be enhanced by changing conditions such as pH, being the tested pH values in the range of 5.25–11, but the specific range depends mainly on the type of substrate used (Dahiya et al., 2015; Fernández et al., 2008).

Volatile fatty acids production in these series of experiments are shown in Figure 5.5, where it is visible that higher product concentration was presented in fermentors with pH levels of 6 and 9. The maximum concentration of VFAs was 1.88 g TVFAs/L on day 21 at pH 9 (VFAs net production 1.76 g TVFAs/L), and the second highest was 0.850 g TVFAs/L at pH 6 and day 21, being the former, higher in more than 2 times the production at pH 6. It is noticeable the steady and almost linear production of VFAs in the fermentor AFpH9, which when analysed, presented a linear trend that could be described with the equation $y=0.0766x+0.098$ (R^2= 0.9683). On the other hand, AFpH6 showed a semi-asymptote in VFAs production from day 2, increasing and decreasing from one day to another in no more than ±18% with respect to the previous day giving evidence of generation and consumption of VFAs happening simultaneously.

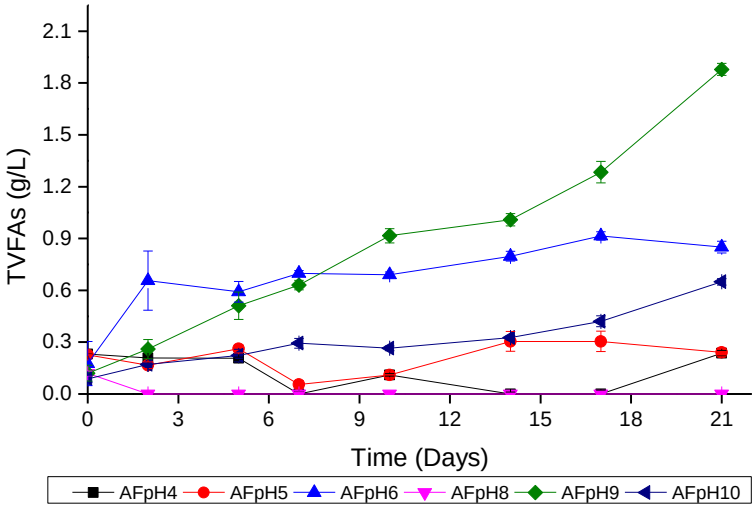

Figure 5.5. VFAs production in acidogenic fermentation of HPTH-WAS at different pH levels.

(Key: Ctrl pH are the blanks of each fermentation with different pH levels (4-10); AFpH reactors are the acidogenic fermentation at different pHs (4-10); thus, AFpH 4 is the acidogenic fermentation at pH 4, and so on until AFpH10 which is the reactor working at pH 10).

In contrast fermentor with pH of 8, did not presented any production or accumulation of VFAs but a consumption of the initial concentration of carboxylic acids, which agree with the biomethane results presented in the previous section, due to the pH was close to the optimum. VFAs data reported is the subtraction of the total VFAs produced in AFpH reactors (g carboxylic acids/L) minus the VFAs produced in their respective control reactors caused by the organic material and the bacteria only from the inoculum.

Fermentors AFpH4, AFpH5 and AFpH10 presented similar trend between each other during the duration of the experiment, with minimal VFAs production and accumulation, probably due to the very adverse conditions for the acidogenic/acetogenic bacteria in the inoculum (even when all inoculums were acclimatized for 21 days prior the experiment) and also negligible consumption, possible because the pH was not the optimum for CH_4 production (Yan et al., 2010). Although few studies have shown the possibility of the production of different species of VFAs with heterogeneous bacteria consortia at low pH due to the different metabolism routes, the affectation on cell morphology and structure, the production of ethanol and/or acetone and, also the shift and elongation of VFAs at low pH, the

reactors in this study working with low pH did not present a high VFAs production (den Boer et al., 2016; Dogan et al., 2009; Grupe and Gottschalk, 1992; Yu and Fang, 2003).

VFAs proportion of the products generated in the fermentation broth in all fermentors was above 95% at day 21 and during the whole process of the fermentation which suggest the poor conversion of organic material to solvents and that pH has little or no influence on the production of ethanol, acetone or butanol. Ethanol was detected mainly at the beginning of the fermentation (0.02-0.013 g/L) in most of the reactors, but its concentration decreased along the time, suggesting a transformation into VFAs because of its ability to act as an electron acceptor for VFAs chain elongation (den Boer et al., 2016; Spirito et al., 2014). In contrast, AFpH10 did not show any drop on the ethanol concentration, probably because of the low utilisation of the substrates for the production/elongation of VFAs by the bacteria at high alkaline conditions.

Although high alkaline pH levels promote the solubilisation of proteins and carbohydrates (Chen et al., 2007; Yu and Fang, 2003; Yu and Fang, 2002), no substantial amounts of VFAs where produced by pH adjustment as the initial VFAs concentration range between 0.08-0.22 g TVFAs/L.

With the information given above, it is clear that fermentations at alkaline conditions in reactor AFpH9, presented the best conditions for VFAs production by some key factors such as, the inhibition of methanogenic bacteria and the avoidance of inhibition by-products (VFAs) as the pH did not decrease below pH 5.

Yields of the fermentors at different pH in acidogenic fermentation of HPTH-WAS were calculated in two different units, g VFAs/g TVS and g VFAs as COD/g COD to understand the VFAs production per gram of substrate in the system and the possible chain elongation of VFAs.

The highest VFAs yield was presented at day 21 at pH 9, being 0.415 g VFAs/g TVS which corresponds to 0.264 g VFAs (in COD terms)/g COD (Figure 5.6). VFAs conversion in terms of COD was previously reported in Chapter 6. Agreeing with the marks of the TVFAs production, AFpH6 presented the second highest VFAs yields reaching an average conversion of about 15% of COD or TVS into carboxylic acids which is lower than the yield of AFpH9 between 1.8 to 2.5 times. Neither AFpH9 nor AFpH6 showed an asymptote on the VFAs yield which suggest that microorganism could

continue the conversion of the feedstock into VFAs if a longer HRT is set and also, that the remaining organic material is still suitable for further resource recovery via conventional anaerobic digestion.

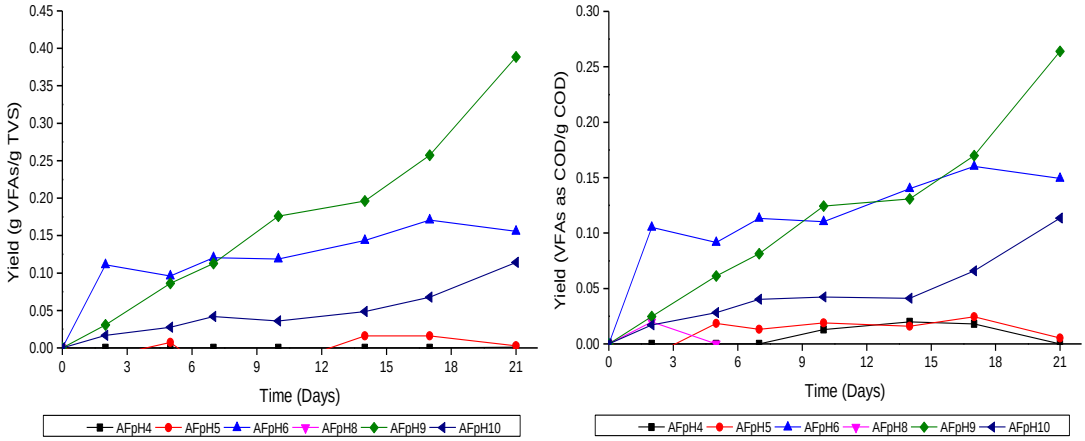

Figure 5.6. Average VFAs yields in mixed acid fermentation of HPTH-WAS at different pH levels.

(Key: Ctrl pH are the blanks of each fermentation with different pH levels (4-10); AFpH reactors are the acidogenic fermentation at different pHs (4-10); thus, AFpH 4 is the acidogenic fermentation at pH 4, and so on until AFpH10 which is the reactor working at pH 10).

Ma et al. (2016) report yields between 0.152 and 0.24 g COD/g TVS when working with untreated and pre-treated sludge in batch reactors at pH 7, 9 days of fermentation and with 2-bromoethanesulfonic acid (BES) for the inhibition of methanogenic bacteria. Studies from den Boer et al. (2016) descript yields of 0.353 g VFAs/g TVS in pure culture batch with *E. coli* and *Klebsiella mobilis*, using kitchen biowaste and potato peels with HRT of 3 days, pH 6.5 and organic load of 91.1 g TVS/L. Also Zhao et al. (2010) achieved yields of 0.27 g VFAs/g TVS in fermentations in batch experiments with mesophilic conditions, pH adjustment at 7, kitchen waste as feedstock at its best HRT of 4 days. Liu et al. (2015) investigations, with the supernatant of pre-treated WAS by thermo-alkaline process (pH 12, 90°C, 2 h), reached its maximum yield of 0.57 g COD/g TVS in 10 days of fermentation and pH 10 in mesophilic conditions. Meanwhile, Wu et al. (2009) indicated yields of 0.30 g COD/g VSS with experiments at pH 10, 5 days of fermentation, primary sewage sludge and room temperature. Another case is the one reported by Yuan et al. (2006) who found a

maximum yield of 0.130 g COD/g VSS when fermenting secondary sludge, room temperature and pH 10. Experiments conducted by Yu and Fang (2002) established yields of 0.32 g COD/g VSS·d in an upflow reactor with a HRT of 12 h and solid loading rate of 8 g COD/L·d. At the same time, Bengtsson et al. (2008) report in acidogenic fermentations with paper mill effluent and cheese whey, yields of 0.59 g COD/g COD and 0.60 g COD/g COD respectively. Also, Xiong et al. (2012) described a yield of 0.23 g VFA (as COD)/g TVS with a production of 5.699 g VFA (as COD)/L after 8 days fermentation using WAS as main feedstock. Taking in consideration the mentioned studies, the VFAs yields of this set of experiments showed a respectable figure which in some cases is 2 to 3 times higher than some reports. Also, few investigations pointed that the highest yield was reached at pH 10, which is similar to the best results of these trials at pH 9 achieving acceptable yields with HPTH-WAS as main substrate.

To distinguish firmly which fermentor reached the highest VFAs at which pH conditions, a statistical analysis was run. The two analysed reactors were AFpH6 and AFpH9 as they achieved the highest yields on day 21. Additionally, a statistical analysis within groups were conducted to know which HRT was the optimal.

Table 5.2. Independent Samples t-Test of mixed acid fermentation of HPTH-WAS at different pH levels.

Yield	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig	t	df	Sig. (2-tailed)	Mean difference	Std. error difference	95% CI of the Difference	
								Lower	Upper
Equal variances assumed	3.071	0.155	-27.98	4	0.00	-0.2398	0.0085	-0.2636	-0.2160

Independent samples t-test was conducted, as in previous chapter, with fermentors AFpH6 and AFpH9 and its VFAs yields at day 21 (in triplicate) with α=0.05. The analysis can be seen in Table 5.2 where Sig. (2-tailed) is equals to 0.00, which is remarkably lower than the significance level (α) and verify that the differences between the yields of each reactor are statistically significant different, consequently, fermentor AFpH9 achieved the best yields

and performance with regards to the production/accumulation of VFAs at day 21.

Additionally, analyses were conducted with the yields of AFpH9 at days 17 and 21 to determine the best HRT in the fermentation. In this case, a paired-samples t-tests were carried out with confidence intervals (CI) of 95% (Table 5.3). The results showed a significant difference in yields AFpH9 day 17 and AFpH9 day 21 with a p = 0.004 (two-tailed). Bearing these results in mind, acidogenic fermentations with pH 9 and HRT of 21 days were the best conditions to reach the highest VFAs yields.

Table 5.3. Paired Samples t-Test of mixed acid fermentation of HPTH-WAS at different pH levels.

	Paired differences					t	df	Sig. (2-tailed)
	Mean	Std. dev.	Std. Error mean	95% CI of the Difference				
				Lower	Upper			
AFpH9D17 - AFpH9D21	-0.1326	0.1516	0.0087	-0.1703	0.9497	-15.15	2	0.004

Bearing in mind these results, the operation of mixed acidogenic fermentations at different pH presented mixed impacts on the production and accumulation of VFAs. A negative effect on the production of VFAs at highly acidic pH (4-5) was presented, whereas reactors at pH 8, accumulation was not noticed although production could be promoted because of the favourable pH for the conversion of VFAs into biogas. The influence of the pH 10 on the acidogenic fermentation was positive in terms on the production and accumulation because there was a patent generation and poor consumption of the VFAs for the conversion into methane or carbon dioxide. Finally, it was found a positive impact of the pH 9 and pH 6 in the carboxylate platform experiments carried out which is confirmed by the sustained generation and accumulation of VFAs but with different speed reactions.

5.4.2 VFAs composition at different pH levels

It is been reported that production and composition of volatile fatty acids is highly influenced by the composition of substrates, although pH values could also affect the type of VFAs by elongating its carbon chain or developing a mixture with few different products (Khan et al., 2016). The main aim of

acidogenic fermentation is to produce VFAs with medium/long carbon chains due to the ease of recovery and extraction in reasonable retention times (den Boer et al., 2016).

Figure 5.7 present the overall VFAs production and composition on day 21 for the different pH levels used in this study. It is notorious that among the fermentors ran, AFpH9 reached a concentration higher than any other. AFpH7 is reported as AF0 from chapter 6 as it was maintained at pH 7 using $CaCO_3$ for neutral value and with no addition of methanogenic inhibitor.

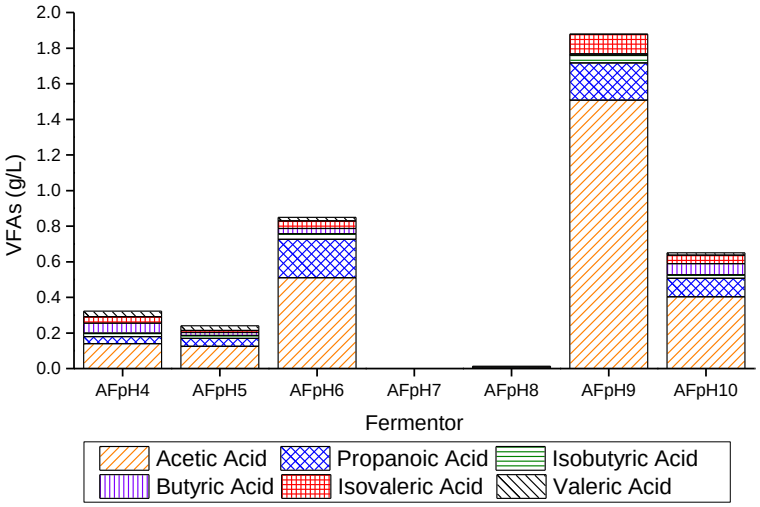

Figure 5.7. Effect of different pH levels on the VFAs generation on day 21 of acidogenic fermentation of HPTH-WAS.

The effect of pH in the volatile fatty acids composition is presented in Table 5.4. Long-medium VFAs (iso-butyric, valeric and iso-valeryc acids) presented higher content in reactors with acidic pH, while alkaline pH fermentors moved to a shorter carbon chain VFAs production, mainly constituted by acetic acid; although this acid was the predominant in all reactors no matter the pH, ranging between 52.4 and 80.3%, with similar concentrations than obtained by Forrest et al. (2010a), Golub et al. (2013), Rughoonundun et al. (2012) and Ucisik and Henze (2008), and in the studies to find the best methanogenic inhibition in this project (Chapter 5), in a range between 43-95%. The second highest carboxylic acid in the broth depended significantly on the pH; whereas in alkaline reactors was propionic acid, in acidic fermentors was iso-butyric or propionic acid. While at alkaline pH the carbohydrates and/or proteins were converted to acetate by acetyl-

CoA as intermediate, acidic pH leaded the reaction towards the conversion of pyruvate to lactate in to propionate and long carbon chain carboxylates (Shanmugam and Horan, 2009; Temudo et al., 2007).

Table 5.4. Percentage of individual VFAs accounted for total VFAs at different pH on day 21 of acidogenic fermentation of HPTH-WAS.

pH	Carboxylic acid (%)					
	Acetic	Propionic	Butyric	Iso-butyric	Valeric	Iso-valeric
4	58.4	16.4	8.06	22.9	15.1	13.6
5	52.3	18.0	7.1	6.4	4.9	10.9
6	60.0	25.4	3.8	3.4	5.1	2.4
7	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
8	62.5	N.D.	37.5	N.D.	N.D.	N.D.
9	80.3	11.1	2.4	0.3	5.9	0.1
10	62.2	15.8	3.2	9.5	7.3	1.9

*N.D.: Not detected.

To understand and figure out the changes in VFAs composition, the products' profile of AFpH4 and AFpH9 where plotted in Figure 5.8, discovering that in reactors operated at pH 4, the acetic concentration dropped from 62% to about 40% of the total VFAs from day 5 until day 21, whereas all carboxylic acids with carbon chain of 4 or more carbons (butyric, iso-butyric, valeric and iso-valeric), increased from 3 to 13 percent, in the case of the iso-valeric concentration; with an overall increase of 5-7 percentage for VFAs with chains of 4 or more carbon atoms on day 21. In comparison, the composition of VFAs in fermentor AFpH9 was prevailed by acetic acid from day 0, reaching its highest concentration on day 17, however, VFAs with more than 4 carbons remained with concentrations lower than 10% at any time of the process.

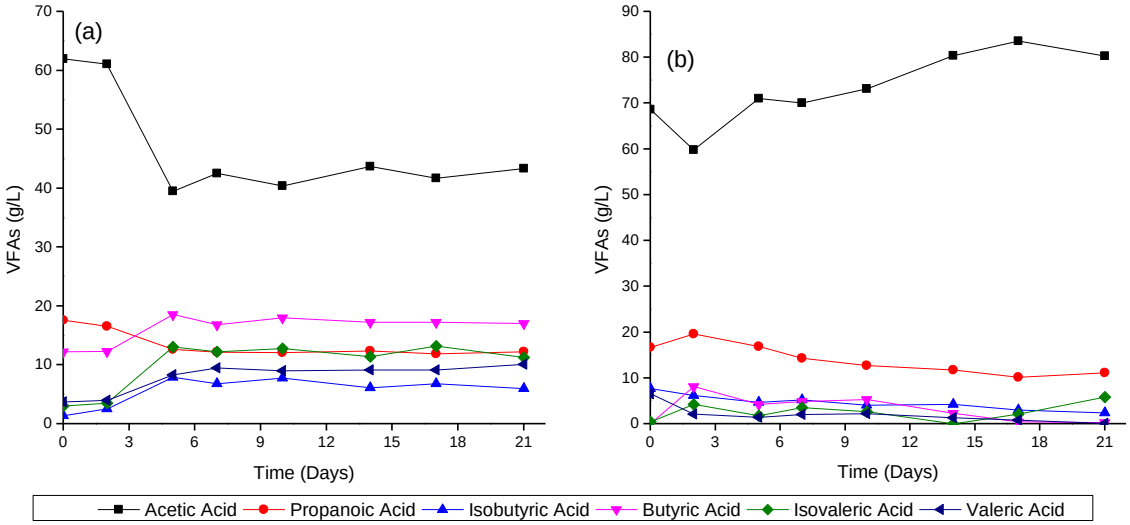

Figure 5.8. VFAs composition in acidogenic fermentation of WAS at pH 4 (a) and pH 9 (b) of acidogenic fermentation of HPTH-WAS.

Wu et al. (2009) found similar trends on VFAs generation and composition; with acidic pH fermentors showing high concentrations of propionic and butyric acids and alkaline pH with predominant acetic acid generation. Also Zhang et al. (2005) found a parallel trend in VFAs production: long-chain fatty acids (propionic and butyric) at low pH (5) and short-chain fatty acids (acetic acid) at high pH (9-11). A study done by Yu and Fang (2002) using pH levels between 4.0 and 6.5 (testing increases of pH of 0.5 points) showed the same behaviour, acetic acid was predominant at high pH (6.0-6.5) and propionic acid was major at pH between 4.0 to 5.0. Finally, Chen et al. (2007) reported low concentrations of acetic acid at low pH levels (12-35%) and proportional increments with pH until a concentration about 73% at pH 11 whereas the opposite behaviour was presented for iso-valeric acid, 35% at pH 4 and 16.6% at pH 11. Despite these findings, authors such as Cagnetta et al. (2016) and Liu et al. (2012a) found no correlation between the pH and the VFAs composition, being the acetic acid the most common in all fermentations done with sewage sludge. Results from this set of experiments support the hypothesis that there is a correlation between carbon elongation of VFAs and fermentations with acidic pH.

The impact of the pH on the speciation of the VFAs produced in mixed acidogenic fermentation is visible, whereas fermentors working with acidic pH tended to produce long-chain fatty acids (butyric and valeric acids),

alkaline or neutral pH generated shorter-chain fatty acids (acetic and propionic acids).

An important fact to be considered is the degree of acidification (VFAs/SCOD relation) to know the amount of soluble organic material being converted to the final product, VFAs (Maharaj and Elefsiniotis, 2001).

Table 5.5. COD conversion on the acidogenic fermentation of HPTH-WAS at different pH levels.

Reactor	Day	Suspended COD (g/L)	$VFAs_{COD}$ (g/L)	SCOD (g/L)	TCOD loss (g/L)	Acidification degree (VFAs/SCOD)
AFpH4	0	14.89	0.31	3.05	0.00	0.10
	21	14.38	0.32	2.05	1.50	0.16
AFpH5	0	13.42	0.31	2.76	0.00	0.11
	21	12.17	0.34	1.78	2.16	0.19
AFpH6	0	12.64	0.23	2.76	0.00	0.09
	21	14.26	1.11	2.17	1.04	0.51
AFpH8	0	12.27	0.16	2.46	0.00	0.06
	21	11.26	0.00	0.68	2.82	0.00
AFpH9	0	13.04	0.15	4.35	0.00	0.03
	21	9.72	2.25	4.85	2.82	0.46
AFpH10	0	11.79	0.11	4.13	0.00	0.03
	21	11.91	0.86	4.22	0.99	0.20

The highest VFAs concentration was reached on fermentor AFpH9 as was mentioned before, with a final amount of 2.25 g VFAs in COD terms, which is similar to the value on fermentor AF6 (2.18 g $VFAs_{COD}$, 0.60 g VFAs/ g SCOD, 0.15 g VFAs/TCOD) in Chapter 5, on day 21 with a degree of acidification of 0.46 (VFAs/SCOD) and 0.15 (VFAs/TCOD). Ma et al. (2016) found higher values of acidification than the reported in this study, with a range that fall between 0.18 to 0.30 VFAs/TCOD when working with an alkaline pre-treated WAS and at pH 7 with methanogenic inhibitor (BES). Results from Bengtsson et al. (2008) showed high degrees of acidification in studies in CSTR in chemostat, 0.83 VFAs/SCOD for whey and 0.76 for paper mill effluent at pH 5.5 with 48 h of retention time, demonstrating the high biodegradability of the substrates employed. WAS is usually a very difficult substrate for microbial digestion, however, Yuan et al. (2009), report high degree of acidification in semi continuous fermentors, with ranges between 71 to 76% with a SRT of 10 days and 84 to 88% with a SRT of 5

days, utilising different organic loading rates. Although more than 45% of the SCOD in the broth was converted to VFAs in the acidogenic fermentations at pH 9 in this set of experiments, this value is lower than the results presented by mentioned authors which suggests there is still area to improve the degree of acidification in acidogenic fermentations of HPTH-WAS.

5.5 Stoichiometric (SMP), Biochemical methane potential (BMP) and mass balance of acidogenic fermentation of WAS at different pH levels

Using the methodology described in Chapter 5, SMP and BMP were determined for reactors AFpH6 and AFpH9 as these fermentors presented the highest production/accumulation of VFAs, in order to compare the potential of production of VFAs in acidogenic fermentation as first step in an anaerobic fermentation system for the establishment of a biorefinery scheme in wastewater treatment works.

As the WAS used as feedstock was the same as in Chapter 5, the empirical formula is $C_{10.3}H_{16.5}NO_{4.3}$ and the SMP resulted in 0.461 litre CH_4/g TVS of HPTH-WAS at STP conditions. Whereas, the BMP for the reactors showed respectable yields, with 0.099 (0.427 L CH_4) and 0.224 L CH_4/g TVS (1.016 L CH_4) for AFpH6 and AFpH9 respectively.

Fermentors at pH 9 reached a VFAs production which represents about 48% of the SMP value and is also, 2.8 times higher than the BMP reactor (AD experiment) from Chapter 4. It is also significant that AFpH9 produced a high content of methane during the whole fermentation process according to the biogas analysis, which suggest that aside from the VFAs production, the biogas produced could be recoverable and consumed.

Studies conducted by Bougrier et al. (2007), Bougrier et al. (2008), Cano et al. (2014) and Qiao et al. (2011), for the production of biogas with hydrothermal pre-treated WAS as feedstock, presented yields of 0.256-0.333 litre CH_4/g TVS, which are similar to the outcomes in this study. Despite of, more than 50% of the SCOD was not converted in to VFAs (52% of SMP), some other molecules could be produced such as pyrroles, indoles and formic, caproic, lactic and succinic acids which were not quantified and also should be considered as acidogenic fermentation products (Bastidas-Oyanedel et al., 2015; Morgan-Sagastume et al., 2011).

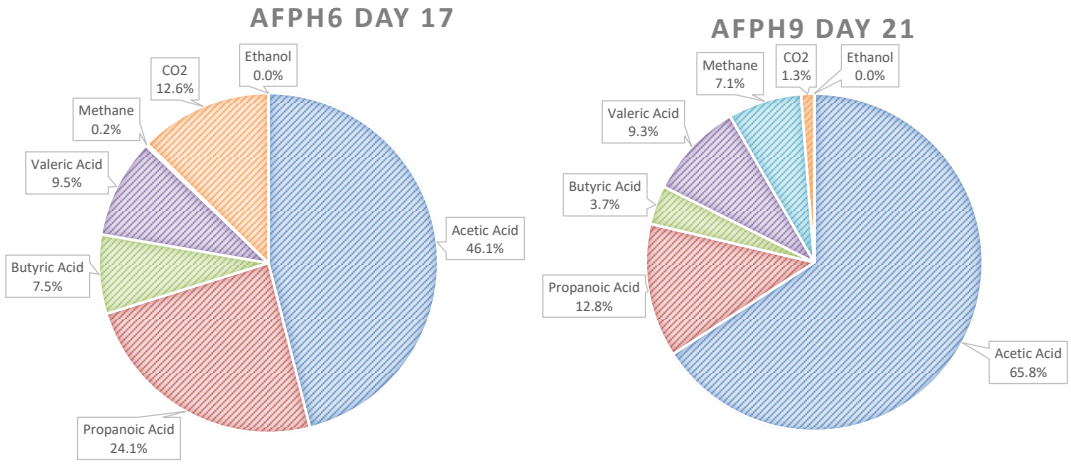

Figure 5.9. Average products of the mixed acidogenic fermentation of HPTH-WAS expressed as mass percentage in terms of COD of AFpH4 and AFpH9.

A mass balance assessment was calculated to find out the conversion of organic material on the acidogenic fermentation at pH 6 and 9 (Figure 5.9). As being observed in the TCOD results, the majority of the organic content remained inside the reactor with the highest loss on fermentor AFpH8 showing a decrease of about 19%, which was mainly converted into methane and carbon dioxide. Most the products in reactors AFpH6 and AFpH9 were mainly acetic acid with values between 46 and 65% respectively. Additionally, gases production in AFpH6 was higher than in fermentor at pH 9, probably due to the mineralisation at pH near neutrality.

5.6 Summary

The effect of the pH on the acidogenic fermentation of pre-treated HPTH-WAS showed that pH 9 and 21 days of HRT reached a concentration of 1.88 g TVFAs/L with a yield of 0.415 g VFAs/g TVS which corresponds to 0.264 g VFAs (in COD terms)/g COD. Fermentation with pH 6 presented the second highest VFAs yields with a conversion of about 15% of COD or TVS into carboxylic acids.

The effect of the pH in the mixed acidogenic fermentations was mixed; experiments ran with highly acidic pH presented a positive impact on the inhibition of the production of methane, a negative effect on the SCOD accumulation inside the reactor and its possible consumption and conversion into gaseous products, a negative influence of the pH on the production of VFAs and an influence of the low pH for the production of long-chain fatty acids. In contrast, alkaline pH (8-9) presented a positive effect on the conversion of organic material for the generation of biomethane or biohydrogen for pH 10, a positive impact on the increasing of SCOD value which denotes the continuous activity of bacteria for the production of soluble products such as VFAs, a positive influence of the pH 9 and pH 10 on the specific production and accumulation of short-chain fatty acids, corroborated by the sustained generation and accumulation of acetate and propionate.

The conversion of SCOD to VFAs in acidogenic fermentation at pH 9 was about 46% (VFAs/SCOD) whereas its methane potential represents about 48% of the stoichiometric methane potential SMP of WAS treated by HPTH, which resulted in slightly more than 50% of unconverted organic material that can be used for further conventional anaerobic fermentation and the production of biogas.

The information presented in this study give a strong evidence that fermentations at alkaline conditions (pH 9), presented the best conditions for VFAs production by some key factors such as, the hydrolysis of organic material from WAS, inhibition of methanogenic bacteria, and the avoidance of inhibition by VFAs as the pH was maintained at high levels.

Chapter 6. EFFECT OF THE CO-FERMENTATION AND SUBSTRATE PRE-TREATMENT OF WASTE ACTIVATED SLUDGE AND FOOD WASTE/MICROALGAE ON THE PRODUCTION/ACCUMULATION OF VFAs IN ACIDOGENIC FERMENTATION.

6.1 Introduction

Bearing in mind the concept of the biorefinery (an analogous refinery to today's petroleum refinery, which produces multiple products from biomass), several bottlenecks must be tackled, such as the diversification of the low environmental impact technologies and the biomass resources, with the aim of the valorisation of inexpensive wastes for its conversion into valuable renewable and more sustainable chemicals and fuels for the progressive replacement of the usage of non-renewable sources such as oil (Sokhansanj et al., 2003). Among the purposes in the of the biorefinery concept development are the accomplishment of global energy demand, enhancement of profits, sustainable management of the biomass and the production of chemicals that are capable to join the existing energy infrastructure with competitive prices versus oil refinery products (Cherubini, 2010; Fernando et al., 2006; IEA, 2009).

Although the carboxylate platform has proven to be an advantageous process for the production of intermediate biochemicals during anaerobic fermentation of WAS as a first approach for the biorefinery concept in the WWTPs, there are several areas to be studied and clarified, such as the benefits arising from the co-fermentation of WAS with other organic substrates; particularly, it is of great interest to explore fermentations using different C/N ratios and the benefits from pre-treatment processes of the biomass involved. The variations of carbon to nitrogen ratios are an important parameter to be considered in anaerobic fermentation, as it has been stated that the optimal C/N ratio value for biogas production is between 20 to 30 units, whereas below this value the degradation of proteins would lead to a high production of free ammonia (NH_3), which could direct to a toxicity of methanogenic bacteria with a consequent VFAs accumulation (McCarty, 1964; Sialve et al., 2009).

On the other side, the hydrolysis and solubilisation of organic material is visualised as the main bottleneck in the anaerobic digestion because of the difficulty of converting fats, complex carbohydrates, proteins and breakage of hard cell walls, to simpler compounds such as glycerol, simple carbohydrates (glucose) and aminoacids, which can be easier to degrade by the microorganisms presented in the anaerobic fermentation system (Fiore et al., 2016; Gonzalez-Fernandez et al., 2015; Ucisik and Henze, 2008; Ward et al., 2014; Zhang et al., 2015).

A healthy carbon-to-nitrogen ratio as well as easy-degradable organic material are important considerations in the development of different pathways for the conversion of biomass into valuable products. Among these factors, there is also the need of diversification of the wastes used as feedstocks for the AD or the acidogenic fermentation, in order to increase the capacity of the biofuels production in the existing WWTP. As mentioned in the literature review, food waste is a significant social and environmental issue, representing an important implication in terms of the cost of production, treatment and its consequent contribution to the greenhouse emissions. As well as food waste, microalgae has taken relevance recently for the production of biofuels due to its high organic content, rich in proteins, which are the main substrates for the generation of long-chain VFAs such as butyrate and valerate, and at the same time microalgae can be cultivated using wastewater in WWTPs for resource recovery (carbon, nitrogen and phosphorus) (Gonzalez-Fernandez et al., 2015; Nagase and Matsuo, 1982).

Bearing in mind the previous information, two additional factors were tested and are reported in this chapter: (a) feedstock pre-treatment and (b) blend ratios of WAS with food waste and microalgae. Food waste (FW) and microalgae (*Chlorella vulgaris*) were chosen as potential feedstocks that can be integrated to existing facilities for sewage treatment (DEFRA, 2016b; Mena et al., 2014; WRAP, 2013) with the benefits of providing carbohydrates and proteins to balance C/N ratios currently found in sewage sludge. The integration of different organic wastes into WWTP systems can be attractive for the production of fuels/chemicals, as part of the valorisation of organic wastes via resource recovery.

Tests were conducted firstly to investigate the effect of the co-fermentation of WAS with additional substrates (microalgae and food waste) with three different blends between the additional substrate and WAS in ratios WAS:substrate of 3:1, 1:1 and 1:3 (75%/25%, 50%/50% and 25%/75%) and

also, to investigate the effect of the C/N ratio for the production of VFAs in acidogenic fermentation. At the same time, the determination of the impact of the different thermal pre-treatments applied to the feedstocks using two different conditions (i.e., standard autoclaving and high pressure thermal hydrolysis) was explored. All these tests are key to evaluate the overall process efficiency when working with pre-treated feedstocks during co-processing, in order to enhance the production/accumulation of VFAs in mixed acidogenic fermentation.

6.2 Characterisation of food waste and microalgae

6.2.1 Raw food waste and microalgae

As mentioned in the methodology (Chapter 4), food waste and microalgae samples were treated by high pressure thermal hydrolysis (HPTH) and conventional autoclaving before being submitted to anaerobic acid fermentation. The results of the characterisation of both organic feedstocks and its treated samples are presented in Table 6.1.

The SCOD/TCOD ratio of the raw food waste in this study (0.219) is comparable with the results reported by Tang et al. (2017) (SCOD/TCOD = 0.23) and identical to values found by Chen et al. (2013) and Li et al. (2014), despite the fact that all these studies used an electrical blender to reduce particle size to 1-2 mm. Whereas food waste in studies from Wu et al. (2016) and Zhang et al. (2005) reported values higher than 0.485, which suggests that the difference in hydrolysis ratios can be attributed to the specific composition of each waste.

Table 6.1. Characterisation of raw and pre-treated food waste and algae samples.						
	Sample					
Parameter	Raw FW	Autoclaved FW	HPTH FW	Raw Algae	Autoclaved Algae	HPTH Algae
TCOD (g/kg)	349.74	411.92	393.78	1417.5	1197.04	1365.97
SCOD (g/kg)	76.42	108.81	173.58	115.61	195.30	544.45
SCOD/TCOD	0.219	0.264	0.441	0.082	0.163	0.398
TS (g/kg)	248.05	244.09	248.05	951.04	859.61	832.12
TVS (% of TS)	95.70	95.70	89.72	88.67	89.41	84.31
TSS (g/kg)	215.57	227.54	187.43	852.87	826.5	385.02
VSS (% of TSS)	98.56	96.36	96.09	93.32	96.61	86.64
Ashes (g/kg)	6.98	6.94	6.94	78.24	71.51	72.77
TKN (g/kg)	8.85	8.01	8.03	78.68	67.43	67.35
pH	4.65	4.00	4.00	4.65	4.00	4.00
C (%)	50.58	49.35	52.12	46.65	46.12	49.55
H (%)	7.24	7.03	7.24	6.85	6.35	6.90
O (%)	35.15	37.25	33.89	28.62	29.98	22.38
N (%)	4.07	3.43	3.64	9.09	8.90	10.82
C/N	12.4	14.4	14.3	5.1	5.2	4.6
Empiric Formula	$C_{14.5}H_{24.9}O_{7.6}N$	$C_{16.8}H_{28.5}O_{9.5}N$	$C_{16.7}H_{27.8}O_{8.1}N$	$C_{6}H_{10.5}O_{2.8}N$	$C_{6}H_{10}O_{2.9}N$	$C_{5.3}H_{8.9}O_{1.8}N$

The concentration of TVS in raw food waste samples used in this study is similar to studies conducted by Wu et al. (2016), who report a value of 0.98 for the ratio TVS/TS in food waste composed mainly by rice, noodles, vegetables and meat. Also, Cheng et al. (2016) and Tang et al. (2017) found ratios of TVS/TS of 0.967 and 0.964 respectively, for food wastes collected from university canteens. Karthikeyan et al. (2016) reported a ratio of 0.971

TVS/TS when conducting the characterisation of simulated food waste comprising bread, boiled rice, cabbage and cooked meat. Statements by Zhang et al. (2005) agreed with these results, presenting a value of 0.968 TVS/TS for waste coming from a university restaurant, which mainly contained cooked rice, vegetables, meat, eggs and potatoes. Working with segregated domestic food waste from a biowaste digester in the UK, Chinellato et al. (2013) reported a TVS/TS ratio similar to the food waste from this study (0.951). Finally, studies from Argelier et al. (1998) and Parawira et al. (2004) found TVS/TS ratios higher than 0.95 for solid wastes from a restaurant and a potato processing factory, respectively.

Other authors reported lower results in terms of TVS/TS ratios in food waste, with values ranging from 0.750 to 0.927, probably due to a lower inorganic content (Chen et al., 2013; den Boer et al., 2016; Fisgativa et al., 2016; Heo et al., 2004; Li et al., 2010; Lissens et al., 2004; Liu et al., 2013a; Qiao et al., 2011; Traverso et al., 2000; Xie et al., 2017; Zheng, 2013)

From the elemental analysis of food waste, it can be seen that the ratio between carbon and nitrogen (C/N = 12.4) is far below the optimum recommended value for AD processes (20 < C/N < 30) (Yen and Brune, 2007), suggesting a low amount of carbohydrates and lipids that could contribute to a higher carbon content and also high content of protein waste such as meat and legumes, in agreement with the composition reported by Li and Jin (2015) for kitchen waste. This values were clearly lower than other studies conducted by Chen et al. (2013) (C/N=32.0), Cheng et al. (2016) (C/N=26.3), Li et al. (2010) (C/N=24.8) and Zhang et al. (2005) (C/N=49.9).

Considering the results obtained from the characterisation of raw microalgae (*Chlorella vulgaris*), the SCOD/TCOD ratio of 0.082 reveals a very low concentration of soluble organic compounds as expected due to the nature of the cell wall that prevents hydrolysis. This value agreed with the ratios reported by Astals et al. (2015) for *Scenedesmus sp.* (SCOD/TCOD = 0.142); Seo et al. (2016) and Suresh et al. (2013) for lipid extracted *Ettlia sp* (0.054); and Zhen et al. (2016) for a microalgae mixture mainly containing *Chlorella vulgaris* and *Scenedesmus sp.* (0.054).

Low concentrations of soluble organic material in algal samples are also supported by the TSS/TS ratio of 0.896, in agreement with Suresh et al. (2013) who reported a value of 0.927 TSS/TS. Untreated *Chlorella vulgaris* contain a high content of organic material as found from the high percentage

of TVS with regard to TS content (88.67%). Research conducted by Zhao et al. (2014) describes a TVS/TS ratio of 0.887 for *Chlorella vulgaris* which agrees with the results of this study, whereas Suresh et al. (2013) and Seo et al. (2016) reported higher values (0.929) for lipid extracted *Ettlia sp.*; in contrast, Neumann et al. (2015) reported a TVS/TS ratio of 0.75 for *Botryococcus braunii.* These differences can be explained due to the fact that some algal samples were pre-processed for lipid extraction and also to the natural composition of every microalga specie.

Along with a high carbon content (46.65%), microalgae samples also contained a high content of nitrogen (9.09%) when compared with food waste, which significantly made an influence on the final C/N ratio (5.1) making this feedstock particularly difficult to produce biogas. Reported values for *Chlorella vulgaris* range between 6.4 and 6.8 (Biller et al. (2012) and Zhao et al. (2014)), which are marginally higher than those found in this study and than in investigations conducted with *Scenedesmus dimorphus* (C/N = 5.95) (Zhao et al., 2016). These results show that raw microalgal samples contain a high amount of nitrogen and low soluble organic matter that make it a harder feedstock to produce biogas when compare with other organic substrates with higher soluble/easier-to-hydrolyse carbon content, but possibly more suitable for the production of VFAs (McCarty, 1964; Sialve et al., 2009; Zhao et al., 2016).

In view of the low anaerobic biodegradability of *Chlorella vulgaris* and the potential of increasing the digestibility of food waste, a pre-treatment step was tested to assess the improvements in VFA production by anaerobic acid fermentation. In that sense, high pressure thermal hydrolysis (HPTH) and conventional autoclaving were tested.

6.2.2 Characterisation of food waste and microalgae thermally pre-treated

Pre-treatment of feedstocks with low anaerobic biodegradability is often essential for making the organic material more accessible to the anaerobic consortia by promoting changes in the physicochemical properties of the macromolecules in the feedstock (Kondusamy and Kalamdhad, 2014; Sialve et al., 2009).

In order to track changes on chemical parameters after pre-treatment of the substrate, it has been suggested that by monitoring the hydrolysis ratio of the substrate is possible to assess the efficiency of the process with regard

to the solubilisation of particulate (non-soluble) organic material after pre-treatment. The hydrolysis ratio can be calculated considering the concentration of proteins, carbohydrates or VSS before and after pre-treatment, according to the formula provided by Liu et al. (2012b):

$$\text{Hydrolysis ratio} = \frac{\text{Parameter}_{\text{before treatment}} - \text{Parameter}_{\text{After treatment}}}{\text{Parameter}_{\text{Before treatment}}} \times 100\% \quad \text{.....Equation 6.1}$$

When analysing the thermally treated samples, it is clearly noticeable that pre-treatment by HPTH led to the hydrolysis of organic material and hence, it increased the amount of SCOD of both substrates when compared with raw samples. SCOD increased from 21.8% to 44.1% for HPTH-food waste and from 8.1% to 39.8% for HPTH-microalgae. Pre-treatment at lower temperature and pressure (autoclaving) contributed to less COD solubilisation with 26.4% and 16.3% SCOD after treatment for food waste and microalgae, respectively.

Works from Kim et al. (2013) report solubilisation of food waste in terms of COD, being around 20% for ultrasonication pre-treatment and 30% for alkaline hydrolysis (alkalinisation) at pH 12. Whereas Elbeshbishy et al. (2011) found solubilisation of 33% of COD when working with food waste using an ultrasonic treatment followed by alkali treatment. Elbeshbishy and Nakhla (2011) found a 9% SCOD increase by ultrasonication (24 min/d) as a pre-treatment for an AD system, using a CSTR for biohydrogen production. Fdez.-Güelfo et al. (2011) found the best conditions for alkaline hydrolysis of the organic fraction of municipal solid waste, being 180°C, 3 g NaOH/L and 3 bar, resulting in SCOD increments of about 246%. Considering these reported findings, the treatment of food waste by HPTH in this study, contributes to a respectable solubilisation of organic matter, which performed better than ultrasonication but not as well as hydrothermal alkaline processes.

For studies on microalgae pre-treatment processes, Keymer et al. (2013) found higher SCOD/TCOD ratios when using HPTH on *Scenedesmus sp.* and for lipid-extracted *Scenedesmus sp.,* obtaining 0.55 and 0.95, respectively. Using lower temperatures, Marsolek et al. (2014) found solubilisation ratios of 0.33, 0.27 and 0.29 for *Nanochloropsis oculata* treated at 90, 60 and 30°C respectively, which shows that the increase in

temperature increases the degree of organic matter solubilisation. Studies from Suresh et al. (2013) working with *Ettlia sp.* and sonication, microwaving and autoclaving in alkaline conditions increased the SCOD in 57%, 52% and 82% respectively, showing that a combination of pressure and temperature is also responsible for the solubilisation of COD. Yang et al. (2011) report an increase on COD solubilisation of about 87% when treating oil-extracted *Scenedesmus sp.* biomass on alkaline conditions at 100ºC for 2.5 h. *Chlorella vulgaris* treated by HPTH in this study showed a respectable solubilisation of COD, but the resulting hydrolysis did not reach values as high as the ones cited previously, possibly due to the lack of alkali addition during the treatment process.

The solubilisation of COD concurs with the figures reported for TSS, which decreased significantly after HPTH treatments with a hydrolysis of 24.4% for food waste and 53.7% for microalgae. VSS hydrolysis performance for food waste and microalgae reported values of 15.23% and 58.08% respectively, showing high solubilisation of organic material from the feedstock. On the other hand, it is important to mentioned that the standard autoclaving pre-treatment process showed very low content of soluble COD at just 6.7% and 3.8% for food waste and microalgae respectively, which is inferior than the soluble content in the raw samples for both feedstocks (Food Waste = 13.1%; microalgae = 10.3%) presumably because of the absorption of water inside the cells and the gelatinisation of starch and other carbohydrates (Gomez and Aguilera, 1983; Gomez et al., 1991).

VSS hydrolysis of the food waste in this study is clearly lower than the hydrolysis reported by Liu et al. (2012b) from studies using kitchen waste and vegetable/fruit residues after thermal treatment at 175ºC for about 50 min, giving values of 38.9 and 38.4% VSS, respectively. The 2-fold difference can be attributed to the higher temperature and time used. On the other hand, studies from Yin et al. (2014) found a hydrolysis of 6.24% TVS when using hydrothermal conditions at 160ºC for 30 min, in samples originally containing 5.24% of TVS. That slight difference on TVS hydrolysis can be attributed to the different composition inherent to the food waste used.

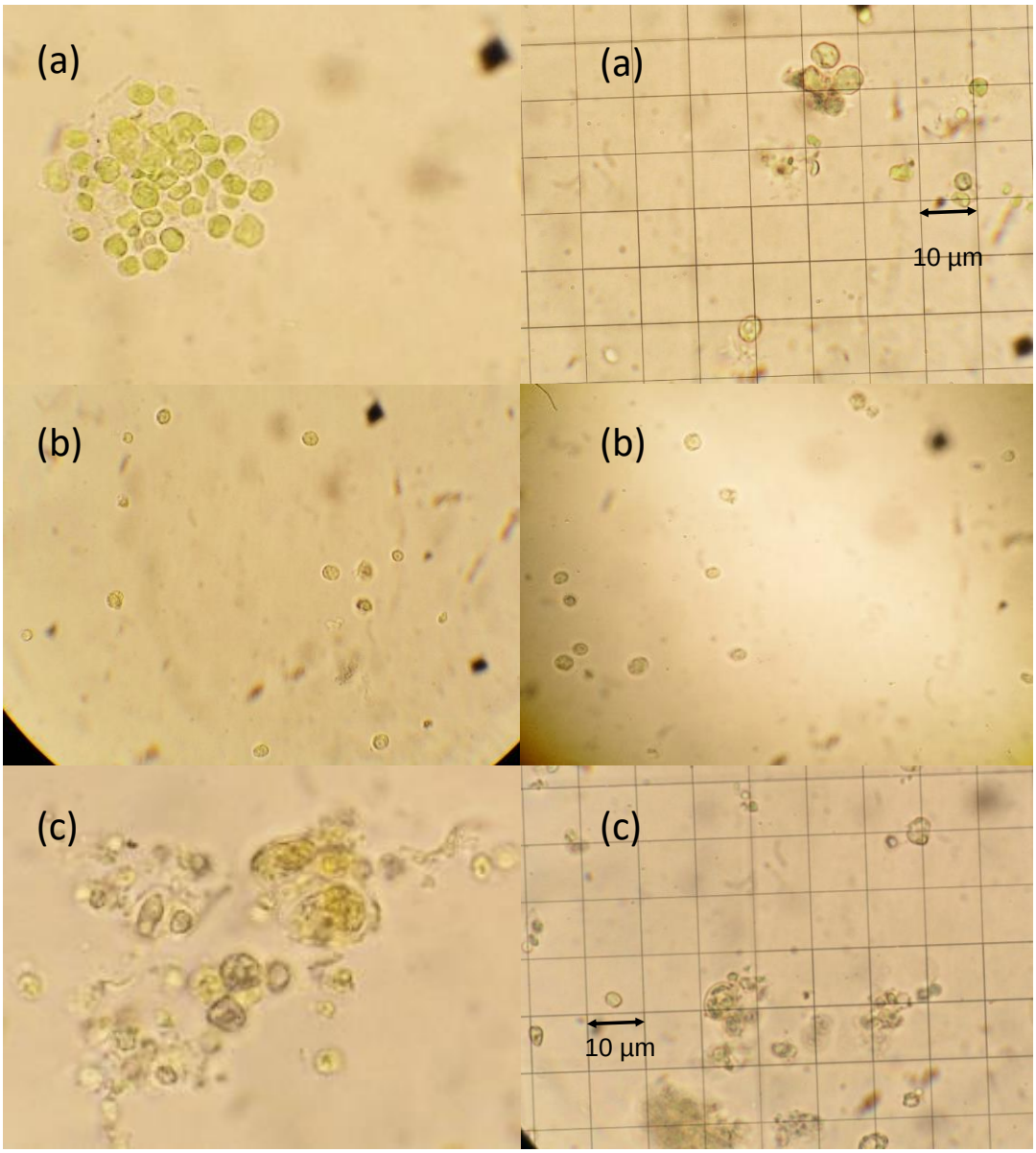

Figure 6.1. Microscopic structural analysis of *Chlorella vulgaris*. (a) Raw microalgae, (b) Autoclaved microalgae and, (c) HPTH-microalgae.

Microscopic analysis of untreated and pre-treated *Chlorella vulgaris* was conducted to observe the changes on microalgae structure and cell wall caused by thermal pre-treatments. Raw and autoclaved algae presented similar structure (a clear round cell shape on both cases) which suggests the poor cell wall disruption of the cell wall caused by the autoclave process (Figure 6.1). In contrast, microalgae treated by HPTH showed few complete algae structures and some possible fragments which can be algae debris caused by the rupture of the cell wall. These findings are in accordance with the reports from Suresh et al. (2013), who also found disruption on the

microalgae cell walls on the microscope when treating Ettlia sp. residue with autoclave or ultrasonic processes in alkaline conditions.

When comparing the elemental composition and the C/N of all feedstocks, it is evident that microalgae samples have a much higher concentration of nitrogen, about 2 to 3 times higher than food waste samples, which derives in lower C/N ratios. Optimal ratios between carbon and nitrogen in anaerobic fermentation systems are reported to be between 20 to 30 units, thus the co-digestion of WAS with microalgae will inevitably affect this ratio and could potentially promote the accumulation of VFAs by the production of free ammonia and its effect on methanogens (McCarty, 1964; Sialve et al., 2009). None of the pre-treatments showed any dramatic change on the C/N ratio as treatments were performed in closed systems, applying temperatures that did not change the composition of the substrate and at the same time, avoiding the releasing of gases. All pre-treatments achieved recovery yields higher than 96% of TS. No attempts were made to analyse the release of nitrogen or phosphorus into the soluble part of the feedstocks.

Statistical analysis to determine the impact of the thermal pre-treatment on the solubilisation of organic material of food waste or microalgal biomass was conducted using the SCOD/TCOD ratio for treated and untreated samples. Firstly, the results for the untreated food waste versus the two different treatments showed that conventional autoclaving did not make a significant difference to the SCOD/TCOD ratio ($p=0.128$, two-tailed), whereas HPTH contributed to a higher solubilisation of the organic material ($p=0.02$).

In contrast, respectable solubilisation of the TCOD from *Chlorella vulgaris* was achieved in both pre-treatment processes. Despite the formation of gels and absorption of water inside algal cells after conventional autoclaving and only partial destruction of microalgal cell wall, there was some releasing of soluble organic material and thus, the SCOD/TCOD ratio showed a significant difference of both treated samples with regards to the untreated microalgae ($p=0.000$ and $p=0.001$, for HPTH and autoclaving respectively). The SCOD/TCOD ratio from HPTH-WAS 2-fold higher than conventional autoclaving of microalgae, which is confirmed by a statistically significant difference when comparing both pre-treatments ($p=0.03$), in terms of organic material solubilisation.

6.3 Carbon/Nitrogen ratio on the co-fermentation of WAS and food waste/Microalgae and its pre-treated samples

The main aim of pre-treating feedstocks in this study was to improve the biodegradability of organic material by the consortia of microorganism in anaerobic acid fermentation. For example, some authors working with microalgae have reported a low conversion, around of 20%, of microalgae organic compounds to bioproducts (methane), which supports the need for the use of pre-treatment processes before anaerobic fermentation/digestion. That could also help to fully exploit the potential of blending additional organic wastes by integrating them into current processes for pre-treatment and co-digestion of WAS in WWTP (Tartakovsky et al., 2015; Tartakovsky et al., 2013; Zamalloa et al., 2012).

Several nutrients are needed for the microbial consortia in the anaerobic digestion. Among the most important nutrients, nitrogen compounds are essential for the preservation, maintenance and metabolism of bacteria (e.g., ammonia, vitamins and proteins). Therefore, having a deep understanding of the role of C/N ratios on process performance is indispensable as it accounts for the balance between energy sources (carbon) and nutrients (nitrogen) (Smith and Holtzapple, 2011).
In order to study the co-fermentation of WAS with other organic substrates and the influence of the resulting C/N ratio, mixtures of WAS with treated and untreated food waste and *Chlorella vulgaris* were tested independently according to Table 6.2. Acidogenic fermentations were based primarily on the content of WAS as this project is aiming to treat and increase the recovery of biochemicals from sewage sludge in current waste water treatment works.

The production of biogas is influenced to a large extent, by the choice of organic material (feedstock) and its carbon to nitrogen ratio (Dioha et al., 2013). In this respect, it is clear that the C/N ratio of all the mixtures of WAS with other substrates are lower than the optimal C/N ratio recommended to sustain the anaerobic digestion process (McCarty, 1964; Sialve et al., 2009; Zhao et al., 2016), mainly because of the very low carbon content found in the original sewage sludge composition. At the same time, none of the co-substrates (food waste or microalgae) presented a carbon content high enough to increase the overall C/N ratio in the final mixture to comply with the optimal value of C/N. Among the mixtures, the blends of HPTH-WAS and

microalgae presented the lowest C/N proportions due to the high content of nitrogen in microalgae, reaching its smallest when 75% of algae was used. In contrast, the highest content of food waste provided the highest C/N ratio. Considering these data, it is predictable that the mixtures of 25% WAS/75% food waste would produce the highest concentration of VFAs in the acidogenic fermentation.

Table 6.2. Carbon/Nitrogen ratios of the mixtures of HPTH-WAS and treated and untreated food waste/microalgae samples for the production of VFAs.

	C from WAS	N from WAS	C from co-substrate	N from co-substrate	C Final	N Final	C/N Final
75WAS/25RFW	1.466	0.167	0.632	0.051	2.098	0.217	9.65
50WAS/50RFW	0.978	0.111	1.264	0.102	2.242	0.213	10.54
25WAS/75RFW	0.489	0.056	1.897	0.153	2.385	0.208	11.46
75WAS/25ACFW	1.466	0.167	0.617	0.043	2.083	0.209	9.95
50WAS/50ACFW	0.978	0.111	1.234	0.086	2.211	0.197	11.24
25WAS/75ACFW	0.489	0.056	1.851	0.128	2.339	0.184	12.71
75WAS/25HPTHFW	1.466	0.167	0.651	0.046	2.118	0.212	9.98
50WAS/50HPTHFW	0.978	0.111	1.303	0.091	2.280	0.202	11.28
25WAS/75HPTHFW	0.489	0.056	1.954	0.137	2.443	0.192	12.72
75WAS/25RA	1.466	0.167	0.583	0.114	2.049	0.280	7.31
50WAS/50RA	0.978	0.111	1.166	0.227	2.144	0.338	6.33
25WAS/75RA	0.489	0.056	1.749	0.341	2.238	0.396	5.64
75WAS/25ACA	1.466	0.167	0.577	0.111	2.043	0.278	7.35
50WAS/50ACA	0.978	0.111	1.153	0.223	2.131	0.334	6.38
25WAS/75ACA	0.489	0.056	1.730	0.334	2.218	0.389	5.69
75WAS/25HPTHA	1.466	0.167	0.619	0.135	2.086	0.302	6.91
50WAS/50HPTHA	0.978	0.111	1.239	0.271	2.216	0.382	5.80
25WAS/75HPTHA	0.489	0.056	1.858	0.406	2.347	0.461	5.08

Key: 25= 25%, 50= 50%, 75= 75%; WAS= Waste Activated Sludge; RFW= Raw Food Waste; ACFW= Autoclaved Food Waste; HPTHFW= High Pressure Thermal Hydrolysed Food Waste; RA= Raw Algae, ACA= Autoclaved Algae, HPTHA= High Pressure Thermal Hydrolysed Algae

Studies of single substrates for anaerobic fermentation/digestion have worked with different C/N ratios. For example, Suresh et al. (2013) and Seo et al. (2016) reported a C/N of 9.5 with *Ettlia sp*, whereas Zhao et al. (2016) reported a value of 5.95 when working with *Scenedesmus dimorphus*. Zhao et al. (2014) reported values of C/N from 5.51 to 6.8 for *Chlorella vulgaris*; 6.36 to 7.55 for *Nannochloropsis sp*.; 8.46 to 14.87 for *Nannochloropsis salina*; 6.89 to 9.47 for *Nanofrustulum sp.*; and 5.68 to 6.86 for *Phaedactylum tricornutum.* Using *Saccharina japonica*, Jung et al. (2015) reported a high C/N ratio of 24.54. Yen and Brune (2007) worked with mono-digestion of algal sludge (*Scenedesmus spp.* and *Chlorella spp.*) and paper pulp with a C/N ratio of 6.7 and 21.5, respectively.

For food waste fermentations, Liu et al. (2012b) worked with HPTH treated (175ºC, 60 min) and untreated food waste and WAS separately, with C/N ratios of 17.3 for kitchen waste, 21.7 for vegetable and fruit waste, and 7.0 for WAS. Surprisingly, works done by Li and Jin (2015) reported C/N ratios on kitchen waste which were similar to the co-fermentation of WAS and food waste in this study, although these values were below the optimum recommended to prevent biogas production (Chen et al. (2008). Employing only sewage sludge from different WWTPs, Liu et al. (2008) investigated the effect of C/N using ratios of 12.22, 15.10 and 5.01 for the production of VFAs. Other study that used sewage sludge was published by Lin and Lay (2004), which aimed to find the effect of C/N on the hydrogen content in the digestion gas and hydrogen production rate, reporting its best at 47 units.

In terms of co-fermentation, there are several studies which work with many kinds of substrates including pig manure and algae (Astals et al., 2015); sewage sludge and glycerol (Athanasoulia et al., 2014); swine manure and corn stover (Chan et al., 2011); food waste with WAS (Chen et al., 2013; Cheng et al., 2016; Dahiya et al., 2015; Dinsdale et al., 2000; Hong and Haiyun, 2010; Kim et al., 2013; Lafitte-Trouqué and Forster, 2000; Wu et al., 2016; Xie et al., 2017); WAS and plants biomass (Huang et al., 2016); agro-wastes (Misi and Forster, 2001); microalgae and WAS (Neumann et al., 2015); leather fleshing and municipal solid waste (Palaniyandi, 2009); microalgae *Nannochloropsis salina* with energy crops (Schwede et al., 2013); sewage sludge and municipal solid waste (Sosnowski et al., 2003); algal sludge and waste paper (Yen and Brune, 2007); mixed microalgae and food waste (Zhen et al., 2016); and WAS and corn straw (Zhou et al., 2013),

although not many of them focus on explaining the influence of the C/N ratio of the mixtures.

Studies investigating the influence of C/N ratios include Yen and Brune (2007), who tested C/N ratios of 11.8, 18.0 and 36.4 for algal sludge and waste paper. Rughoonundun et al. (2012) investigated the carbon to nitrogen ratio of WAS and pre-treated bagasse with C/N ratios from 6.62 to 64.58. Smith and Holtzapple (2011) researched the influence of C/N ratio of paper pulp waste and wet manure and urea, testing 30 different blends in a range from 5 to 107.3. Wang et al. (2012) tested C/N ratios ranging from 15 to 35, and reported an optimal ratio of 27.2 for methane production using co-digestion of dairy, chicken manure and wheat straw. Murto et al. (2004) investigated the co-digestion of pig manure and various industrial wastes (i.e., slaughterhouse waste, restaurant, fruit and vegetable wastes) to increase the carbon content of pig manure and tested C/N ratios of 8, 10 and 11 for the production of biomethane. One of the first studies working with the co-digestion of algae and sewage sludge was conducted by Samson and LeDuy (1983), finding that mixtures of 50 algae/ 50 WAS increased methane yield and productivity over 2-fold than only working with HPTH-WAS.

Because of this deficiency of information about the determination and understanding of the best blend of WAS and food waste or microalgae, it is important to test the co-fermentation these substrates in order move one step closer to the prediction of the fermentation process and engineering acidogenic fermentation systems.

6.4 Effect of co-fermentation and substrate pre-treatment on the chemical oxygen demand

Following the same procedures considered in previous chapters, SCOD and TCOD are important monitoring parameters as they show the initial and final concentration of organic content in the broth to determine any solids losses (biogas production) and the hydrolysis of complex organic compounds.

As mentioned previously, mixtures of treated and untreated food waste and microalgae with WAS (based on TVS) were 75% WAS/25% food waste or microalgae, 50% WAS/50% food waste or microalgae and 75% WAS/ 25% food waste or microalgae. In the subsequent figures, the key used to read the legends is as follows: firstly the content of the supplementary substrate to WAS (25, 50 or 75%), which in this case is food waste or microalgae,

followed by the abbreviation of the treated/pre-treated substrate involved which are: RFW= raw food waste, ACFW= autoclaved food waste, HPTHFW= high pressure thermal hydrolysis food waste, RA= raw algae, ACA= autoclaved algae, HPTHA= high pressure thermal hydrolysed algae. Thus, if the key is 75 HPTHFW, it refers to 25% WAS and 75% HPTH pre-treated food waste. Figure 6.2 shows the TCOD content and its progress during 21 days of fermentation. The fermentors with blends of WAS and food waste presented solid losses ranging from 13% (25% WAS/75% autoclaved food waste or 25% WAS/75% HPTH-food waste) to 34% (25% WAS/75% raw food waste). These results show that higher losses of COD in raw food waste mixtures were due to the high biodegradability of the blend and affinity with the inoculum.

In contrast, mixtures of WAS and *Chlorella vulgaris* presented mixed results; while raw algal blends showed TCOD decreases between 6.8-22.6% (with its highest reduction when algal biomass was just 25%), both pre-treated microalgae presented low or null COD losses, probably to the incapacity of the inoculum to hydrolyse and transform the microalgae, either because of the impossibility of breaking the cell wall or because the inoculum was not the most suitable for the digestion of microalgae. With this regard, it has been reported in studies from González-Fernández et al. (2012) and (Passos et al., 2014) that cell walls could obstruct the access to the organic material by the microbial consortia. Apparently control fermentors showed poor conversion of COD into biogas, which is sustained by the low losses of COD which in all fermentors were below 10% with regards to the initial TCOD value.

The co-fermentation of HPTH-WAS with food waste or microalgae presented a different effect on the TCOD mineralisation with regards to the mixture employed. Whereas high losses of TCOD were presented in reactors working with mixtures with food waste, small or null TCOD mineralisation was observed in fermentations working with microalgae (treated or untreated). In terms of pre-treatment, there was not a clear impact of each pre-treatment process on the final TCOD mineralisation as all the fermentors working either with food waste or microalgae, presented similar trends between each other.

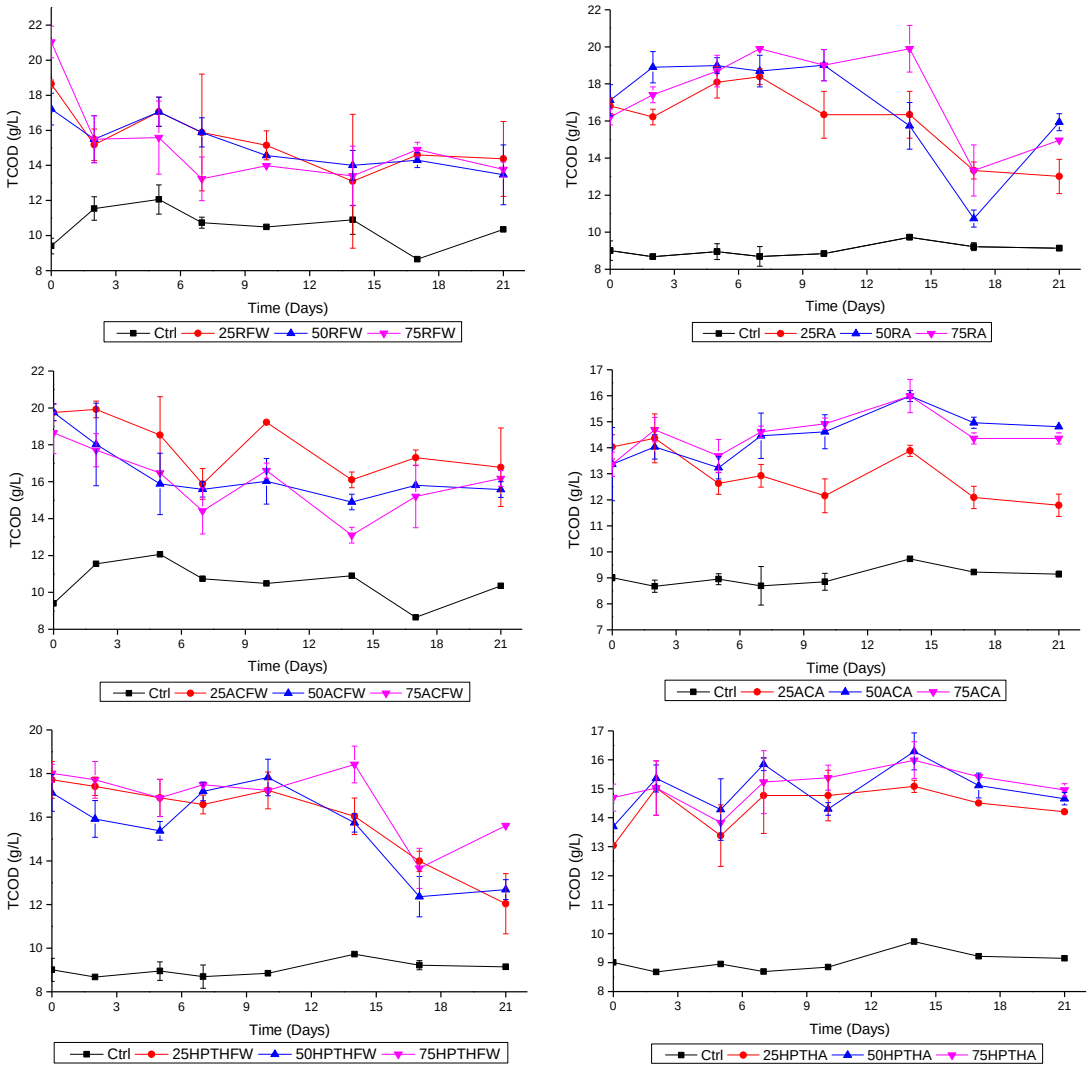

Figure 6.2. Total COD results from acidogenic anaerobic co-fermentation of HPTH-WAS with FW and/or microalgae at pH 9.

(*Key: 25= 25%, 50= 50%, 75= 75%; WAS= Waste Activated Sludge; RFW= Raw Food Waste; ACFW= Autoclaved Food Waste; HPTHFW= High Pressure Thermal Hydrolysed Food Waste; RA= Raw Algae, ACA= Autoclaved Algae, HPTHA= High Pressure Thermal Hydrolysed Algae).*

Opposite to the TCOD progress during the acidogenic fermentation, the soluble COD increases along the time in the majority of the mixtures tested, which showed the activity of microbial consortia hydrolysing non-soluble/hard to digest COD and increasing the SCOD as a result (Figure 6.3).

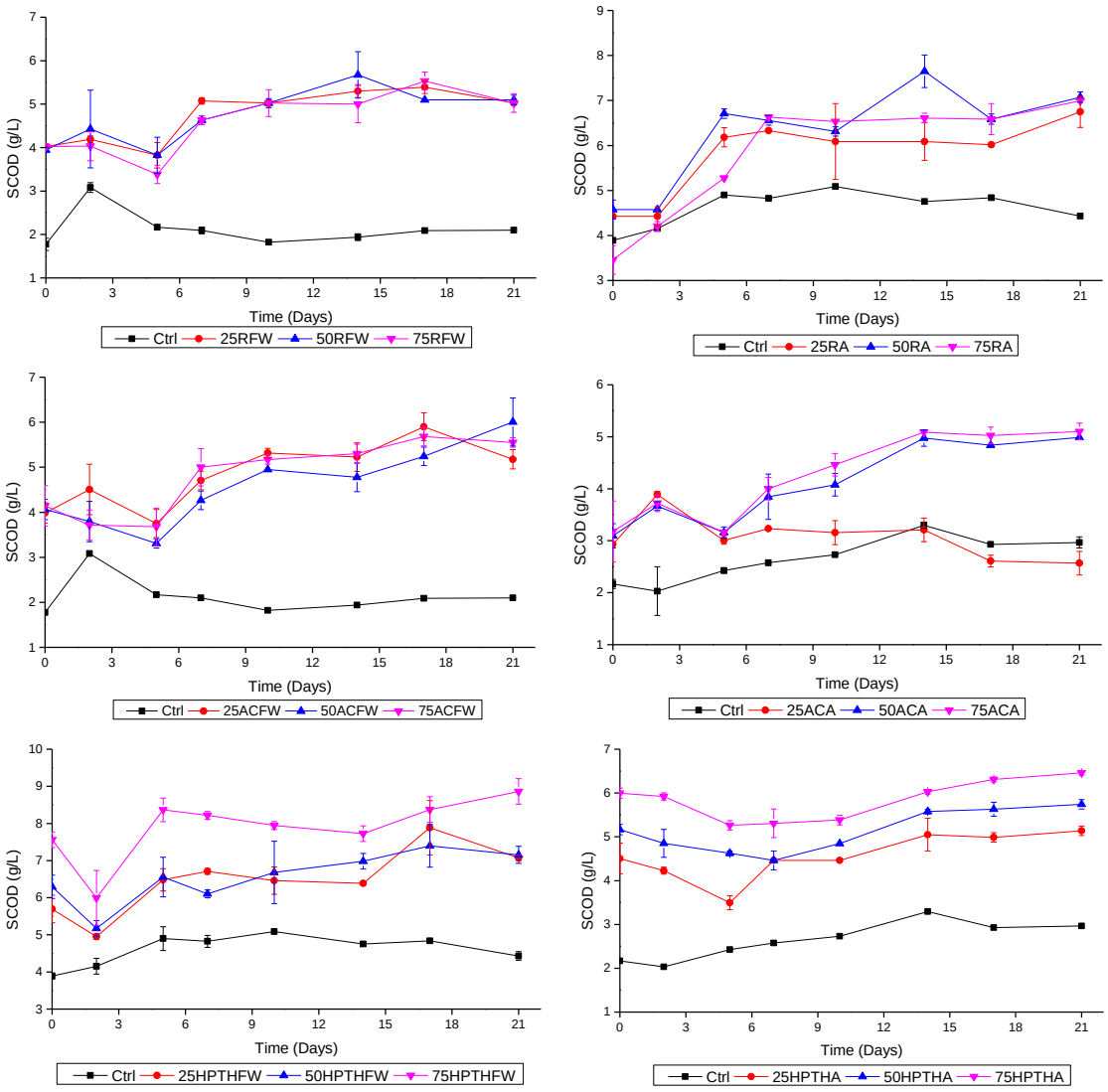

Figure 6.3. Soluble COD results during acidogenic anaerobic co-fermentation of HPTH-WAS with FW and/or microalgae at pH 9.

(*Key: 25= 25%, 50= 50%, 75= 75%; WAS= Waste Activated Sludge; RFW= Raw Food Waste; ACFW= Autoclaved Food Waste; HPTHFW= High Pressure Thermal Hydrolysed Food Waste; RA= Raw Algae, ACA= Autoclaved Algae, HPTHA= High Pressure Thermal Hydrolysed Algae).*

Also, it was noted that SCOD in fermentors with raw and autoclaved food waste presented similar concentrations of initial SCOD (3.94–4.02 g SCOD/L) which agrees with the low SCOD/TCOD values for both substrates. In contrast, the HPTH-food waste reactors presented higher initial concentration of SCOD in comparison with the autoclaved and raw food waste fermentors; a dissimilar initial SCOD among its different mixtures, being the lowest when HPTH-food waste represented just 25% percent of the substrate mixture (5.69 g SCOD/L), and increased with the

rise on its content to reach its maximum at 75% of HPTHFW (7.56 g SCOD/L). This disagreement on the initial soluble COD concentrations are evidently caused by the addition of soluble organic material from the pre-treated food waste by HPTH and not by the WAS in the system.

After day zero, SCOD concentrations in all fermentors increased during the fermentation process, which indicates hydrolysis caused by the activity of the microbial consortia. The mixed acidogenic fermentations presented different trends with regards to the SCOD evolution which may perhaps be attributed to the feedstocks and its different pre-treatments.

Primarily, fermentors with mixtures of WAS/raw food waste showed the highest SCOD increasing during the first seven days (15.1 - 26.0%) and then presenting a semi-plateau, with variations of 0.1 to 1.0% of the SCOD/TCOD ratio until day 21, when they reached COD hydrolysis between 24.8 to 29.3% with respect to the initial SCOD. These results suggest that higher contents of raw food waste did not impact on the final concentration of the soluble COD as the final SCOD/TCOD ratio ranged between 0.35 and 0.37 for all the raw food waste mixtures (Table 6.3).

Contrary to these results, reports from Tang et al. (2017) showed mixed results on the SCOD content in the experiments that were ran with three different inocula with three acidic pH levels for the production of lactate from food waste. On the other hand, the methanogenic sludge inoculum consumed the SCOD probably for the production of biogas, the fresh food waste and anaerobic sludge inoculum increased the SCOD along the seven days of fermentation at any pH.

Table 6.3. SCOD/TCOD ratios on the acidogenic co-fermentation at pH 9 of HPTH-WAS and food waste and/or *Chlorella vulgaris* on different days.

	Fermentation days			
Fermentor	0	7	14	21
25RFW	0.21	0.31	0.40	0.35
50RFW	0.22	0.29	0.40	0.37
75RFW	0.19	0.35	0.37	0.36
25AFW	0.20	0.29	0.32	0.30
50AFW	0.20	0.27	0.32	0.38
75AFW	0.22	0.34	0.40	0.34
25HPTHFW	0.32	0.40	0.39	0.58
50HPTHFW	0.36	0.35	0.44	0.56
75HPTHFW	0.42	0.47	0.41	0.56
25RA	0.26	0.34	0.37	0.51
50RA	0.26	0.35	0.48	0.44
75RA	0.21	0.33	0.33	0.46
25ACA	0.20	0.25	0.23	0.21
50ACA	0.23	0.26	0.31	0.33
75ACA	0.23	0.27	0.31	0.35
25HPTHA	0.34	0.30	0.33	0.36
50HPTHA	0.37	0.28	0.34	0.39
75HPTHA	0.40	0.34	0.37	0.43

Studies from Wu et al. (2016) reported hydrolysis (SCOD/TCOD) above 0.55 on day seven when co-fermenting mixtures of 83% food waste and 17% excess sludge in semi-CSTR whereas Parawira et al. (2004) found SCOD increments during the first seven days of acidogenic fermentation of solid potato waste of about 28% and 36% when using 500g or 1000g of food waste in a leach bed reactor. Yin et al. (2014) used food waste treated by hydrothermal processes (140º, 160º, 180º and 200ºC) for VFAs production, finding similar trends on SCOD evolution than the ones encountered in this study, but discovered a quicker solubilisation of organic material and a SCOD plateau from day three until day nine of the fermentation, with organic matter solubilisation as high as 91% of the initial SCOD value. Finally, Kim et al. (2006) reported SCOD highest value from day 5 or 6 during mixed acid fermentation, which represents an increase of 34.9% of the initial SCOD

amount in experiments for VFAs production using raw food waste treated by pure enzymes. SCOD hydrolysis mentioned in the above studies were higher than the one found in this study, which is probably caused by the composition of the substrate and/or the type of reactor and operation conditions used that are different to the fermentations in this project (batch experiments).

For the tests performing fermentation of WAS with food waste treated by conventional autoclaving, SCOD presented a consistent increment at in all sampling days during the test and reached its maximum at day 21 with hydrolysis ratios (SCOD/TCOD) ranging between 0.30 and 0.38, which represents an increment of 29.9% for the mixture containing 25% ACFW and 48.9% for the blend with 50% ACFW with regards to the initial value for each fermentor.

Although there was a steady increment on the solubilisation of COD in ACFW reactors, final values were similar to the soluble COD figures obtained from the fermentation with raw food waste. A statistical analysis comparing SCOD/TCOD ratios from both wastes and their blends was performed by running a t-test for independent samples resulting in a $p=0.462$, which implies that there was no significant difference between the two groups (raw vs autoclaved food waste).

Results of SCOD from the fermentations with food waste treated by HPTH displayed mixed results; whereas the mixture of 25 WAS/75 HPTHFW always exhibited the highest SCOD during the time of the fermentation process, the other two blends presented parallel SCOD progress, finalising with values of 7.07 to 7.15 g SCOD/L. Increments in SCOD caused by microbial hydrolysis in fermentors with HPTHFW as substrate were between 13.63 and 24.15% which are lower than the hydrolysis obtained from raw and autoclaved food waste. This phenomenon can be explained due to the conditions set during thermal treatment at 160°C that could solubilise organic material that was then hydrolysed by the microbial consortia in the fermentations with raw and autoclaved food waste. A t-test for independent samples was carried out with the SCOD/TCOD ratios from raw food waste and HPTHFW at day 21, finding a $p=0.000$ (Sig. two-tailed), which means that SCOD/TCOD ratios were significantly different between pre-treatment processes being HPTHFW the one with higher solubilisation.

Yin et al. (2014) explored the influence of hydrothermal pre-treatment on food wastes at different temperatures (140º, 160º, 180º and 200ºC) in mixed acid fermentation showing a quick increment of SCOD concentration within the first 3 days with no further solubilisation, but a decrease after day 11, in a 15-day experiment. Also, it is reported that hydrolysis of FW at 160ºC in the mentioned study was higher than at lower temperatures. These findings are opposite to the results revealed in this series of experiments were hydrolysis of organic material in treated and untreated food waste was uninterrupted during the 21 days that the fermentation was held, showing an incessant hydrolytic bacterial activity. HPTH pre-treated samples did not reach the same percentages of SCOD increments along the experimental time than raw and autoclaved food waste.

Although microalgae has been proven to be a difficult substrate to hydrolyse and digest by anaerobic bacteria (Song et al., 2015; Neumann et al., 2015; Mendez et al., 2013; Lee et al., 2014; Pham et al., 2012; Pham et al., 2013; Tartakovsky et al., 2013; Kinnunen et al., 2014), it is clear that there was an increase on the SCOD values during the fermentations which suggests the solubilisation of organic material by hydrolytic bacteria in the consortium. All the fermentations with untreated microalgae mixtures presented a rapid hydrolysis in the first seven days of the process when reached SCOD increments of 43% for mixtures of 25 and 50% raw algae and 92% for 75% raw algae. After day seven of the fermentation, was found a semi-plateau on the SCOD concentrations in all reactors working with raw algae, when they reached their maxima, with final increments of 52, 54 and 102% for mixtures of 25, 50 and 75% of untreated microalgae on day 21. This behaviour can be explained because of rapid hydrolysation of WAS and further low hydrolysation of microalgae content because of the difficulty of algal biomass to be hydrolysed. These results are higher than the SCOD increments on the mono-digestion of untreated *Scenedesmus sp.* in an CSTR reported by Gruhn et al. (2016), which were 27.3% after one week of fermentation and without further increase after 3 weeks of the process. This discrepancy could be attributed to the co-fermentation of microalgae and WAS, which probably played a synergistic effect on the hydrolysis of the organic material.

For autoclaved microalgae, interesting results were found. Whereas the blends with 50 and 75% of microalgae showed similar trends and increase on hydrolysis values, mixture with 25% of raw *Chlorella vulgaris* showed a slight increase at day 5 and further decrease until the end of the

fermentation (12.25% reduction), which could only be attributed to a conversion of the SCOD to gaseous products even when alkaline pH is used in the acidogenic fermentation. The increases of SCOD in blends of 50 and 75% of untreated microalgae with WAS were in the region of 60–61% for both cases, which is higher than the studies from Gruhn et al. (2016), and this behaviour could be endorsed to the effect of the co-fermentation and/or the pre-treatment of the algal biomass used in this research.

With regards to the fermentations using HPTH pre-treated microalgae, the initial SCOD concentration showed different values as the higher amount of microalgae in the blend provides a higher solubilised organic material. Along the fermentation, all mixtures of WAS/HPTH-microalgae presented similar progress in terms of COD hydrolysis at all times, with a final value higher of SCOD with respect to its initial value (7.8-14.1% of solubilisation).

Final values of SCOD/TCOD ratios in fermentors with pre-treated and untreated microalgae were compared using a t-test for independent samples resulting in a $p=0.023$, which indicates that there is a significant difference between the raw versus autoclaved pre-treatment, being the prior, higher than the treated microalgae. On the contrary, t-test results for the comparison between raw and HPTH pre-treated microalgae showed a $p= 0.58$, which is an indicator of no significant difference on the solubilisation of COD in both samples.

Hence, in the experiments with HTPH-WAS and food waste there was not a noticeable effect of the co-fermentation on the solubilisation of the suspended COD for all the food waste mixtures as they presented similar trends on the increasing of SCOD. In contrary, there was a positive effect from the pre-treatment as experiments with HPTH-food waste reached higher final solubilisation when compared with autoclaved or untreated food waste.

With regards to experiments using microalgae as feedstocks, it was clear the negative effect of the pre-treatments of microalgae on the hydrolityc bacteria for the solubilisation of COD in the acidogenic fermentation as, neither autoclaved nor HPTH-microalgae presented a substantial increase on the SCOD content during the fermentation process. The co-fermentation of HPTH-WAS with microalgae did not show a positive impact on the COD solubilisation as the final SCOD/TCOD values were similar in all the mixtures tested for treated or untreated microalgae.

As it can be seen in Figure 6.2 and Figure 6.3, COD hydrolysis caused by microbial consortia presented a similar trend to the previous experiments when WAS samples were fermented at pH 9, which suggests a slow but sustained hydrolysis of the COD. Additionally, this tendency gives idea that the conversion of total and soluble COD could be modelled by kinetic approaches, thus order zero and order one kinetics were tested to determine the hydrolysis constant rate; however, the results did not fit to any kinetic model tried perhaps due to the high variability on the experimental COD results and the complexity of simultaneous processes and the actual nature of the samples.

6.5 Effect of co-fermentation and substrate pre-treatment on the solids content

Similarly to the total COD results, TVS content showed a tendency to decline in all fermentors with any feedstock and/or pre-treatment (Figure 6.4). For raw food waste, the maximum TVS loss was exhibited when the mixture contained 25% of HPTH-WAS and 75% of food waste, with a 22.2% TVS reduction, whereas mixtures with 25 and 50% of raw FW only reached a TVS removal of 14%. This performance corresponds with the highest TCOD removal found in the blend of 25 WAS/75 RFW, evidencing that raw food waste is easier to digest by the bacteria consortia in the fermentor (Heo et al., 2004) and could produce H_2, CO_2 and CH_4 as there is evidence supporting the generation of those gases in fermentations with alkaline pH (Dahiya et al., 2015; Dong et al., 2010a; Nath and Das, 2004). For autoclaved food waste, there was not a mixture that clearly presented a much higher concentration than other blends, as the three mixtures WAS/ACFW presented TVS destructions from 11 to 18.8%, which are lower than the results for fermentations with raw food waste. The TVS destruction on fermentors using food waste treated by high pressure thermal hydrolysis was fairly minimal, ranging between 2.5% for 75WAS/25 HPTHFW and 4.3% for 25WAS/75 HPTHFW.

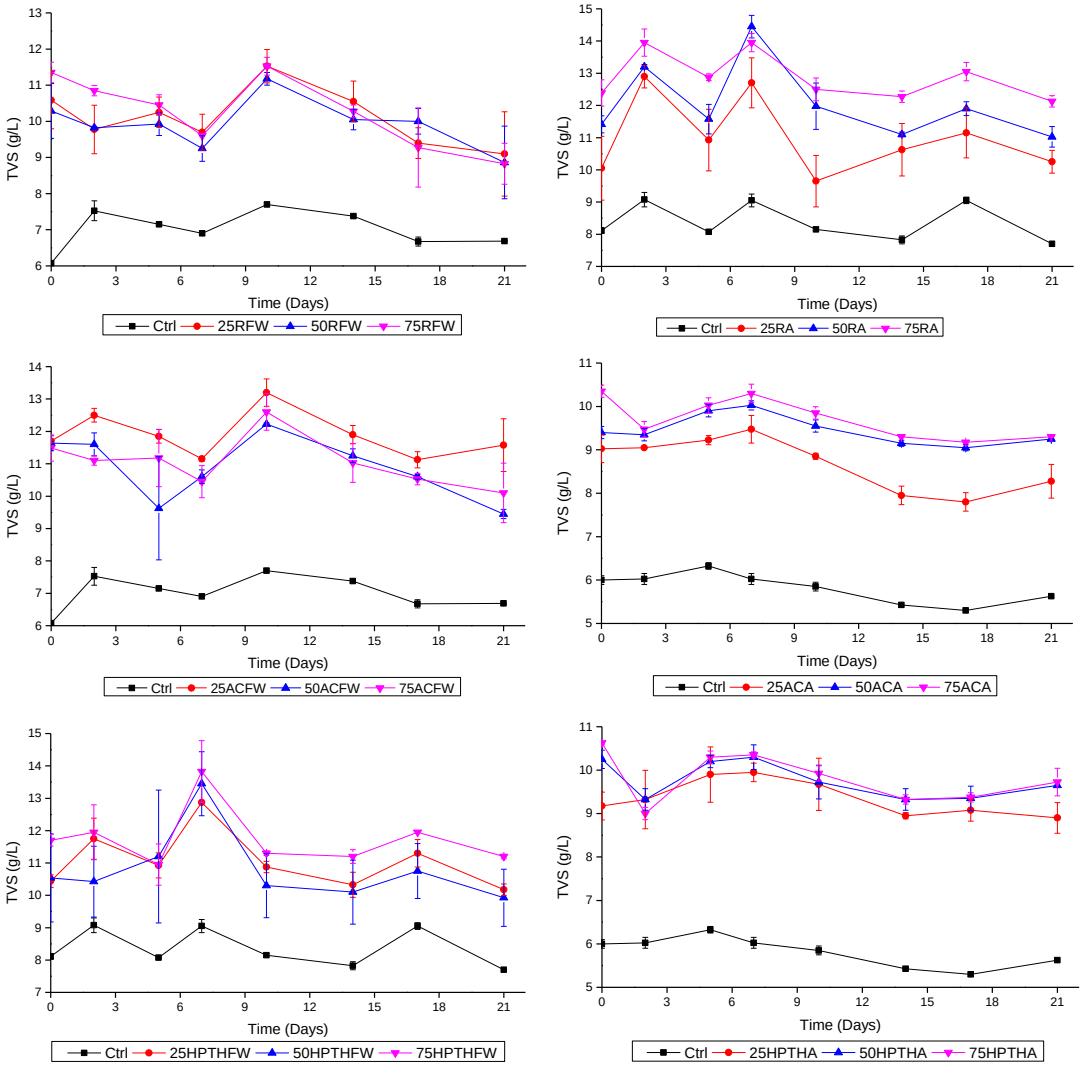

Figure 6.4. TVS results from the acidogenic co-fermentations of WAS with FW and/or microalgae at pH 9.

(*Key: 25= 25%, 50= 50%, 75= 75%; WAS= Waste Activated Sludge; RFW= Raw Food Waste; ACFW= Autoclaved Food Waste; HPTHFW= High Pressure Thermal Hydrolysed Food Waste; RA= Raw Algae, ACA= Autoclaved Algae, HPTHA= High Pressure Thermal Hydrolysed Algae).*

Additionally, the higher TVS destruction on raw food waste fermentations in comparison with thermal treated food waste reactors suggests a strong conversion to biogas probably because raw food waste was an easier substrate to be converted into liquid by-products followed by quickly gaseous products. This evidence supports the poor effect of conventional autoclaving pre-treatment (120°C, 30 min, 1.5 bar) on the TCOD destruction, COD hydrolysis and TVS destruction during acidogenic fermentation, whereas HPTH pre-treatment of food waste showed respectable results on COD

solubilisation but poor performance on TCOD and TVS destruction, showing that hydrothermal processes might not be the best method for the pre-treatment of food waste to achieve high COD hydrolysis and biogas production but might be important on the production of intermediate products such as VFAs and solvents (i.e., alcohol, acetone, butanol, etc.). These findings are similar to the reports from Liu et al. (2013a), who reported TVS removal efficiencies between 16.4 and 22.1% when co-digesting mixtures of 60% WAS and 40% food waste. For blends of WAS/FW with contents of food waste of 85%, the fermentations reached a maximum of 55.7% of TVS destruction. Results from Liu *et al.* (2013) also showed higher TVS destruction values than the TVS removal from the current research, probably because the aim of this project was to produce liquid products such as VFAs instead of biogas.

It is important to point that the pre-treatment of food waste presented a negative effect on the destruction of TVS which suggest that raw food waste was more liable to be converted into gaseous products and that hydrothermal processes might not be the best method for the pre-treatment of food waste. Co-fermentation in experiments with HPTH-WAS and food waste did not present an impact on the TVS behaviour as the mineralisation recorded was similar in the mixtures tested of raw, autoclaved or HPTH-microalgae fermentations.

Microalgae fermentors showed similar behaviour to the FW reactors, with very small TVS reductions in all cases. Untreated microalgae presented the lowest TVS destruction with percentages between 2.0 and 3.4% whereas pre-treated microalgae presented slightly higher percentages of TVS removal with ranges between 1.6 to 10.1% for mixtures of WAS and autoclaved microalgae and 3.0 and 8.5% for blends of WAS and HPTH-microalgae. The difference on the TVS removal among reactors can be explained due to the different pre-treatments applied to the microalgae although the pre-treatments did not show a dramatic improvement of mineralisation, COD hydrolysis or destruction when compared with untreated microalgae. It is important to mention that the concentration of TS and TVS in all control fermentors remained relatively stable along the duration of the experiment, reporting losses always below 10.1% which suggests that the products from the fermentations were derived mainly from the substrate digestion.

Among the different proportions of algae, there was a non-reasonable behaviour on the TVS destruction as the highest mineralisation was on the mixtures of 75% WAS/25% of raw algae and 25% WAS/75% of autoclaved or HPTH-microalgae. These findings are similar to the results from fermentations with food waste which showed their highest TVS removal with blends of 25% WAS/75% raw food waste and 50% WAS/50% autoclaved or HPTH-food waste, thus, considering these outcomes, it can be said that there is not a strong effect of the co-fermentation and pre-treatment of organic substrates and different C/N ratios on the TVS removal.

In general, results from this batch of experiments concur with the low TVS losses and mineralisation found in the fermentations of WAS at the same pH (pH 9) caused by the high alkaline pH, which shows the impact of the pH on the inhibition on the destruction of solids and the production of biogas.

6.6 Effect of the co-fermentation and substrate pre-treatment on the production, accumulation and composition of volatile fatty acids

6.6.1 Production and accumulation of VFAs

The co-digestion of different organic substrates is being recognised as an advantageous mode for the enhancement of the production of biochemicals or biogas during the fermentation/digestion of different organic material based on the balance of the C/N ratio and the use of other wastes with high content of C or N (Kondusamy and Kalamdhad, 2014; Weiland, 2010), or for the selective production of specific VFAs as it is been stated that the specific range of products depends mainly on the type of susbtrate (Arslan et al., 2016; Dahiya et al., 2015; Fernández et al., 2008).

The total VFAs concentration in the fermentors with mixtures of WAS and FW and microalgae are shown in Figure 6.5. Fermentations of raw and autoclaved food waste presented similar trends on their degree of acidification with its highest ramp during the first two days of the process, when the concentration increased from 3.6-fold to 3.9-fold for the mixtures with 25% of food waste; 5.5- to 5.7-fold for the mixtures with 50% food waste; 7.37-fold for the blend with 75% of raw food waste; and 10-fold for mixtures with 75% of autoclaved food waste. Kim et al. (2013) monitored VFA production in mixtures of FW and WAS (25, 50 and 75% FW) during fermentation for the production of hydrogen; they reported that when

increasing the content of FW, VFAs were produced in much higher concentrations, reaching their maxima on mixtures of 25% WAS/75% untreated food waste, which agrees with the results from this set of experiments. That performance shows that when increasing the concentration of raw or autoclaved food waste and hence the C/N ratio as well, there is a proportional and very rapid production of VFAs. Total VFA data reported in Figure 6.5 is the subtraction of the total VFAs produced in the fermentors with organic material (WAS, FW, microalgae) as substrate minus the VFAs produced in the fermentor control which only contains the inoculum previously adapted in the laboratory.

These findings concur with the studies conducted by Chinellato et al. (2013), who reported the highest production of VFAs after just 50 hours of fermentation using food waste as substrate under semi-continuous conditions for the production of biohydrogen and using different initial organic loadings and thermophilic conditions. As that study was conducted in a semi-CSTR for the production of biogas, VFAs concentration declined dramatically after 150 h, which is opposite to the results found in this project due to the different operational conditions (batch reactor) and the main aim of this research being the production of liquid biochemicals. Same behaviour was corroborated by the research carried out by Zheng (2013), who used fruit and vegetable waste for the production of acidification intermediates with different acidic pH; they found that at pH 6, the highest VFAs concentration was produced during the first 2 days of the process and for pH 4 and 5 the maximum VFA production was from day 2 to day 4. Babel et al. (2004) also found a fast VFA production reaching a semi-plateau after the first 3 days of fermenting pineapple peels at pH 7.

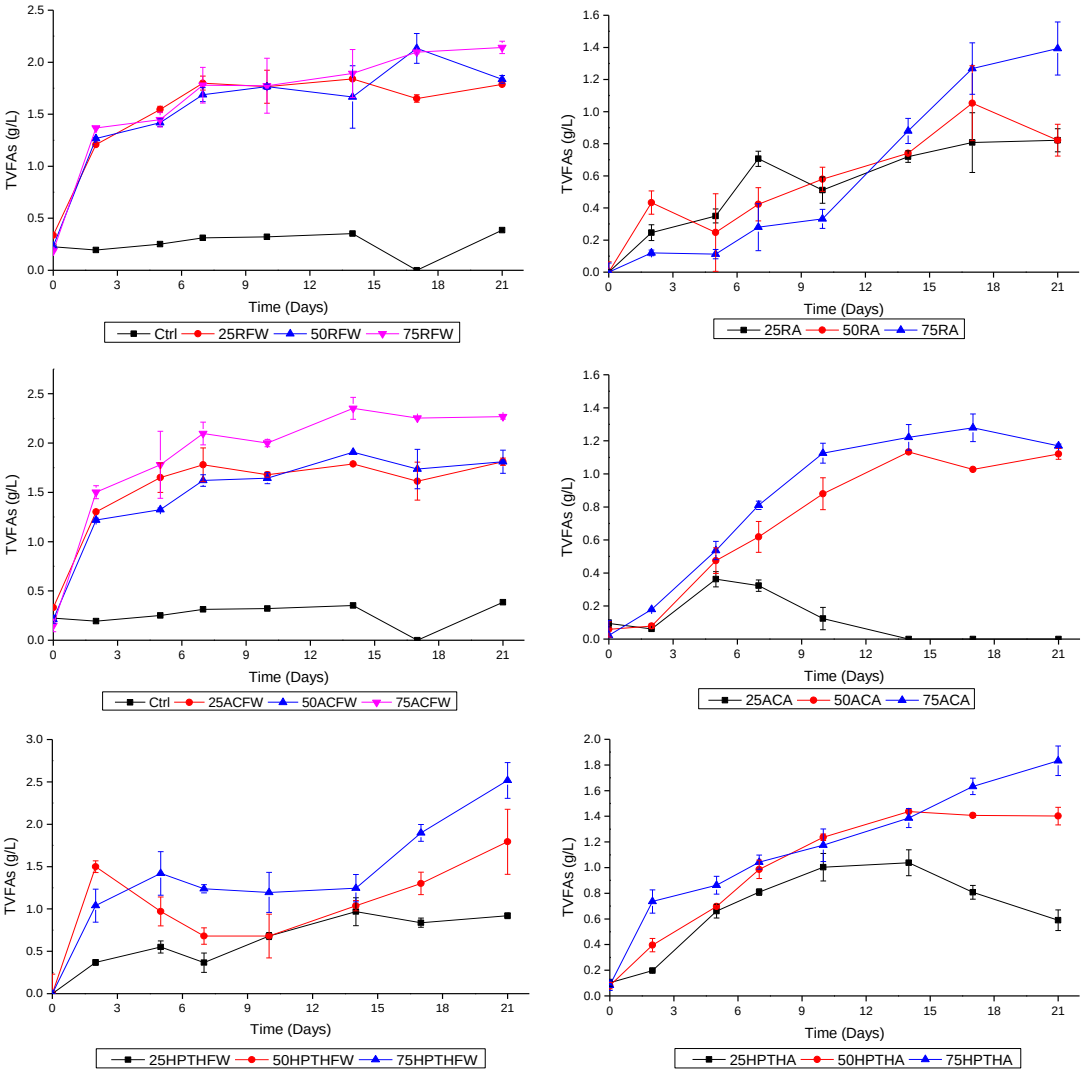

Figure 6.5. VFAs production in acidogenic co-fermentation of WAS and pre-treatments of food waste and microalgae at pH 9.

(*Key: 25= 25%, 50= 50%, 75= 75%; WAS= Waste Activated Sludge; RFW= Raw Food Waste; ACFW= Autoclaved Food Waste; HPTHFW= High Pressure Thermal Hydrolysed Food Waste; RA= Raw Algae, ACA= Autoclaved Algae, HPTHA= High Pressure Thermal Hydrolysed Algae).*

After the second day of my experiments, VFAs increased with much slower rates in FW fermentors, with changes as high as 27.9% of acidification degree from day 2 to day 5 for 75% WAS/ 25% raw food waste. After day 7, the VFA production tended to the stabilisation and showed changes on VFAs concentration lower than 15% and in most of the cases, lower than 10% especially between days 17 and 21. Maximum production of VFAs was presented in fermentors with 75% of raw waste (1.95 g VFAs/L) and 75% of autoclaved food waste (2.12 g VFAs/L). Comparisons between VFAs

production are reported later in this chapter were VFAs yields are reported (Figure 7.6).

On the other hand, food waste treated by HPTH showed different behaviour than autoclaved and raw food waste, presenting an steady increase of VFAs during the first 5 days and then a semi-plateau from day 5 to day 14 and after, experiencing a rapid VFAs production to reach its maximum on day 21 (2.52 g VFAs/L). This late production of VFAs is similar to the studies conducted by Yin et al. (2014), who worked with raw and hydrothermally treated food waste and showed that the treatment at 160ºC for 30 min achieved the highest VFA production on day 15, whereas samples treated at 140ºC presented the highest VFAs concentration from day 3 to day 11. That slow performance on the VFA production could be explained by the difficulty in the conversion of carbohydrates in FW samples that were possibly transformed to amadori or melanoidins compounds (coming from the Maillard reactions), which are recalcitrant to bacterial digestion (Li and Jin, 2015). Thus, it is visualised that for ensuring efficiency in the economics of the process with short solids retention times for VFA production, HPTH at 160ºC for 30 min might not be the best option.

The fermentations of microalgae presented different behaviours according to each individual pre-treatment. Firstly, untreated microalgae rapidly produced VFAs from the mixtures with higher content of WAS (50 and 75%) and steady products generation along the entire time of the fermentation reaching a maximum of 0.821 g VFAs/L on day 21 for both blends. In contrast, mixtures of 25% WAS/75% microalgae exhibited and very low and slow VFAs generation during the first 10 days of the fermentation process and then a very abrupt acidification, reaching values of 1.393 g VFAs/L on day 21. This slow VFAs production correspond with the results from Cho et al. (2015) who used mixtures of different raw microalgae (*Desmodesmus* sp*.,* *Scenedesmus* sp*.,* and *Chlamydomonas* sp*.)* and reported degrees of acidification (TVFAs/TCOD) below 10%. Also, Gruhn et al. (2016) reported a very low increase of VFAs concentration in a CSTR during the first three weeks of the process on mesophilic conditions, probably due to the use of untreated microalgae and the inability of the bacterial consortium in the fermentor to break the cell wall and convert algal organic material into VFAs.

With regards to the fermentations with microalgae treated by conventional autoclaving, the VFA concentration (1.17 g VFAs/L on mixtures of 25% WAS/75% autoclaved microalgae) was surprisingly higher when the content

of microalgae was the highest, which could suggest the positive effect of the co-fermentation of different substrates, even when the C/N ratio was low in mixtures with high microalgae content. These results also agree with the results from fermentations with HPTH pre-treated samples, where the highest VFA production was reached on day 21 (1.832 g VFAs/L) with a blend of 25% WAS/75% microalgae. VFAs production from HPTH pre-treated samples presented a semi-linear trend given by the equation $y=0.0716x+0.4138$ with a R^2= 0.9174 (y = VFAs in g/L; x = time in days). Most of the studies working with microalgae as substrate are focused on the production of biohydrogen or biomethane and hence, it is difficult to compare with results for the production of VFAS from this study.

Comparing the three different treatments, it seems that HPTH could break the microalgae cell, making the organic material more available for its conversion by bacterial consortia, giving as a result, a higher final production of VFAs. Finally, it is clear that fermentations of WAS with FW presented higher production of VFAs in comparison with the experiments using microalgae as substrate, probably due to the higher carbon to nitrogen ratio of the fermentations with food waste, which shows the significant effect of the C/N ratio on the activity of microbial consortia in the fermentors. Furthermore, it is important to note that VFA production during co-fermentations of WAS with FW or microalgae at pH 9 did not show major decreases on the concentration of VFAs, possibly due to the inhibition of methanogenic bacteria at high alkaline pH, but with exceptions only in the mixtures of 75% WAS/25% autoclaved/HPTH-microalgae which could suggest a conversion of VFAs to other by-products such as long-chain fatty acids, solvents or biogas (Yan et al., 2010).

It is also important to mention that VFAs were the main type of products of the fermentation of WAS with FW and microalgae, with minimal generation of ethanol detected in fermentations with concentrations about 19 mg ethanol/L for food waste and 12 mg ethanol/L (≤1% when compared with the VFAs content) for microalgae, which shows that there was not any specific tendency related to the pre-treatment or solid retention time on the production of solvents. Those results are similar to the findings from fermentation of WAS at pH 9 with, concentrations as low as 13 mg/L of ethanol. Studies from Traverso et al. (2000) reported values of ethanol about 7% in the final blend of products after fermentations of fruit and vegetable waste after 6 days of the process. This discrepancy could be endorsed to

the difference on the substrate, whereas the mentioned study used very easy degradable organic material, this project worked with the co-fermentation of WAS and FW with low C/N ratios. High ethanol concentrations were found in fermentations ran by Yu and Fang (2001), who used synthetic dairy wastewater for the production of VFAs, reporting about 60 mg ethanol/L after 4 days. That could be caused by the simple substrate used in the experiment. On the other hand, experiments conducted by Liu et al. (2013a) showed a much higher concentration of ethanol when co-fermenting WAS and food waste for the production of biohydrogen; the maximum content of ethanol in that study was slightly higher than 12% for mixtures of 15% WAS/85% FW, probably because the experiments were run in a two-stage fermentation system, first stage for biohydrogen (acidogenesis) and the second phase for methane production (methanogenic stage). The results of ethanol generation in this study coincide with the results from Zheng (2013), who reported a very low production of ethanol during semi-continuous experiments using mixtures of 66.6% of FW and 33.3% of WAS at pH 5-6; that could be justified due to pH conditions are not in line with optimal figures reported for solventogenesis process (Agler et al., 2011; Grupe and Gottschalk, 1992). These comparisons showed a visible room for the improvement of ethanol and VFA production by changing and proving different HRTs, organic loading rates, mixtures of organic substrates, different C/N ratios and/or reactor configurations.

In order to be able to compare the production of VFAs regarding the substrate added to each reactor and its pre-treatment, the yields on each reactor were calculated in two different units, g VFAs/g TVS added and g VFAs as COD/g COD added (Figure 6.6).

Among the fermentors with food waste as co-substrate, it is evident that fermentors using food waste in co-fermentation with WAS showed respectable yields on day 21, reaching values of 0.370, 0.391 and 0.496 g VFAs/g TVS for raw, autoclaved and HPTH pre-treated food waste, respectively. Also, it is evident the difference between raw/autoclaved and HPTH pre-treated food waste mixtures on VFA production during the entire process. Fermentations with untreated and autoclaved food waste substrates showed a quick increase on the VFA content, especially during the first seven days of the process, reaching average yields of 0.346 and 0.360 g VFAs/g TVS for mixtures of 50% WAS/50% untreated food waste

and 25% WAS/75% autoclaved food waste, whereas HPTH-food waste mixtures reached a value of 0.231 g VFAs/g TVS which is 35% lower in comparison with the first two pre-treatments.

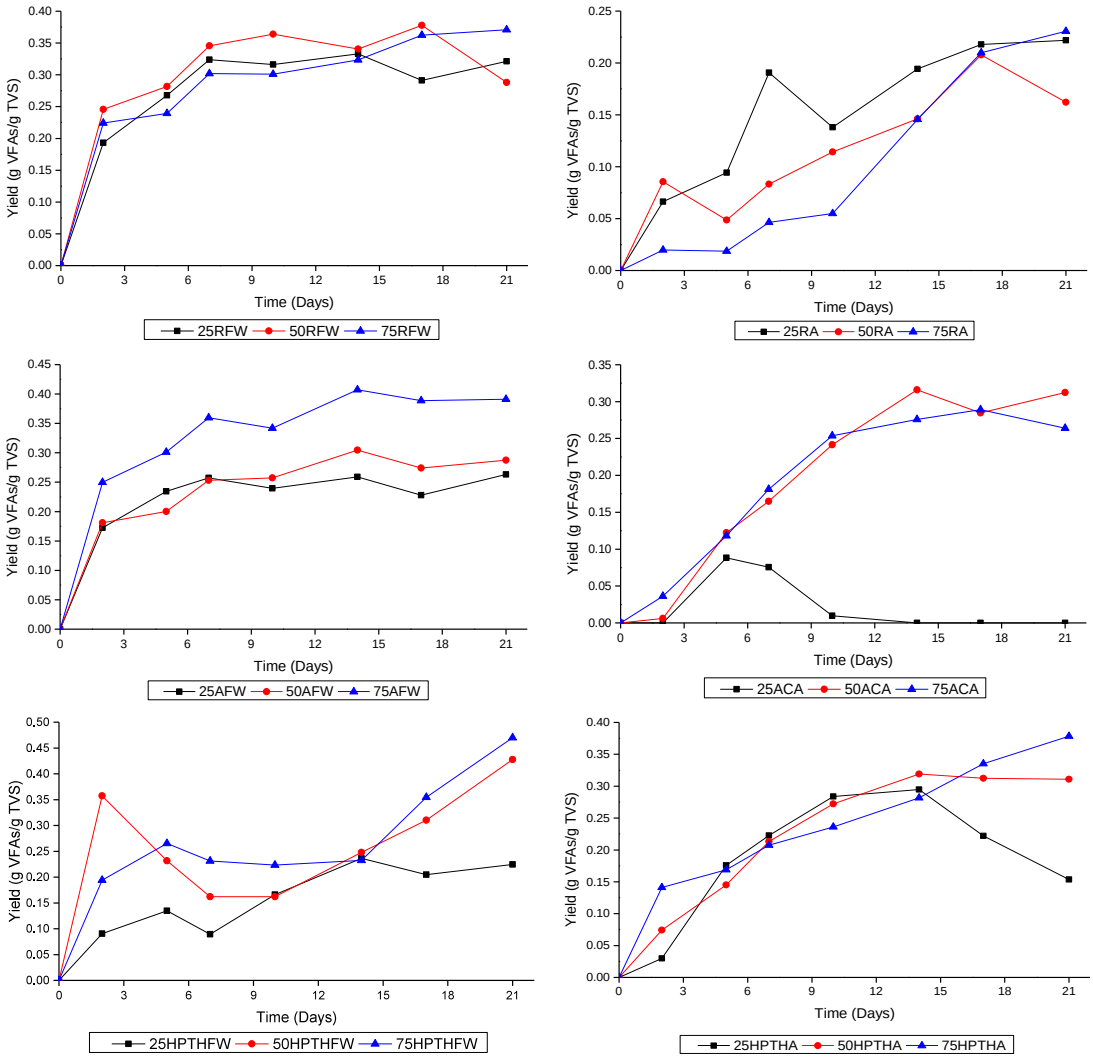

Figure 6.6. Average VFAs yields from mixed acidogenic co-fermentation of HPTH-WAS with FW and/or microalgae at pH 9.

(*Key: 25= 25%, 50= 50%, 75= 75%; WAS= Waste Activated Sludge; RFW= Raw Food Waste; ACFW= Autoclaved Food Waste; HPTHFW= High Pressure Thermal Hydrolysed Food Waste; RA= Raw Algae, ACA= Autoclaved Algae, HPTHA= High Pressure Thermal Hydrolysed Algae).*

After day seven, fermentations with raw and autoclaved food waste presented a semi-plateau on VFA production, which is supported by the maximum increase of about 12% to 19% on the VFAs content respectively, with regards to the concentration in its correspondent previous day. In

contrast, HPTH-food waste fermentations showed a slower increase on VFA concentration during the first five days, followed by a plateau from day 5 to day 14 and finally, a rapid VFA production to reach its maximum on day 21. This behaviour agrees with the findings reported by Li and Jin (2015), who found that pre-treatment processes at high temperature and pressure could convert carbohydrates and proteins in food waste into Amadori compounds via the Maillard reaction, making the substrate more difficult to be fermented and converted to VFAs or to other by-products by the action of anaerobic bacteria. Despite the presence of those recalcitrant compounds, HPTH mixtures with contents of 50 and 75% FW reached similar final VFA yields (0.428 and 0.496 g VFAs/g TVS), which suggests that with high FW proportions and hence higher C/N ratios, the pre-treatment process could induce to a formation of slow degradable compounds causing a late conversion of food waste components into VFAs. It is important to mention that reactors with mixtures of 25% WAS/75% HPTH-food waste did not reach an asymptote for the resulting VFA yields, which suggests that the remaining organic material is still suitable for further resource recovery via the carboxylate platform or conventional anaerobic digestion.

Studies carried out by Dinsdale et al. (2000) showed low VFA yields (0.09 g VFAs/ g TVS added) when working in a two-stage anaerobic co-fermentation of WAS and untreated fruit/vegetable waste with a SRT of 3-4 days in mixtures of 75% WAS/25% FW (TVS content). These results are evidently lower when compared with the co-fermentations in the current project, which reached yields 4 times higher (0.268 g VFAs/ g TVS) for mixtures of 75% WAS/25% untreated FW with HRT of 5 days. This discrepancy in yields could be attributed to a single stage reactor configuration used in the current project and the long HRT employed. Other authors reported VFA yields based on its COD equivalent with respect to the TVS or VSS in the broth. That is the case for Feng et al. (2009), who reported a VFA yield of 0.520 g COD/g VSS after 8 days of fermentation with WAS and rice, which is similar to the values encountered in this study which were 0.511 g COD/g TVS for the mixture of 25% WAS/75% raw food waste and 0.580 g COD/g TVS for the blend of 25% WAS/75% autoclaved food waste. That clearly shows that the digestion of WAS could be benefited from the addition of other substrates with higher content of carbohydrates and easy degradable compounds such as the ones present in food waste.

On the other side, den Boer et al. (2016) reported high yields for VFAs (0.276 VFAs /g TVS) and for ethanol (0.353 g ethanol/g TVS) when working with kitchen waste and potato peels in fed-batch reactors operated at 47 and 72 h, respectively. Parawira et al. (2004) reported a yield of 0.260 g VFAs/ g TS when working with potato waste in a leach-bed reactor with a SRT of 300h (12.5 d). These differences could be attributed to differences in reactor configurations, as results are reported when the system reached pseudo-steady state conditions in configurations for fed-batch and leach-bed reactors, respectively. Works carried out by Hong and Haiyun (2010) and Chen et al. (2013) also report high VFA yields of 0.39 g VFAs/g VSS with a HRT of 8.92 days, an OLR of 8.31 g VSS/L d, and pH 6.99; and 0.692 g COD/g TVS using WAS and kitchen waste with pH 8, C/N ratio 22, temperature 37ºC and fermentation time 6 d, being both optimal conditions obtained from a response surface model and higher carbon-to-nitrogen ratio which can be the reasons why these studies showed a higher yield for VFA production than the present study. Also Tang et al. (2017) reported respectable VFA yields with values as high as 0.375 g VFAs/g TVS when using anaerobic activated sludge as inoculum, pH 6, 7 days of fermentation and untreated food waste as the main substrate. Results from those studies showed that VFA yields from the co-fermentation of WAS and food waste found in this project showed an acceptable production of VFAs and that the addition of food waste and the increase on the carbon-to-nitrogen ratio, benefited the action of the bacterial consortium.

Yields for VFA production from microalgae reactors showed different behaviours; for example, raw algae presented lower yields than autoclaved or HPTH-microalgae. Fermentors using raw microalgae showed a steady increase on VFA yields, stabilising on day 17 and 21. Yields from raw microalgae fermentors presented a non-synergistic effect for the co-fermentation with WAS during the entire time of the process as fermentation with higher VFA yields were observed when the content of microalgae in the mixture was low, reaching a maximum of 0.221 g VFAs/g TVS in the blend 75% WAS/25% microalgae on day 21, whereas mixtures with 50 and 75% of microalgae reached 0.162 and 0.230 g VFAs/g TVS, respectively. These results are similar to the values reported by Zhao et al. (2016) with a VFA yield of 0.237 g VFAs/ g TVS fed in a anaerobic sequencing batch reactor, working with *Scenedesmus dimorphus,* 8 d of HRT, an OLR of 3.96 g/L·d and 0.433 g VFAs/ g TVS with a 16 d of HRT. The discrepancy from the

results in both studies could be attributed to the different type of microalgae used as *Scenedesmus dimorphus* has proven to achieve higher yields on biomethane production versus *Chlorella vulgaris* (Frigon et al., 2013; Zhao et al., 2014) or endorsed to the configuration of the reactor used, as the current study worked in batch reactors. Studies carried out by Gruhn et al. (2016) reported a VFA yield of 0.171 g VFAs as COD/g TVS in experiments using untreated *Scenedesmus sp.-AMDD* in mono-substrate, mesophilic conditions, pH 5, semi-CSTR and 10 days of incubation, which shows that the results from the current study were higher probably due to the type of microalgae used, or the usage of a pH that could potentially inhibit the acidogenic bacteria in the inoculum. Also, Cho et al. (2015) carried out fermentations with *Desmodesmus sp., Scenedesmus sp. and Chlamydomonas sp.*, under batch conditions, 13 days of SRT, in mesophilic and thermophilic conditions with yields of 0.10 g and 0.34 g VFAs/g TVS; the results from mesophilic conditions are clearly lower than the results obtained in the current study, but the yields under thermophilic conditions were higher probably due to the high temperature and higher hydrolityc activity from the inoculum. Finally, Jung et al. (2015) reported high VFAs yields (0.5 g VFAs/g TVS) in studies with *Saccharina japonica*, under batch conditions, OLR of 3.5 g of substrate with β-cyclodextrin as inhibitor of methanogenic bacteria and C/N ratio of 24.54. The discrepancy between the studies cited and the current work seems to be the usae of a response surface methodology and the high C/N ratio in the study with *Saccharina japonica*, which could benefit the attack of acidogenic bacteria.

On the other hand, the fermentations with autoclaved and HPTH-microalgae presented similar trends on VFA yields along the experimental timeframe, which agrees with the increases in COD solubility caused by pre-treatment processes and microbial hydrolysis inside the fermentor. The highest VFA yields were 0.312 and 0.264 g VFAs/g TVS for the mixtures of 50 and 75% of autoclaved microalgae respectively; and, 0.319 and 0.378 g VFAs/g TVS on days 14 and 21 for the blends of 50 and 75% of HPTH-microalgae respectively. These results show that despite of the decrease in the C/N ratio in the mixtures with high content of HPTH-microalgae, the co-fermentation of WAS with the pre-treated organic substrate could benefit the action of the acidogenic bacteria giving as a result, similar yields in all the mixtures tested.

This results are comparable with the outcomes from Suresh et al. (2013), who reported a VFA yield of 0.27 g VFAs/g TVS when working with *Ettlia sp.*, previously treated by alkali-sonication; methanogenic bacteria was also inhibited by adding iodoform (0.016 g), batch experiments conducted with an organic loading rate of 10 g TS/L and 7 days of fermentation. Studies from Yang et al. (2011) presented yields of 0.14 g VFAs/g TVS when working with *Scenedesmus sp.* treated by thermo-alkali process in mesophilic conditions with initial pH 6.5 in repeated batch cultivation. Results from those studies are evidently lower than the outcomes from this project, with differences between 1.4 to 2.7-fold when compared with the VFA yields for 75%HPTH-microalgae mixtures, which evidences the importance of the co-fermentation and the pre-treatment of microalgae in the production of VFAs in mixtures of organic material with low C/N ratio.

Statistical analyses were also conducted, firstly to determine the best HRT for each fermentation with both wastes and its pre-treatments using paired-samples t-tests and comparing the highest yield versus the second, third and/or fourth highest yields of each specific reactor, with confidence intervals (CI) of 95% and finally selecting the highest yields with the shortest HRT. Results of these tests are presented in Table 6.4 and the extended results are shown Table A.1.

Values of p higher than 0.05 in Table 6.4, represent that samples are not statistically significant different, whereas the opposite, $p \leq 0.05$, denotes a difference statistically significant among the two samples/results analysed. This analysis helped to determine the best HRT (SRT) by comparing the yields at different times on each reactor and finding if the amount of VFAs per gram of substrate was statistically different or not. The statistical analysis and the determination of the best HRT for mixtures of food waste and microalgae and its pre-treatments did not present any tendency that could help to define an average HRT for an specific substrate and/or pre-treatment in all its proportions in the mixtures, but is clear that increasing the percentage of the co-substrate (FW or microalgae), made the process longer with examples such as the results from autoclaved microalgae, with 5 d, 10 d and 14 d of HRT when algae content was 25, 50 and 75% respectively. This outcome might be explained due to the poor adaptation of the inoculum to each co-substrate.

Table 6.4. Paired samples t-test results of the average VFAs yields from mixed acidogenic co-fermentation of HPTH-WAS with FW and/or microalgae at pH 9.

FOOD WASTE	Best HRT	MICROALGAE	Best HRT
25RFW	7 d	25RA	7 d
50RFW	17 d	50RA	17 d
75RFW	14 d	75RA	17 d
25ACFW	5 d	25ACA	5 d
50ACFW	14 d	50ACA	10 d
75ACFW	14 d	75ACA	14 d
25HPTHFW	14 d	25HPTHA	10 d
50HPTHFW	17 d	50HPTHA	14 d
75HPTHFW	21 d	75HPTHA	21 d

Following with the statistical analysis, it is important to determine which fermentations of substrates and its mixtures report the highest yield with the lowest HRT. For that purpose, t-tests for independent samples with confidence intervals (CI) of 95% were run using the yields of the best HRT on each substrate and its blends. Again, values of $p \geq 0.05$ represent that samples are not statistically significant whilst p lower than 0.5, represents a significant difference among analysed samples. From Table 6.5 it can be seen that the mixtures with the highest yields in both substrates (letters in bold) presented differences statistically significant in all cases compared, except with regards to the comparison between the 50 vs 75% of untreated food waste (day 17 and day 14 respectively) and 50 vs 75% of autoclaved microalgae (day 10 and day 14 respectively). These results show contrary performances, whereas in the formerly example, higher FW content showed better VFAs yields in shortest time, the autoclaved microalgae presented higher yields with low microalgae content at a shorter HRT. This behaviour can be explained firstly by the higher C/N ratio presented in the fermentations with 75% of FW which allowed the bacteria to convert the high easy degradable organic material from the raw food waste. On the other side, the low content of non-complex compounds in the mixture with 75% of

autoclaved microalgae delayed the action of the acidogenic bacteria, reaching its highest yield until day 14.

Table 6.5. Independent samples t-test of the average VFAs yields from mixed acidogenic co-fermentation of HPTH-WAS with FW and/or microalgae at pH 9.

FOOD WASTE	t	df	p	**MICROALGAE**	t	df	p
25RFW_d7 vs 50RFW_d17	-4.759	4	0.009	25RA_d7 vs 50RA_d17	-4.094	4	0.015
25RFW_d7 vs **75RFW_14**	-5.865	4	0.004	25RA_d7 vs **75RA_d17**	-15.806	4	0.000
50RFW_d17 vs **75RFW_14**	-2.666	4	0.056	50RA_d17vs **75RA_d17**	-6.234	4	0.003
25ACFW_d5 vs 50ACFW_d14	-4.576	4	0.010	25ACA_d5 vs **50ACA_d10**	-13.750	4	0.000
25ACFW_d5 vs **75ACFW_d14**	-10.413	4	0.000	25ACA_d5 vs 75ACA_14	-16.901	4	0.000
50ACFW_d14 vs **75ACFW_d14**	-10.826	4	0.000	**50ACA_10** vs 75ACA_14	1.444	4	0.222
25HPTHFW_d14 vs 50HPTHFW_d17	-7.585	4	0.002	25HPTHA_10 vs 50HPTHA_14	-1.108	4	0.330
25HPTHFW_d14 vs **75HPTHFW_d21**	-11.284	4	0.000	25HPTHA_10 vs **75HPTHA_21**	-4.667	4	0.009
50HPTHFW_d17 vs **75HPTHFW_d21**	-4.890	4	0.008	50HPTHA_14 vs **75HPTHA_21**	-4.822	4	0.009
50RFW_d17 vs 75ACFW_d14	-0.104	4	0.923	75RA_d17 vs 50ACA_d10	3.0736	4	0.020
50RFW_d17 vs 75HPTHFW_d21	-3.564	4	0.023	75RA_d17 vs 75HPTHA_d21	-2.129	4	0.100
75ACFW_d14 vs 75HPTHFW_d21	-4.062	4	0.015	50ACA_d10 vs 75HPTHA_d21	-6.212	4	0.003
50RFW_d17 vs 75RA_d17	3.801	4	0.019	**75HPTHFW_d21 vs 75RA_d17**	7.176	4	0.002

Letters in bold notes the days with the highest yields.
Key: 25RFW_d14 denotes the mixture of 25% raw food waste on day 14, thus, 25HPTHFW_day21 is the mixture of 25% HPTH-FW on day 21.

It is also important to mention that when comparing the yields of fermentations using mixtures of 50% WAS/50% raw food waste vs 25% WAS/75% autoclaved food waste versus 25% WAS/75% HPTH-FW, the pre-treatment with HPTH appeared to have an effect on the final production of VFAs, reaching values slightly higher than 0.496 g TVFAs/ g TVS even when the C/N ratio was similar in the mentioned mixtures.

Comparisons among different pre-treatments of the each substrate and also a further comparison between the highest yields resulting from both substrates and its pre-treatments were performed, showing that yields of untreated and autoclaved food waste fermentations were not different statistically but these both were different significantly to the yields of fermentations of 75% FW treated by HPTH, which can be seen as the mixture of FW which presented the maximum VFAs yield. On the other side, the statistical assessment of the VFAs yields of fermentations with microalgae as co-substrate showed that processes with blends with 75% raw algae at day 17 and 75% HPTH-microalgae at 21 days showed no difference statistically significant which suggests that despite of the high temperatures and pressure applied for the pre-treatment of the *Chlorella vulgaris*, the fermentations with raw algae were able to achieved similar yields than experiments using HPTH-microalgae.

Finally, it is important to compare the results from all the fermentations ran in this project in order to find the best conditions encountered and the advantages and disadvantages that the carboxylate platform could face if operated in a WWTP. Table 6.6 shows the yields obtained in fermentations of WAS with iodoform used as methanogenic inhibitor, using different pH levels and also using co-fermentation with food waste or microalgae and its pre-treated samples. With this information is clear that the lowest yields were reached in fermentations of WAS at pH 6 for 21 days and fermentations using 3 mg CHI_3/g VSS for 10 days. To determine which fermentations were the most prolific in terms of grams of VFAs produced per gram of $TVS_{substrate}$, independent t-tests were performed finding that only yields from fermentations AF3_d10 vs AF6_d21 and, AFpH9_d21 vs 50RFW_d17 were statistically similar between each other (p=0.14 and 0.06, respectively). The maximum yield was determined to be reached by fermentations with 25% WAS/75% HPTH-FW with SRT of 21 days, followed by fermentations of WAS with 21 of SRT and 50% untreated food waste on day 17. In terms of percentage, yields on the reactor using 75% HPTH-food waste (HRT=21 d)

presented a value 20% higher than the fermentations with WAS at pH 9 (HRT=21 d) and 32% with regards to the fermentations of untreated food waste in mixtures of 50% WAS/50% FW (HRT=17 d).

Table 6.6. Highest yields of acidogenic fermentation of WAS, FW and microalgae and its pre-treatments.

Yields (g TVFAs/g TVS)						
AF3_d10	AF6_d21	AFpH6_d21	AFpH9_d21	50RFW_d17	75HPTHFW_d21	75RA_d17
0.1958	0.2128	0.1931	0.4206	0.4022	0.5190	0.3380
0.2207	0.2636	0.1827	0.4097	0.3540	0.4730	0.2760
0.2082	0.2382	0.1879	0.4152	0.3781	0.4960	0.3070

Key: AF3_d10= WAS with 3 mg CHI_3/ g VSS on day 10; AF6_d21= WAS with 3 mg CHI_3/ g VSS on day 21; AFpH6_d21= WAS at pH 6 on day 21; AFpH9_d21= WAS at pH 9 on day 21; 50RFW_d17= 50% Raw food waste on day 17; 75HPTHFW_d21= 75% High pressure hydrothermal hydrolysis food waste on day 21; 75RA_d17= 75% Raw algae on day 21.

With regards to the yields from fermentations of WAS and its co-fermentation with raw microalgae (C/N=5.64-7.31), it is visible that mono-substrate fermentation, with higher C/N ratio (C/N=8.80), presented higher VFAs yields which can be attributed to either the quick action of the inoculum particularly adapted to HPTH-WAS or the higher carbon-to-nitrogen ratio in the process but not to the effect of the co-fermentation. Adewale (2014) discovered that higher algae content (*Chlorella vulgaris*) in anaerobic co-digestion with WAS, which led to a higher carbon-to-nitrogen ratio, showed a final synergistic effect of the co-digestion conducting to a higher CH_4 production (484.57 mL CH_4/g $TVS_{destroyed}$) which agrees with the current research in terms of high C/N ratio conducts to higher generation of products. Studies from Ehimen et al. (2011) found that the best C/N ratio when working with microalgae residues from the biodiesel production for biomethane production was 8.53 with a yield of 0.302 m^3 CH_4/kg TVS and lower yields with C/N ratio of 12.44 showing that very low or very high C/N ratios could lead to a negative effect on the biomethane production.

The main outcome from the statistical analysis is given by the evident effect that the co-fermentation of WAS and the pre-treatment of food waste by

HPTH (C/N ratio=12.72) have on the final production of VFAs in 21 days which agrees with the statements from Edward et al., 2015; Hansen and Antizar Ladislao, 2013 and Schwede et al., 2013, who report increases on products yields when co-digesting WAS with other organic substrates and increasing the carbon-to-nitrogen ratio.

According to the results previously presented, it is apparent the positive effect from the co-fermentation of HPTH-WAS and the pre-treatment of food waste by HPTH as reactors with this substrates reached the highest VFAs production and yields. On the contrary, neither pre-treatment nor co-fermentation of HPTH-WAS with microalgae presented a positive effect on the VFAs yields as all mixtures tested, with treated or untreated microalgae, achieved similar yields.

Further economical and viability analyses are recommended to be run to determine if the energy input in the HPTH process could be recovered after the carboxylate platform process in wastewater treatment works and its potential to substitute the current anaerobic digestion process for the production of biogas.

6.6.2 VFAs composition on the co-fermentation of WAS and substrate pre-treatment of food waste and *Chlorella vulgaris*

As the production and composition of volatile fatty acids is highly predisposed by the composition of substrates and hence, the co-fermentation of different organic material as acetic, proponic and butyric acids can be produced directly from the fermentation of carbohydrates, proteins and lipids whereas iso-valeric and valeric acids are formed by the transformation of proteins (Khan et al., 2016; Wang et al., 2014a; Horiuchi et al., 2002; McInerney, 1988). In this section, the effect on the VFAs pattern by using different substrates was investigated as it is been hypothesised that the acidogenic fermentation of different organic substrates could produce medium/long carbon chains which are easier to extract from the fermentation broth (den Boer et al., 2016).

The effect of the pH in the volatile fatty acids composition is presented in Figure 6.7. Fermentations of untreated and autoclaved food waste presented similar trends on the production of VFAs, with 75.8 and 91.4% of acetic acid at the beginning of the fermentation, decreasing in percentage on day 2 as more propionic acid was produced and finally showing a tendency to slightly

increase to reach values of 70.8 and 77.2% respectively. Few differences were encountered among these reactors, such as the higher production of propionic acid in fermentations with raw food waste, especially on day 2 when it reached 30.8% of the VFAs mixture and then tend to decrease towards the end of the fermentation whereas the autoclaved food waste reached concentrations no higher than 23.2% during the entire fermentation time with a semi-constant concentration from day 2. In both cases, acetic acid was the predominant followed by propionic acid which agrees with the results from Karthikeyan et al. (2016) who reports acetic acid proportions of 95% in the VFAs mixture in fermentations with a reactor solid-liquid separation CSTR, food waste as main substrate, 600 rpm, mesophilic temperature and alkaline pH, suggesting that high pH could cause the acetic acid production. Also, studies from Babel et al. (2004), Chen et al. (2013), Dong et al. (2010a), Henry et al. (1987), Parawira et al. (2004), Traverso et al. (2000), Weimer (2015), presented a fermentation leading to acetic acid production with percentages between 29.9 to 62% in the VFAs content. Also Zhang et al. (2005) presents similar trend on VFAs production, using FW at pH 9, achieving concentrations above 45%.

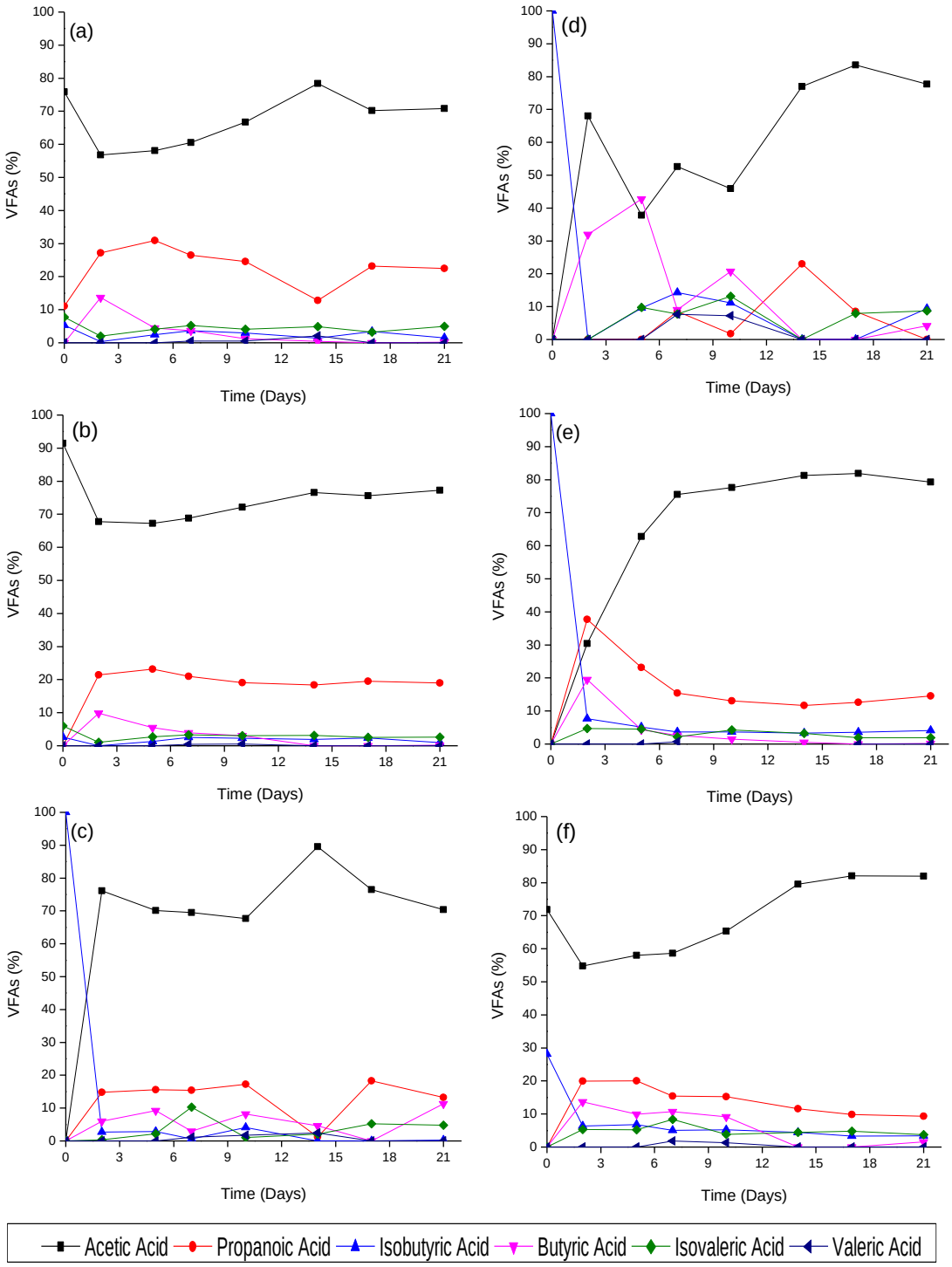

Figure 6.7. VFA profiles in reactors AF75RFW (a), AF75AFW (b), AF75HPTHFW (c), AF75RA (d), AF75RFW (e) and, AF75AFW (f).

(*Key: 25= 25%, 50= 50%, 75= 75%; WAS= Waste Activated Sludge; RFW= Raw Food Waste; ACFW= Autoclaved Food Waste; HPTHFW= High Pressure Thermal Hydrolysed Food Waste; RA= Raw Algae, ACA= Autoclaved Algae, HPTHA= High Pressure Thermal Hydrolysed Algae).*

Contrary to these findings, studies from Yin et al. (2014) showed a production of VFAs towards the butyric acid generation when fermenting hydrothermally treated food waste at different temperatures and pressures for 15 days, suggesting the reaction of lactate to other by-products, specifically to butyric acid as can be seen in equations 7.4 to 7.6, but further investigations to elucidate the metabolic pathways could be investigated. Same metabolic route has been proposed by Kim et al. (2009) which worked with high temperature, acid or alkali pre-treatment of food waste, finding that the lactate produced in the reactor could be converted to butyric acid. Other studies with the same tendency for the production of butyrate are from Yu and Fang (2001), den Boer et al. (2016) and Liu et al. (2013a).

Low production of butyric and propionic acid can be attributed to the poor generation of lactate as is the main precursor for the synthesis of mentioned C3-C4 fatty acids according to the equations 7.2 to 7.6 (Horiuchi et al., 2002; Klijn et al., 1994; Saint-Amans et al., 2001; Thauer et al., 1977b). No long-medium chain fatty acids were detected in concentrations above 10% of the total VFAs mixture which suggest a low content and/or poor conversion of proteins in the organic substrate into valeric and iso-valeric acids (McInerney, 1988). No attempts were made to determine the concentration of proteins and/or carbohydrates in any organic substrate.

$$Lactate + H_2 \rightarrow Propionate + H_2O \quad \text{.....Equation 6.2}$$

$$Lactate + H_2O \rightarrow Acetate + CO_2 + 2H_2 \quad \text{.....Equation 6.3}$$

$$Lactate + 0.4\ Acetate + 0.7H^+ \rightarrow 0.7\ Butyrate + CO_2 + 0.6H_2 + 0.4H_2O \quad \text{..Equation 6.4}$$

$$Lactate + Acetate + H^+ \rightarrow Butyrate + 1.4CO_2 + 0.8H_2 + 0.6H_2O \quad \text{.....Equation 6.5}$$

$$2Lactate + H^+ \rightarrow Butyrate + 2CO_2 + 2H_2 \quad \text{.....Equation 6.6}$$

Analysing the behaviour of the fermentations with HPTH-food waste it can be seen that iso-butyric acid was the most predominant at the beginning of the fermentation which suggests the concentration of that acid could come from the inoculum used. Same behaviour has been recorded in the fermentations with raw algae due to both processes were run simultaneously. After day 2, the content of acetic acid reached a 76.1% of the VFAs mixtures and presenting changes between of ±11.2 to 17.6% until

the end of the process when presented a value of 70.3% of the total VFAs content. Li and Jin (2015) also explained that thermal pre-treatment temperatures have a robust effect on the composition of the VFAs produced, with low temperatures producing more acetic acid during the first 5 days of fermentation whilst on fermentations with FW pre-treated at 160ºC produced acetic acid until the eighth day. These results disagree with the fermentations of food waste in this study as acetic acid was found to be predominant in all the fermentations showing a not significant effect of the temperature of the pre-treatment on the composition and appearance of the VFAs.

Overall all the reactors co-fermenting WAS with food waste presented acetic acid as the main product followed by propionic acid with concentrations majorly around 20% of the VFAs total content which are similar outcomes to the reported by Argelier et al. (1998) who worked with solid food waste in a semi-CSTR with mesophilic conditions and different organic loads (from 2 to 25 kg COD/m^3) and found contents of acetic acid and propionic of about 47.3 and 32.8%, respectively. Also, Cheng et al. (2016) found the same trend on VFAs production, when working in batch co-fermentations of WAS and untreated food waste (mixtures 75%/25, 50%/50 and 25% WAS/75% FW) [C/N ratios 8.6, 11.1 and 15.6 respectively] for the production of biohydrogen at pH 6 and 48 h of SRT, with percentages up to 52.7% for the case of acetic acid and 42.7% for propionic acid in the fermentation with 50% WAS/50% FW. These findings suggest either long HRT (like in this project) or short HRT, could lead to similar products profile which are acetic and propionic acids that could come directly from the fermentation of soluble carbohydrates (Wang et al., 2014).

With regards to the fermentation of HPTH-WAS and microalgae it is visible that main acid in fermentations with untreated and pre-treated microalgae, acetic acid was the predominant along the entire process time, with values as high as 83.5% in fermentations with HPTH algae. Untreated microalgae fermentations showed not conclusive results on the tendency of the composition of VFAs whilst the autoclaved microalgae fermentations presented a rapid increase on the VFAs content during the first seven days of the process and then showing an semi-asymptote (changes no higher than 8%) until the end of the fermentation when it reached a concentration of 79.0% of the total VFA content. The second highest short-chain fatty acid was propionic reaching its highest content on day 2 (37.6%) and reducing

from then until showing a semi-constant content from day 7 until the end of the fermentation. Other acids were found in contents generally lower than 10% of the entire mixture of VFAs. The fermentations of HPTH showed similar VFAs composition to the autoclaved fermentations, with acetic acid increasing from day 2 (54.7%) till the end of the fermentation (81.9%). The increasing on the acetic acid content could be attributed mostly to the high specific production of this acid as the propionic acid concentration in the broth remained constant after day 2 and also to the unlikely conversion of propionic to acetic acid due to the conversion is not favourable energetically (Equation 7.7) (Thauer et al., 1977b).

$$\text{Propionate} + 3H_2O \rightarrow \text{Acetate} + HCO_3^- + 3H_2 + H^+ \quad \text{.....Equation 6.7}$$

Results from Pham et al. (2013) working with fermentations of enzymatic or alkaline pre-treated macroalgae (*L. japonica, P. elliptica* and *E. crinita*) in batch experiments with a SRT of 5 days and iodoform in ethylic solution for the production of VFAs, showed acetic and propionic acids as the main products with ranges between 53-59% and 23-27%, respectively, which elucidate that pre-treatment has a very minor or negligible effect on the VFAs composition. Similarly, Zhao et al. (2016) presented results were acetic acid was predominant in fermentations using *Scenedesmus dimorphus* in mono-substrate process operating an anaerobic sequencing batch reactor with different HRT (4.2, 8, 12 and 16 days), whereas butyric acid was the second most prevalent for all the HRT tested. In mentioned study was visible the positive effect of increasing the HRT on the valeric acid production as the percentage of this acid on the VFAs composition raised from 10.43 to 17.12% on 4.2 to 16 days, respectively. In the case of the current project, long HRT did not presented any increasing on the production of long-medium chain VFAs, possibly as it was run in batch mode. Additionally, Cho et al. (2015) reported high percentages of acetic acid from acidogenic fermentations of mixtures of *Desmodesmus* sp*.*, *Scenedesmus* sp*.,* and *Chlamydomonas* sp*.*, with 85.6% in mesophilic conditions and decreasing to 65.8% in thermophilic operation, which proposes a tendency on the production of long-medium chain fatty acids when using high operational temperatures and that VFAs produced from the organic compounds of microalgae are mainly simple products such as acetic and propionic acids.

Contrary to these findings, Yun et al. (2014) described a butyric acid pathway when submitting enzymatic pre-treated *Chlorella vulgaris* to acidogenic fermentation for the production of biohydrogen, with organic productions between 53 to 61% of the total VFAs content. This behaviour was endorsed to the oxidation of lactate to butyrate by *Clostridium acetobutylicum* coupled with the reduction of acetate to make the reaction energetically favourable as stated in equation 7.4.

It is also important to mention that in mixed acid fermentations at pH 9, all the reactors with WAS or co-fermentation with food waste or microalgae (untreated or pre-treated), the main product was mainly acetic acid followed by propionic acid which agrees with the thesis of the conversion of carbohydrates and/or proteins predominantly to acetate by acetyl-CoA as intermediate (Shanmugam and Horan, 2009; Temudo et al., 2007) and also that there was not major difference on the VFAs composition regardless of the organic material or co-fermentation used. Overall, it can be said that there was no impact of the co-fermentation or the feedstock pre-treatment on the speciation of the VFAs produced in the mixed acidogenic fermentors.

Another factor under consideration is the degree of acidification as it shows the amount of soluble COD that can be converted to acidic products (Maharaj and Elefsiniotis, 2001). Figure 6.8 presents the conversion of SCOD into VFAs for the co-fermentations which reached the highest degree of acidification for FW or microalgae.

Figure 6.8. COD mass balance of the acidogenic co-fermentation of HPTH-WAS with FW and/or microalgae at pH 9.

(*Key: 75= 75%; RFW= Raw Food Waste; ACFW= Autoclaved Food Waste; HPTHFW= High Pressure Thermal Hydrolysed Food Waste; RA= Raw Algae, ACA= Autoclaved Algae, HPTHA= High Pressure Thermal Hydrolysed Algae).*

The highest conversions of SCOD to VFAs were reached in reactors with untreated food waste for the fermentations with FW and with HPTH algae in co-fermentations with microalgae which shows two different behaviours; although the thermal treatment increased the overall initial soluble COD on both types of substrates, the major conversion of SCOD to VFAs (acidification degree=0.524 g VFAs as COD/SCOD and 0.191 g VFAs as COD/TCOD) was detected in the reactor with mixtures of 25% WAS/75% raw food waste on day 21 as it is mainly composed of simple carbohydrates and proteins that are easy degradable by the action of the inoculum. The quick biodegradability of raw food waste is also sustained by the high conversion of soluble organic content inside the reactor to biogas by reaching a 34.5% conversion with regards to the COD inlet (biogas composition was not determined). These outcomes present the possibility of the co-fermentation of raw food waste and WAS by increasing the carbon-to-nitrogen ratio and thus, the production of biohydrogen, VFAs and biomethane.

Reactors using mixtures of thermally treated food waste presented similar COD conversions with acidification degrees of 0.484 and 0.358 g VFAs as COD/SCOD (0.166 and 0.203 g $VFAs_{COD}$/TCOD) with TCOD losses of 13.21 and 13.32% for samples of autoclaved and HPTH-food waste respectively. These findings show that physical and chemical properties of the substrate could be changed by the effect of high pressure and temperature, making the organic material possibly more difficult for the microbial activity and the further conversion into biogas, as all fermentors were operated with same temperature and pH conditions. Although the fermentor with mixtures of HPTH-food waste presented a high initial SCOD content, the inoculum was not able to convert all the soluble organic material from the substrate after 21 days of fermentation which indicates that some organic compounds formed after HPTH were not easy to convert into VFAs or biogas, such as recalcitrant compounds from Maillard's reaction (Li and Jin, 2015) and that more products can be formed when using longer HRT or by supplementary processing by conventional anaerobic digestion.

Degrees of acidification from all fermentations with food waste, reached similar values to the results from Kim et al. (2013) who reports values between 0.011 to 0.199 g VFAs as COD/TCOD for the co-fermentation of untreated food waste and pre-treated WAS (75% WAS/25% FW), reaching its highest value in samples with WAS treated by ultrasound and alkaline

process; and by Kim et al. (2006) who found VFAs/SCOD ratios up to 0.428 g VFAs as COD/SCOD in acidogenic fermentation of enzymatic pre-treated food waste in mono-substrate with short HRT of 10 days. Other investigations with similar degrees of acidification in fermentations with food waste are from Parawira et al. (2004) (≅ 0.4 g VFAs as COD/TCOD, mono-substrate, raw solid potato waste, HRT 12.5 days) and Traverso et al. (2000) (≅ 0.5 g VFAs as COD/TCOD, mono-substrate, untreated vegetable and fruits waste, HRT 10 days). Contrary to these findings, studies from Wu et al. (2016) using a response surface methodology, report a high effect of the co-fermentation of WAS or food waste in mono-substrate fermentations reached only 0.161 and 0.252 g VFAs as COD/TCOD for WAS and untreated food waste, respectively, and 0.834 when co-fermenting untreated food waste and excess sludge. This high degree of acidification could be attributed to the response surface methodology used in mentioned research. Best HRT from the current study is clearly higher than the mentioned investigations which suggest that more work is needed to decrease the solid retention time and optimise the fermentation system.

The degree of acidification in fermentors with microalgae showed lower degree of acidification than fermentors with FW, with values of 0.467, 0.355 and 0.432 g VFAs as COD/SCOD (0.117, 0.096 and 0.145 g VFAs as COD/TCOD) for raw, autoclaved and HPTH-microalgae on day 21 with mixtures of 25% WAS/75% microalgae, which shows that the pre-treatment with high pressure and temperature presented a low decrease (8.1%) on the conversion of VFAs to SCOD when compared with the untreated microalgae. With respect to the conversion of COD into biogas, surprisingly, the fermentations with raw and autoclaved algae presented similar results (6.7-7.7%) on the COD losses while reactors with HPTH-microalgae showed a small destruction of COD into biogas, reaching a value of just 1.99%.

Similar results are reported in studies from Cho et al. (2015) who ran fermentations with mono-substrate using a mixture of untreated *Desmodesmus sp., Scenedesmus sp., and Chlamydomonas sp.* finding degrees of acidification about 0.137 for fermentations at 35ºC, 0.20 at 45ºC and 0.5 in processes at 55ºC in batch mode, which suggest that operations in thermophilic temperatures can increase the conversion of TCOD into VFAs, up to 72.5%. Seo et al. (2016) state also an increase of 54.3% on the degree of acidification (VFAs/SCOD) when rising the temperature from 35ºC to 55ºC in fermentations with lipid-extracted *Ettlia sp.* Opposing to the

discoveries in this project, Gruhn et al. (2016) who used raw *Scenedesmus sp.*-AMDD in batch for 10 days, testing four different inocula (granular biomass, activated sludge, manure and blend of all inocula) finding that all fermentations presented similar degrees of acidification ranging from 0.322 to 0.362 g VFAs as COD/g SCOD which proposes that acidification degree is not related to the adaptation the inoculum to the substrate. These results show that, with the conditions used in these experiments, the pre-treatments and/or co-fermentation of microalgae did not present a positive effect on the degree of acidification and the yields on the production of VFAs with regards to the results from fermentations with untreated microalgae, which makes the thermal pre-treatment not energetically feasible and not beneficial on the generation of final bioproducts.

6.7 Stoichiometric methane potential (SMP) and Biochemical methane potential (BMP) of the co-fermentation of WAS with food waste and *Chlorella vulgaris*

SMP and BMP were calculated using the formula proposed by Buswell and Mueller (1952) in order to assess the final amount of biogas that could be recovered from the acidogenic fermentation of WAS (Table 6.7), food waste and microalgae, its pre-treatments and co-fermentations and also, to determine the feasibility of the development of an acidogenic reactor in WWTPs and thus, contribute to the creation of biorefinery from WAS.

Firstly, it is clear that all blends in fermentations with untreated food waste showed similar efficiencies on the acidogenic fermentation which suggests that despite the amount of raw FW added to the co-fermentation, the efficiency could have reached a maxima that is about 35% percent of the calculated stoichiometric methane potential. Similar behaviour was presented in fermentations with mixtures of WAS/Autoclaved food waste, were only a third of the SMP was accomplished on the acidogenic fermentation tests, which suggest that conventional autoclaving did not present any increase or energetic advantages with regards to the VFAs production when compared with the fermentations with raw food waste. As only a third of the SMP was obtained in the experimental results of fermentations either with raw and autoclaved food waste, it is envisaged that the remaining organic material in the broth could be converted to biogas in a further anaerobic digestion process. These performances could also indicate

the low or nil effect of the increasing of the C/N ratio in mixed acid fermentation of food waste when values were below 15. Opposite to these results, HPTH-food waste fermentations showed a higher efficiency with values above 40% for mixtures with 50 and 75% of HPTHFW, which although are perceived as satisfactory results, it is lower than the values reached when working in mono-substrate of WAS at pH 9. This behaviour could be explained due to the short period of time the inoculum was adapted to the HPTH-food waste as it was taken from an anaerobic digester in a WWTP.

Table 6.7. Methane potential of the co-fermentation of WAS and substrate pre-treatment of food waste and *Chlorella vulgaris* at pH 9.

Food waste	**Methane potential (STP litre CH_4/g TVS)**			**Chlorella vulgaris**	**Methane potential (STP litre CH_4/g TVS)**		
	SMP	BMP	Efficiency (%)		SMP	BMP	Efficiency (%)
25RFW	0.512	0.175	34.2	25RA	0.515	0.130	25.2
50RFW	0.562	0.135	24.0	50RA	0.568	0.130	22.9
75RFW	0.613	0.222	36.2	75RA	0.622	0.175	28.1
25ACFW	0.509	0.178	35.0	25ACA	0.513	0.022	4.3
50ACFW	0.556	0.178	32.0	50ACA	0.564	0.121	21.5
75ACFW	0.604	0.204	33.8	75ACA	0.616	0.128	20.8
25HPTHFW	0.514	0.127	24.7	25HPTHA	0.524	0.079	15.1
50HPTHFW	0.567	0.230	**40.6**	50HPTHA	0.586	0.150	25.6
75HPTHFW	0.620	0.269	**43.4**	75HPTHA	0.649	0.200	**30.8**
100RFW	0.663	-		100RA	0.675	-	
100ACFW	0.651	-		100ACA	0.667	-	
100HPTHFW	0.672	-		100HPTHA	0.712	-	
100WAS AF	0.461	0.224	**48.6**	100WAS AD (BMP)	0.461	0.079	17.1

Surprisingly, the average BMP tests of mixtures of untreated microalgae resulted in a 25.4% efficiency of the SMP which is higher than the average results of experiments ran with autoclaved microalgae, which agrees with the lower solubilisation of the COD in the autoclaved algae, confirming a low hydrolysis and acidification in those reactors. Lastly, the behaviour in the fermentors with HPTH-microalgae presented an increase in the SMP efficiency when augmenting the amount of microalgae in the mixture which shows that despite of having a lower C/N ratio, the solubilisation of the organic material in the sample, produced by the HPTH treatment, could improve the final VFAs productivity.

Fermentors with WAS and WAS plus HPTH-FW reached VFAs productions which is 2.8-fold and 3.4-fold higher than the BMP reactor (AD experiment). Although respectable concentrations of VFAs were produced in all co-fermentations of WAS, it is clear that fermentations with WAS in mono-substrate could produce almost a 50% of the SMP via the carboxylate platform and this behaviour could be attributed to the inoculum used as it was taken from the same WWTP than the WAS was sampled.

As biomethane has been the main target product for energy and resource recovery in the past years, is important to compare the production of VFAs and the carboxylate platform with the anaerobic digestion of WAS, FW and microalgae (Table 6.8). It is clear that the BMP results of FW and microalgae are extensive and different results are mainly originated from the different type of organic material and the pre-treatment used. For example, results from HPTH-FW are up to 44.24% higher than other studies but also could be as lower as 66.33% when compared with co-fermentations of food waste and WAS, whereas the average yield was only 23.7% higher than the HPTH-FW yields in this project. With regards to the microalgae fermentation, yields were higher up to 53.50 and lower to 35.92%, with an average of just 26.67% higher than the yield from this study. Although BMP tests presented efficiencies lower than 50% with respect to the SMP, other valuable molecules such as formic, caproic, lactic and succinic acids, which were not quantified, should be considered as acidogenic fermentation products (Bastidas-Oyanedel et al., 2015; Morgan-Sagastume et al., 2011).

The acidogenic fermentations in mono-substrate and co-fermentation in this project seem to have respectable yields in terms of VFAs as CH_4 with the main advantage of being the first stage of energy recovery and could be

followed by a VFAs recovery and the submission of the remaining organic material for biomethane production.

Table 6.8. Researchs on biomethane potential test of food waste or microalgae.

Reference	Type of reactor	BMP (L CH_4/g TVS)	Other info	HRT (d)	T (ºC)	Substrate
(D'Addario et al., 1993)	Batch	0.4		12	35	OFMSW
Liu et al. (2012b)	Batch	0.157		15	35	WAS
	Batch	0.568		15	35	Kitchen waste
López Torres and Espinosa Lloréns (2008)	Batch Fill-and-draw	0.15		19	-	OFMSW with chemical hydrolysis
Heo et al. (2004)	Batch	0.159	0.489	40	35	WAS
		0.489	0.542	40	35	Food waste
Maya-Altamira et al. (2008)	Batch	0.36		60	35	Vegetable wastes
		0.23			35	Vegetables fats and oils
Sosnowski et al. (2003)	Batch	0.198			56	1 WAS:2 FW
Kim et al. (2003a)	Batch	0.215			35	50 WAS/50 FW
Cheng et al. (2016)	Two-stage	0.264		30	35	1 WAS:3 FW
Xie et al. (2017)	Batch	0.652		13	35	FW
		0.799				WAS+FW
Liu et al. (2013a)	Batch	0.321		41	37	FW
		0.333				60 WAS/40 FW
		0.350				46 WAS/54 FW
Ehimen et al. (2011)	Batch	0.302	C/N 8.53	15	35	Chlorella residue
		0.295	C/N 12.44			
Suresh et al. (2013)	Batch	0.176	C/N 9.5	117	35	Autoclaved *Ettlia sp* residue
Frigon et al. (2013)	Batch	0.361		34	35	*Chlorella vulgaris*
		0.397				*Scenedesmus dimorphus*
		0.283				*Chlorella sorokiniana*
		0.258				*Scenedesmus sp.-PN2*
Edward et al. (2015)	Batch	0.093	SMP 0.335	32	35	Fresh *Laminaria digitata*
		0.105	SMP 0.334			Fresh *Laminaria hyperborea*
Astals et al. (2015)	Batch	0.163		65	37	*Scenedesmus sp.*
Mendez et al. (2013)	Batch	0.267		28	35	Autoclaved *Chlorella vulgaris*
Neumann et al. (2015)	Batch	0.386	SMP 0.393	65	35	WAS + *Botryococcus braunii*
Marsolek et al. (2014)	Batch	0.434		12	37	Thermally treated *Nanochloropsis oculata*
Zhao et al. (2014)	Batch	0.337	SMP 0.604	30	35	*Chlorella vulgaris*
		0.357	SMP 0.682			Nannochloropsis sp.
Keymer et al. (2013)	Batch	0.15		35	38	HPTH Scenedesmus

6.8 Summary

Among the pre-treatment processes applied to food waste and microalgae, HPTH pre-treatment improved the organic material hydrolysis of untreated substrate with increments from 21.8% to 44.1% for food waste and 8.1% to 39.8% for microalgae of the SCOD content. Whereas FW presented high C/N ratio, *Chlorella vulgaris* samples presented a higher content of nitrogen of about 2 to 3-fold superior to food waste which lead to a reduction on the C/N ratio in the fermentations.

Fermentors using food waste in co-fermentation with WAS showed respectable VFAs yields, reaching values of 0.370, 0.391 and 0.496 g VFAs/g TVS for raw, autoclaved and HPTH-food waste respectively on day 21 which advises that pre-treatment with HPTH had a positive effect on the final production of VFAs even when the C/N ratio was similar than fermentations with untreated or autoclaved FW.

Yields of fermentations with microalgae were 0.312 g VFAs/g TVS for the mixture of 50% of autoclaved microalgae and 0.378 g VFAs/g TVS for the blend of 75% of HPTH-microalgae. These results show that the easy degradable substrate played an important role on the generation of VFAs when comparing experiments using microalgae as substrate and, despite of the decrease on the C/N ratio in mixtures with high content of microalgae, the co-fermentation with WAS could have a synergistic effect on the product formation by the acidogenic bacteria. Statistical analyses demonstrated that yields from fermentations with 75% FW treated by HPTH presented the maximum VFAs yield among all the fermentations ran.

The effect of the co-fermentation of HPTH-WAS and food waste presented mixed results: high TCOD mineralisation was presented in reactors working with any mixture of food waste (treated or untreated) and a small impact on the solubilisation of the suspended COD for all the food waste mixtures fermentations.

From fermentations using microalgae as co-substrate, it was found a small or null effect on the TCOD mineralisation from the pre-treatment and co-fermentations of HPTH-WAS and microalgae, a null effect of the pre-treatments of microalgae on the COD solubilisation as the final SCOD/TCOD values were similar in all the mixtures tested and the null effect of the pre-treatment and co-fermentation of HPTH-WAS with microalgae on the final VFAs yields. Overall, there was no impact of the co-fermentation or the

feedstock pre-treatment on the speciation of the VFAs produced in the mixed acidogenic fermentors.

Although fermentations using food waste treated by HPTH presented a high VFA yield, the percentage of SMP reached by the BMP was only 43.4% whereas experiments using WAS at pH 9 as mono-substrate, reached 48.6% of the theoretical biomethane potential from mono-substrate fermentation of HPTH-WAS. Finally, it can ben stated that acidogenic fermentations in mono-substrate and co-fermentation in this project seem to have decent VFA yields (in terms of biomethane), with the main advantage that the carboxylate platform is envisaged only as a first stage for resource recovery with further processing by the submission of the remaining organic material for biomethane production in a second stage in order to build the basis for the biorefinery concept on the existing WWTPs.

Chapter 7. MIXED ACIDOGENIC FERMENTATION OF HPTH-WASTE ACTIVATED SLUDGE IN SINGLE-STAGE SEMI-CONTINUOUS STIRRED-TANK REACTOR FOR THE PRODUCTION OF VFAS AS PART OF THE BIOREFINERY CONCEPT IN WWTPs.

7.1 Introduction

In previous chapters, acidogenic fermentations were run with alkaline pH (pH 9) in batch reactors finding that high pH levels inhibited the generation of methane and promoted the production and accumulation of VFAs when using WAS in mono-substrate fermentation and also when co-digesting WAS with food waste or microalgae with a final conclusion that pH presented an effect on the formation of a specific type of VFAs. These experiments were operated in batch fermentations due to its simplicity on operation and data analysis, although other configuration of reactors could be considered critical for the performance on the production of VFAs, especially when processes are meant to be used for industrial purposes. Different reactors configurations are mainly used to investigate the influence of the reactor microenvironment, dominant bacterial consortia, hydrodynamic behaviour, etc. One of the most used type of reactors are in continuous method, such as fed or sequencing batch or a Continuous Stirred-Tank Reactor (CSTR) due to popularity on industrial application, simplicity of construction, operation and homogeneous mixing and also the ease on the pH and temperature control (Ntaikou et al., 2010).

Several studies have been conducted in batch and/or continuous configuration for the production of VFAs using WAS with different HRT, reactor configurations, pre-treatments, co-fermentation, bacteria consortia, pH and temperatures. For example, Chen et al. (2007) investigated the effect of the pH on the production of VFAs using protein-rich WAS with incubations of 190 h with pH levels from 4 to 11, 13 g TCOD/L as substrate concentration in batch mode with yields between 0.02 and 0.207 g TVFAs as COD/g TCOD whereas Zhu et al. (2008) tested the effect of different substrates and co-digestion of primary sludge, WAS and/or food waste for the production of biohydrogen, resulting as a by-product from the production of VFAs in mesophilic conditions with low yields between 0.021 and 0.03 g

TVFAs/g TVS. These low yields are similar to the values reported by Yuan et al. (2011) when investigating the influence of the temperature on the acidogenic fermentation with lower yields at 4ºC (0.04 g TVFAs/g TCOD) and higher yields at 25ºC (0.16 g TVFAs/g TCOD), showing that high temperatures could impact on the final production of TVFAs.

The organic loading rate (OLR) is a very important parameter as it presents the possibility of changing the distribution profile of the short-chain carboxylic acids and alcohols and also the opportunity of increasing the productivity of the system by optimising the products generation via the amount of substrate fed into the reactor per day and the solids residence time. In this regard, Morgan-Sagastume et al. (2011) carried out fermentations on fed-batch mode with HPTH pre-treated WAS from different WWTP, uncontrolled pH, with different initial OLR (16, 19, 42 and 49 g COD/L·day) and temperatures of 37 and 42ºC with short HRT of 48 h and reaching its maximum yield with 16 g COD/L·day at 42ºC with values of 0.24 g VFAs/g TCOD which suggest that low organic OLR and short HRT are key parameters on the VFAs production.

Continuous fermentations were ran by Banerjee et al. (1999) with primary sludge using different OLR (4 and 7 g TS/L·day) and HRT (18 or 30 h) at 22ºC achieving yields as high as 0.10 g TVFAs/ g TS with 4 g TS/L·day and 30 h. Later, Yang et al. (2014) carried out experiments with pre-treated WAS on alkaline conditions (pH 12); and then recovering the supernatant to use it as the main substrate in acidogenic fermentations with mesophilic conditions and HRT of 8 h for 45 days, reaching yields as high as 0.365 g VFAs/g VSS. Yu et al. (2008) operated experiments in CSTR in acidic or alkaline pH and mesophilic or thermophilic conditions showing its highest VFAs production at 20 days of HRT at pH 10, showing that pH is a parameter that presents a strong effect on the production and accumulation of short-chain carboxylates. In agreement with these findings, Li et al. (2011) tested a pilot-scale alkaline fermentation at pH 10, using thickened WAS from a municipal WWTP with HRT of 8 days, achieving VFAs production of 2.82 g VFAs/L.

These results present an interesting area of research on the production of VFAs from WAS and specifically from WAS pre-treated by HPTH as an alternative for energy recovery, instead of the current biomethane in UK WWTP, via mixed acidogenic fermentation using alkaline pH which can inhibit the action of methanogenic bacteria consortia and at the same time, prevent the inhibition by the products, due to the quick drop on the pH to

values about 4.5. Simultaneously, the fermentations with alkaline pH have proven the feasibility of the generation of biogas, especially hydrogen a methane which can be other important products that can be eventually recovered and marketed.

Therefore, the main objectives of this chapter are:

1. to understand the effect of OLR in a continuous system with alkaline pH, on the production, accumulation and speciation of the VFAs using WAS in mono-fermentation,
2. to understand the behaviour of a CSTR fermentor in the TVS concentration,
3. to determine the best OLR in a semi-CSTR digester for the highest production of VFAs.

Bearing this in mind and considering the highest yields and operational conditions found in the batch experiments reported in previous chapters, mixed acidogenic fermentations in CSTR were run with WAS in mono-digestion in a AMPTS II (Bioprocess Control Sweden AB) with a HRT of 14 days and pH 9 with OLR of 0.3, 0.6 and 1 g TVS/L·d, fed three times a week, during 56 days in order to examine carboxylic acid and biomethane production, yields and selectivity of the WAS in the system.

Organic loading rates were calculated according to the formula given by Dinopoulou et al. (1988):

$$OLR = \frac{\text{Influent concentration}}{\text{HRT}} \quad \text{.....Equation 7.1}$$

Thus, as the influent concentration and HRT were set up based on previous results from batch experiments, the OLR were calculated to be 0.3, 0.6 and 1 g TVS/day.

The reactors ran were ctrl fermentor which is based only on 5 g TVS/L from inoculum and was considered as the blank to determine the effect of bacterial consortia in the acidogenic fermentation, BMP reactor which was run to understand the behaviour of an standard test for the production of biomethane and contains 5 g TVS inoculum/L plus 5 g TVS WAS/L which was further fed with an OLR of 0.3 g TVS/L·d without pH adjustment.

Fermentors CSAF1, CSAF2 and CSAF3 were run with OLR 0.3, 0.6 and 1 g TVS/L·d respectively with adjustment to pH 9 to understand the effect of the OLR in the mixed acidogenic fermentation in alkaline conditions.

7.2 Effect of organic loading rate on the acidogenic fermentation of HPTH-WAS in a single-stage semi continuous stirred-tank reactor

7.3 Effect of the OLR on the chemical oxygen demand

Analyses of TCOD and SCOD were run in order to track the progress of the organic material content in the fed-batch fermentors during the batch and semi-continuous flux, to determine the effect of the process in the hydrolysis of complex compounds in the organic substrate and the further conversion into biogas or liquid biochemicals. Results of the TCOD of the five fermentors are shown in Figure 7.1.

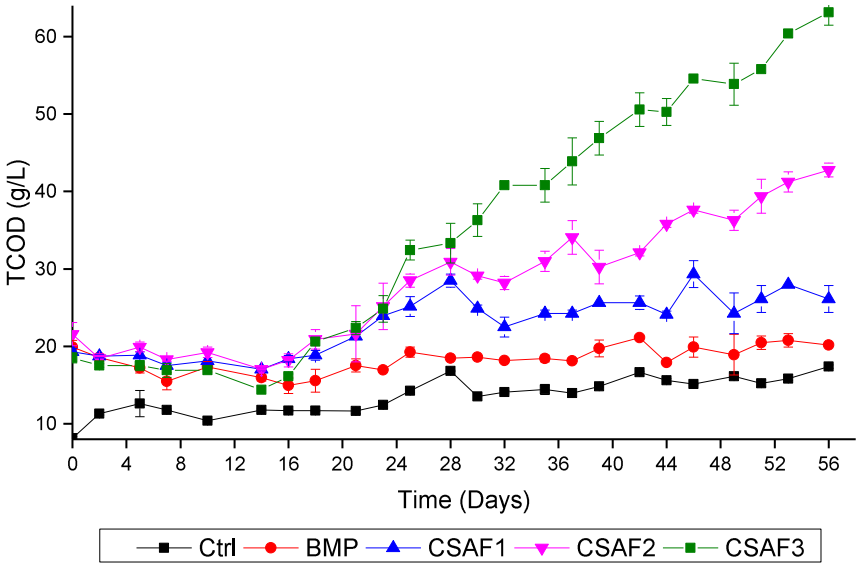

Figure 7.1. Total COD results from acidogenic anaerobic fermentation of HPTH-WAS in semi-CSTR mode at pH 9.

It is visible all fermenters presented similar behaviour during the first 14 days of fermentation as each of them were fed with an initial organic solid content of 5 g TVS WAS/L. Total COD in BMP fermentor presented a quick decrease reaching TCOD removals of about 20% during the first 7 days of

the experiment and reaching an average 22% on day 14. On the other side reactors CSAF1, CSAF2 and CSAF3 presented COD removals lower than 15% on day 7 and a further organic material mineralisation on day 14 only in fermenters CSAF2 and CSAF3, reaching and TCOD removal slightly above 20% in both cases which can suggest the poor adjustment of pH in both fermenters causing the disappearance and conversion of the VFAs produced into biomethane. Evidently, this drop on the VFAs content could affect the final yield of the VFAs on day 14 in mentioned fermenters in their batch period.

After the batch cycle, the TCOD values were clearly differentiated one from the other, reactor showing an evident higher COD concentration in fermenters CSAF3 followed by CSAF2 and CSAF1 but the former two did not presented any asymptote which suggest a steady-state in terms of COD was not reached in 56 days and more time could be needed to possibly determine the yields and productivity of VFAs per day. In contrary, reactors BMP and CSAF1 (0.3 g TVS WAS/L·d) reached a semi-steady state as those reactors were fed with the minimum OLR found in 14 days in previous batch experiments in Chapter 6.

The method carried out for the determination of COD was a colorimetric technique which could explain the variability on the TCOD removal results which are shown in Figure 7.2. It is visible that some mineralisation of the organic material added took place within the boundaries of -10 to +10%, especially during the second half of the continuous fermentation process which could suggest a behaviour tending to a steady state.

The values on COD removal in this study were evidently lower than the values from studies from Wang et al. (2009) who reports removals of 67.7% and 61.6% when using HPTH-WAS in an anaerobic sequencing batch reactor for the production of biogas with HRT of 10 and 20 days, respectively. Similar conditions to the current project were used by Silvestre *et al.* (2011) who worked on the digestion of sewage sludge with OLR of 1.2 g COD/L·d and 20 days of SRT but without the adjustment of pH reporting average TCOD removals of 46%. TCOD removals in the current project are clearly lower than other studies as the main aim was the production of VFAs and not the generation of gaseous products.

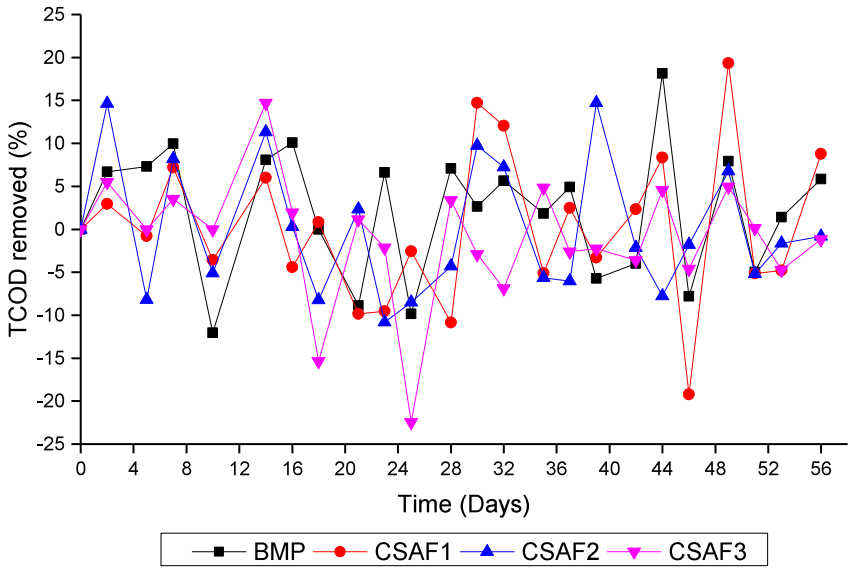

Figure 7.2. TCOD removal results from acidogenic anaerobic fermentation of HPTH-WAS in semi-CSTR mode at pH 9.

(*Key: BMP: Biomethane potential test; CSAF1: Continuous Acidogenic fermentation 0.3 g TVS_{WAS}/L·d; CSAF2: Continuous Acidogenic Fermentation 0.6 g TVS_{WAS}/L·d; CSAF3: Continuous Acidogenic fermentation 1 g TVS_{WAS}/L·d).*

On the other side, SCOD results are in concordance with the TCOD progress; firstly, during the batch cycle, CSAF fermentors presented the same behaviour during the first week of the process but in the second week CSAF2 and CSAF3 showed a quick drop on the SCOD content, possible due to the mineralisation of the organic material in the reactor which could be caused by the poor adjustment and meticulous maintenance of the alkaline pH due to the nature of the pH adjustment process (Figure 7.3).

After the batch phase, the SCOD content on the fermentor BMP increased from day 18 to day 28 possibly due to the lack of adaptation of the microbial consortia to a semi-continuous process, however after day 28, SCOD concentration decreased to values lower than 2 g/L which were maintained until the end of the fermentation process. These values presented similarities with the results encountered in the ctrl-blank reactor which indicates the majority of the SCOD from the inlet WAS could be consumed, mineralised and converted into biogas. Whereas SCOD in CSAF3 reactor increased quickly and achieved a semi-linear tendency from day 25 until the end of the fermentation which can be defined by the equation y=0.4439x - 3.6214, with a R^2=0.9799 and where x represents the time of fermentation in

days. Thus, it shows that the system did not reach a steady state and SCOD that was not converted into biogas remained available for the VFAs production.

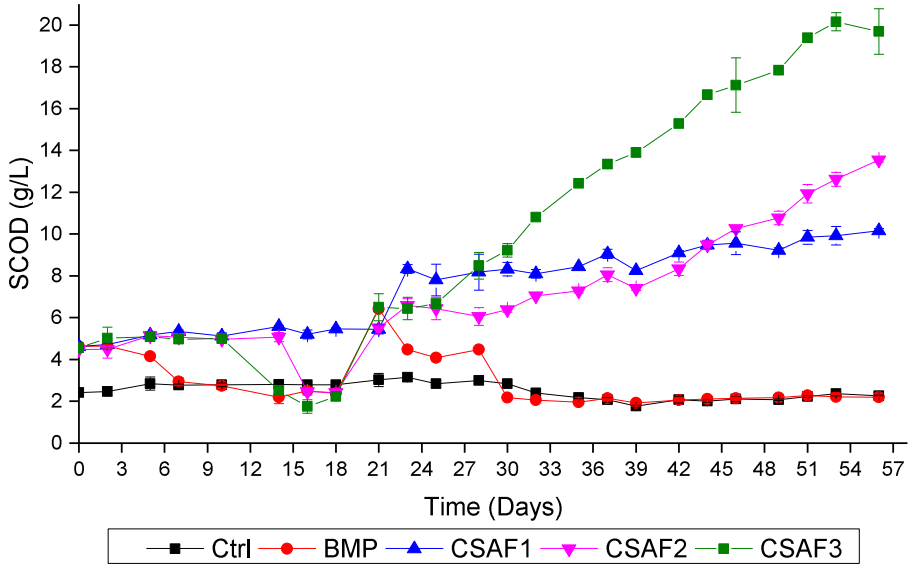

Figure 7.3. Soluble COD results from acidogenic anaerobic fermentation of HPTH-WAS in semi-CSTR mode at pH 9.

(*Key: BMP: Biomethane potential test; CSAF1: Continuous Acidogenic fermentation 0.3 g TVS_{WAS}/L·d; CSAF2: Continuous Acidogenic Fermentation 0.6 g TVS_{WAS}/L·d; CSAF3: Continuous Acidogenic fermentation 1 g TVS_{WAS}/L·d).*

On the other side, fermentors CSAF1 and CSAF2 exhibited analogous progress on the SCOD content, showing that 0.3 and 0.6 g TVS/L·d acted similarly, along the entire fermentation, on the conversion of soluble organic material to VFAs or biogas which will be discussed later.

Progress on SCOD content is reported in studies ran in batch from Wu *et al.* (2009) who worked with primary sludge and pH 9, showing the highest COD solubilisation on day 5 and decreasing on the subsequent days. This quick solubilisation can be endorsed to the characteristics of the primary sludge which typically contains high concentration of soluble carbohydrates and proteins and the later reduction of SCOD can be attributed to the production of the mineralisation of the organic material and the plausible production of biogas. Whereas, Huang *et al.* (2016) report similar trends on COD solubilisation in fermentations with WAS at pH 8 with high COD solubility

from day 4 to day 15 and then decreasing until day 21 when TVFAs decreased and possible biomethane was produced due to the experiments were conducted at a viable pH for methanogenic activity.

Percentages of SCOD removals showed high variations especially throughout the middle of the fermentation process which agrees with the drop on the SCOD concentration. More stable SCOD removals were found from day 25 towards the end of the fermentation falling within the boundaries of ±15% of content. Wang *et al.* (2009) reports SCOD removals of about 91.8% whereas Braguglia *et al.* (2015) shows values between 68 and 73% which are clearly much higher than the reductions in the current study probably because of both investigations aimed for the production of biogas in anaerobic digestion with WAS as mono-substrate.

An important value to be considered is the ratio SCOD/TCOD, otherwise called as the degree of solubilisation, which provides an idea of the progress of suspended COD and its possible conversion into soluble compounds and/or products. The degree of solubilisation progress is shown in Figure 7.4 where is visible that the SCOD/TCOD values in BMP reactor presented a tendency to decrease, reaching a semi steady-state from day 30 until the end of the fermentation and showing that around 10% of the SCOD was not converted into biogas and remained in the reactor and could be recalcitrant compounds for the methanogenic bacteria. On the other side, fermentor CSAF1 presented a tendency to increase from day 0, reaching its maxima on day 44 (0.394) which represents an overall increase of 61.9% increase with regards to the initial SCOD/TCOD ratio. Fermentors with higher OLRs presented a lower degree of acidification with 0.347 on day 51 and 0.317 on day 56, which represents increments of 52.5 and 26.9% for reactors CSAF2 and CSAF3 respectively. This behaviour can be attributed to the effect of the higher loading of organic material in the reactor and its possible lower solid retention time which could impact on the activity of the anaerobic bacteria to convert suspended organic compounds to liquid biochemicals. Thus, the OLR of 0.3 g TVS/L·d presented the highest efficiency on the COD solubilisation at alkaline pH levels, while the other OLR studied in this project, presented also respectable OLR that can be considered for the operation of mixed acidogenic fermentation in pilot or full-scale reactors.

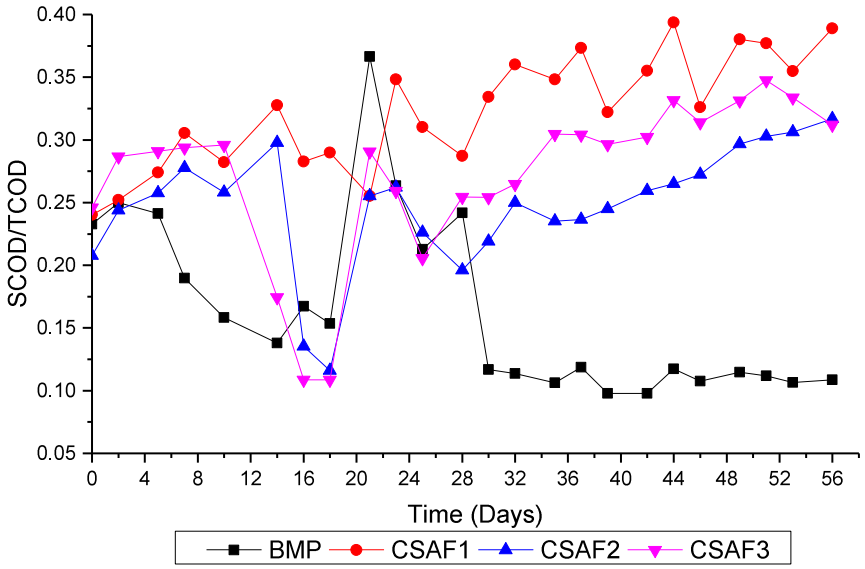

Figure 7.4. SCOD/TCOD results from acidogenic anaerobic fermentation of HPTH-WAS in semi-CSTR mode at pH 9.

(*Key: BMP: Biomethane potential test; CSAF1: Continuous Acidogenic fermentation 0.3 g TVS_{WAS}/L·d; CSAF2: Continuous Acidogenic Fermentation 0.6 g TVS_{WAS}/L·d; CSAF3: Continuous Acidogenic fermentation 1 g TVS_{WAS}/L·d).*

Ucisik and Henze (2008) reported SCOD/TCOD ratios of 0.190 and 0.06 for fermentations on semi-continuous with HRT of 5 days of primary sludge and WAS respectively. Yuan *et al.* (2009) investigated the effect of solids retention and the biomass concentration on fermentations of untreated WAS with SRT of 5, 7 and 10 days and OLRs ranging between 4.33 and 11.62 g VSS/L·d, finding degrees of acidification of 18% when testing SRT 10 days and VSS fed of 4.06 which suggest that longer HRT in combination with low OLR benefits the solubilisation of the organic material, which agrees with the results in this study. On the contrary, Wan *et al.* (2016) reported high COD solubilisation ratios when conducting mixed acidogenic fermentations in mesophilic (0.36) and thermophilic (0.61) in alkaline conditions (pH 10) which can be endorsed to the usage of only the soluble part of WAS which could contain mainly easy degradable organic material. Hao and Wang (2015) reported solubilisations lower than 10% on the carbohydrates and proteins content in semi-CSTR fermentations with 10 days of HRT, untreated dewatered sludge with no pH control and with different OLR; the low solubilisation can be endorsed either to the short SRT or the low hydrolysis of the WAS due to the lack of pre-treatment.

A statistical analysis to compare the SCOD/TCOD ratios of the three semi-continuous fermentations was performed by running a t-test for independent samples, resulting in a p=0.000 for CSAF1 vs CSAF2, and p=0.000 for fermentors CSAF1 vs CSAF3, implying that there is a significant difference between the CSAF1 and the other two fermentors, being the former reactor, the one that presented the highest COD solubilisation.

Finally, it is clear that the soluble part of the COD overall increased along the experiments, suggesting that the microorganism in the inoculum at pH 9 showed a good activity on most of the OLR tested in this project

7.4 Effect of the OLR on the solid contents on the acidogenic fermentation of HPTH-WAS in a single stage semi-CSTR

Similar to the behaviour encountered in the SCOD content, TVS presented a clear tendency to increase in reactors CSAF1 to CSAF3 from day 14 onwards. During the batch cycle, fermentor BMP was evidently the only reactor that showed a decrease on the TVS concentration, which represented a 12.7% TVS reduction with regards to the initial concentration of solids probably due to the lack of adjustment of pH which contributed to the production of VFAs and its quick conversion into biogas. Fermentors with control of alkaline pH showed mainly a slight decrease on the TVS content, with percentages mostly below 5% of the initial TVS concentration (Figure 6.4).

After the batch cycle, concentration of TVS in fermentor BMP showed a slow increase due to the feeding in the reactor with a final increase of 43.93% when compared to the concentration of TVS on day 14. Reactors, CSAF1 and BMP, presented parallel increments with regards to the TVS content, but with a final increase of 60.69% in the fermentor working in mixed acidogenic conditions. As BMP and CSAF1 reactors were fed with the same amount of solids per day (0.3 g TVS/L·d), these results showed that the BMP consumed around 13 percent of the organic content and converted to biogas when compare it with the TVS increase on the reactor CSAF1 at pH 9, which did tend to accumulate organic material inside for the production of biomass and especially, organic biochemicals.

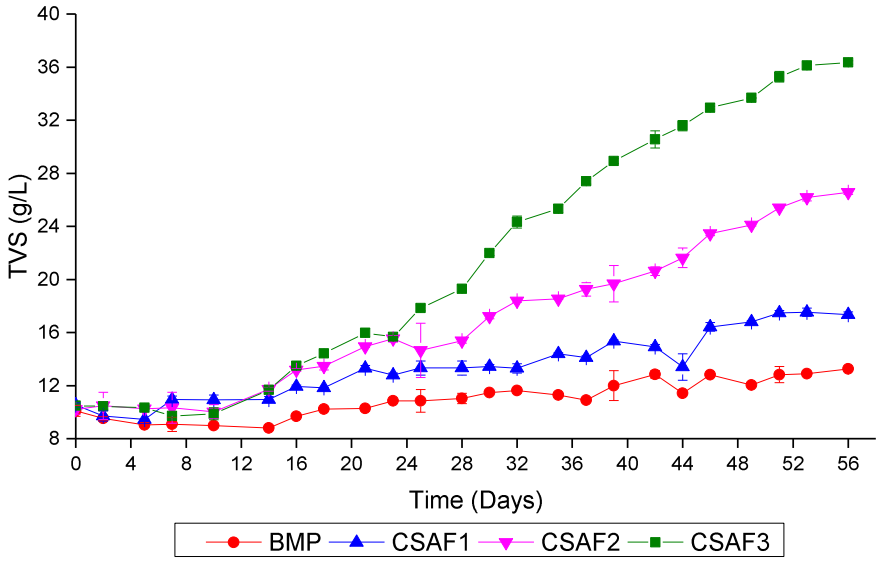

Figure 7.5. TVS results from acidogenic anaerobic fermentation of HPTH-WAS in semi-CSTR mode at pH 9.

(*Key: BMP: Biomethane potential test; CSAF1: Continuous Acidogenic fermentation 0.3 g TVS_{WAS}/L·d; CSAF2: Continuous Acidogenic Fermentation 0.6 g TVS_{WAS}/L·d; CSAF3: Continuous Acidogenic fermentation 1 g TVS_{WAS}/L·d).*

When comparing the concentrations of TVS on CSAF1 to CSAF2 and CSAF3, it is clear than there is a difference between 9.22 to 9.78 g TVS/L which agrees with the change on the amount of solids fed into each reactor. It is also important to point that the content of TVS in the reactors working in acidogenic fermentation conditions did not reach a steady-state which can be attributed to the accumulation of organic material from the feeding and the low or infimums volatile solids destruction and conversion to biogas. In view of these, it can be say that more time is needed to reach steady-state conditions on the experiments ran.

In order to understand the changes on the TVS inside the fermentors, the destruction of TVS was calculated (Figure 7.6). Total volatile solids destruction was calculated according to the modified formula given by Abe *et al.* (2013):

$$\text{The TVS destruction efficiency (\%)} = \left(1 - \frac{\text{Final VSS (g/L)}}{\text{Initial TVS (g/L)}}\right) \times 100\% \quad \text{.....Equation 7.2}$$

The TVS removal agreed with the results of SCOD during the batch cycle as is visible that the destruction of solids was more acute on day 14 which coincides with the dramatic SCOD drop in fermentors CSAF2 and CSAF3, which, as mentioned before, could be due to a non-successful control on the pH in the reactors.

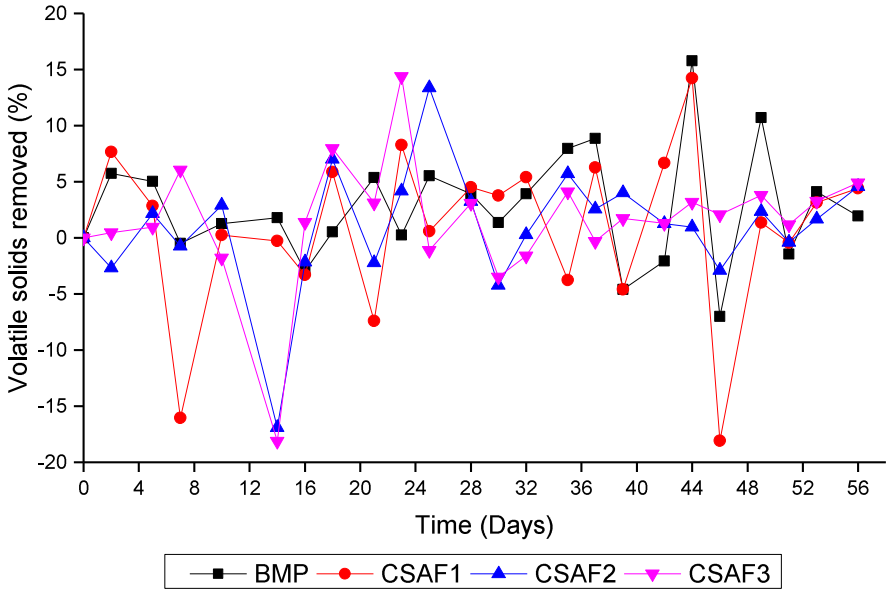

Figure 7.6. TVS removal results from acidogenic anaerobic fermentation of HPTH-WAS in semi-CSTR mode at pH 9.

(*Key: BMP: Biomethane potential test; CSAF1: Continuous Acidogenic fermentation 0.3 g TVS_{WAS}/L·d; CSAF2: Continuous Acidogenic Fermentation 0.6 g TVS_{WAS}/L·d; CSAF3: Continuous Acidogenic fermentation 1 g TVS_{WAS}/L·d).*

After the batch cycle, all the fermentors working on acidogenic conditions presented similar trends on the TVS destruction, showing results that varied between the ranges of ±20%. Some of the negative results can be endorsed either to the TVS analysis technique employed as it can be not as accurate as other techniques such as COD, or to the not completely mixed samples caused by the amount of solids in the broth as it contains more than 10 g/L solids.

Studies from Yang *et al.* (2014) worked with mixed acidogenic fermentation in semi-continuous at pH 9 and 8 days of HRT with untreated WAS, presenting overall removals of TVS of 31.1% which is higher than the results from this project, possibly due to it is reported as a cumulative value and not

as specific values from one day to another during the whole fermentation process. Similar values were found by Yuan *et al.* (2009) with average TVS destruction of 35.8% for fermentations of WAS in semi-continuous reactors, also by Silvestre *et al.* (2011) with a 36% removal for mixed acidogenic fermentations of sewage sludge on anaerobic digestion and OLR of 1.2 g COD/L·d and 20 days of SRT. More prolific TVS removals were found by Wang *et al.* (2009) with higher destruction percentages in reactors operated in anaerobic sequencing batch reactor (ASBR) than in CSTR conditions for the production of biogas with 63.77 and 54.32% TVS removal, respectively. Another study reporting TVS destruction is from Braguglia *et al.* (2015) who informed percentages of 40% in anaerobic digestion of WAS treated by ultrasound process for biogas production.

Finally, Zhou *et al.* (2014) reported higher VSS destruction than the TVS values from this project, with an average of 35% after 28 days of fermentation of alkaline-thermal pre-treated WAS for the production of VFAS which can suggest the solubilisation of the VSS content and also the poor inhibition of the methanogenic bacteria which consumes the VFAs produced during the acidogenic fermentation.

A main outcome from the fermentation in mixed acidogenic fermentation of HPTH-WAS in CSTR in this project was the finding of the low TVS reductions which agrees with the production ad accumulation of VFAs and the low conversion of VFAs into biogas which is most probably due to the inhibition of the methanogenic activity via the fermentation with alkaline conditions.

7.5 Effect of the OLR on the production, accumulation and composition of volatile fatty acids on the acidogenic fermentation of HPTH-WAS a single stage semi-CSTR

7.5.1 Production and accumulation of VFAs

For the escalation of bench-lab experiments to batch or continuous full-plant scale, several factors must be considered such as the temperature conditions (Zhuo *et al.* 2012), monitoring, operational stability, the design of a the reactor, among others (Zhang *et al.,* 2016). The design of the reactor is an extremely important aspect to study as there are many configurations of reactors that have been tested for the production of biogas or VFAs reporting different yields, for example, operation in single or two-stage

reactor, membrane bioreactors, upflow anaerobic sludge blanket (UASB), etcetera, in order to reach the optimisation on organic material hydrolysis and the VFAs production. Among all the reactors used, the most common configuration is the CSTR because it represents a low-cost technology, low economical investment on the construction and a possible adaptation to the current structure of the WWTP (Lafitte-Trouqué and Forster 2000; Bastidas-Oyanedel *et al.* 2015); also provides a completely mixed biomass in the broth, keeping a good contact between the microorganisms and the organic material (Ntaikou *et al.* 2010), easy-operational conditions as the SRT is the same than the HRT, which can help on the selection of the microbial population.

The aim of this part of the project was to understand the OLR in a continuous system with alkaline conditions, on the production, accumulation and speciation of the VFAs using WAS in mono-fermentation.

The progress on the production of VFAs in acidogenic fermentations and BMP is reported in Figure 7.7. During the first 14 days of the fermentation, all semi-continuous acidogenic fermentation reactors showed similar trends on the VFAs production, as all of them were operated using the same conditions (5 g TVS inoculum/L + 5 g TVS WAS/L) which indicates the repeatability of the test in alkaline pH. On the other side, the BMP reactor showed a very typical behaviour of the anaerobic digestion process with a quick production of VFAs during the first two days and then decreasing due to the consumption of the carboxylic acids for the production of biogas.

It is also clear that the concentration of VFAs in fermentor CSAF3 dropped dramatically on day 10, which coincides with the increase on the percentage of TVS and TCOD destruction reported previously in this chapter.

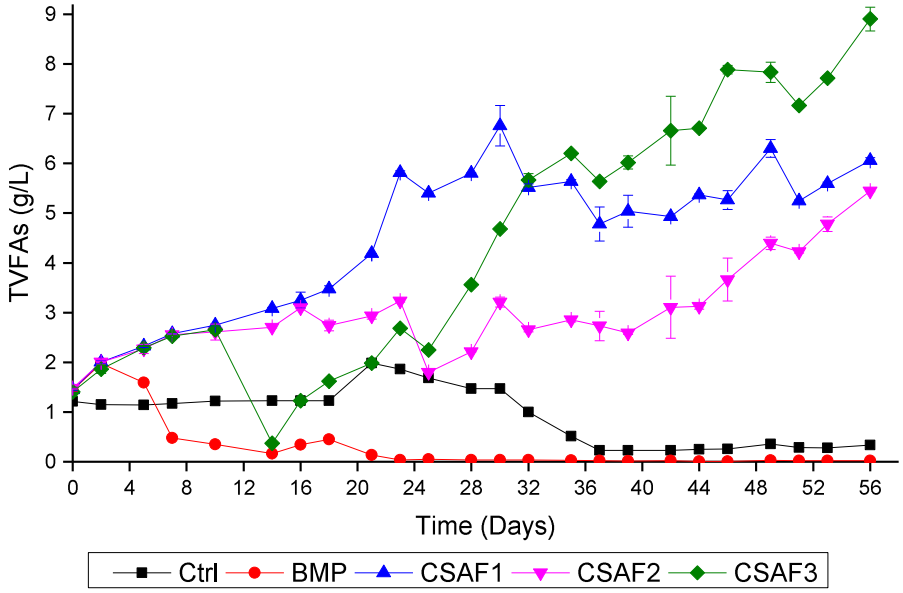

Figure 7.7. VFAs production results from acidogenic anaerobic fermentation of HPTH-WAS in semi-CSTR mode at pH 9.

(*Key: BMP: Biomethane potential test; CSAF1: Continuous Acidogenic fermentation 0.3 g TVS_{WAS}/L·d; CSAF2: Continuous Acidogenic Fermentation 0.6 g TVS_{WAS}/L·d; CSAF3: Continuous Acidogenic fermentation 1 g TVS_{WAS}/L·d).*

After the batch mode, all the CSTR reactors were operated 3 more HRT to reach 56 days of fermentation. BMP reactor presented very low concentrations of VFAs, with concentrations below 47 mg/L from day 25 towards the end of the process. In contrast, the fermentor with OLR of 0.3 g TVS WAS/L·d (CSAF1) showed its highest VFAs concentration on day 30 with a value of 6.758 g VFAs/L, but with a plausible steady-state from day 23 (5.816 g VFAs/L) until the end of the fermentation on day 56 (6.059 g VFAs/L). Reactor CSFA2 showed a different behaviour to the other fermentors, with a semi-asymptote on the production of VFAs from day 7 (2.556 g VFAs/L) until day 39 with a concentration of 2.588 g VFAs/L, and then, an semi-linear increase towards the end of the fermentation with a maxima of 5.449 g VFAs/L, which is 2.1-fold more than on day 39. Finally, the reactor with the highest OLR presented a more erratic evolution of VFAs, with a remarkable drop on day 14 with a minimum value of 0.374 g VFAs/L but increasing from day 14 until day 56 with an almost linear behaviour than can be described with the equation: VFAs concentration (y)=0.1944 days (x)-1.7555, with an R^2=0.9456 and a final VFAs content of 8.905 g VFAs/L. It is also clear that neither CSAF2 nor CSAF3 reached an steady-state in terms

of VFAs concentration and this behaviour can be attributed to the short fermentation period that avoided the complete adaptation of the microbial population in the broth for the increasing of biomass and at the same time, the promotion of high rate yields and production of VFAs. The higher OLR in the CSTR at pH 9 with HPTH-WAS tested in this study, presented the highest concentration of VFAs when compared with the other two different OLR tested.

Furthermore, the main type of products of the fermentation of HPTH-WAS were carboxylic acids with a very minimal generation of ethanol, reaching its maxima on the reactor BMP on day 14 with an average of 67.5 mg ethanol/L, which confirms the thesis of the low solvent production at pH 9 and long HRT (14 days) (Grupe and Gottschalk 1992; Kleerebezem and van Loosdrecht 2007). Considering the results presented, there is not enough evidence to determine the effect of the OLR on the VFAs production as mixed results were obtained, such as the second highest VFAs production/accumulation was obtained when working with the lowest OLR tested (0.3 g TVS WAS/L·d).

This later discovery can be taken into consideration to modify the conditions of the acidogenic fermentation in order to lead the process into a solventogenesis state to produce ethanol, butanol or acetone.

Yields of the VFAs production in terms of g VFAs/g TVS_{fed} were calculated for the complete process as can be seen in Figure 7.8, according to Equation 7.3 for the batch cycle and the Equation 7.4 for the semi-continuous process.

$$\text{VFAs yield} = \frac{VFAt_f - VFAt_i}{OLR_i} \quad \text{Equation 7.3}$$

$$\text{VFAs yield} = \frac{VFAt_f - (VFAt_i + \text{VFA WAS})}{OLR} \quad \text{Equation 7.4}$$

Where $VFAt_f$ is the final concentration of VFAs on time t; $VFAt_i$ is the initial VFAs concentration on the fermentation; OLR_i is the initial organic load; VFAs WAS is the concentration of VFAs in the waste activated sludge; and OLR is the amount of TVS fed to the reactor in g TVS/L·d.

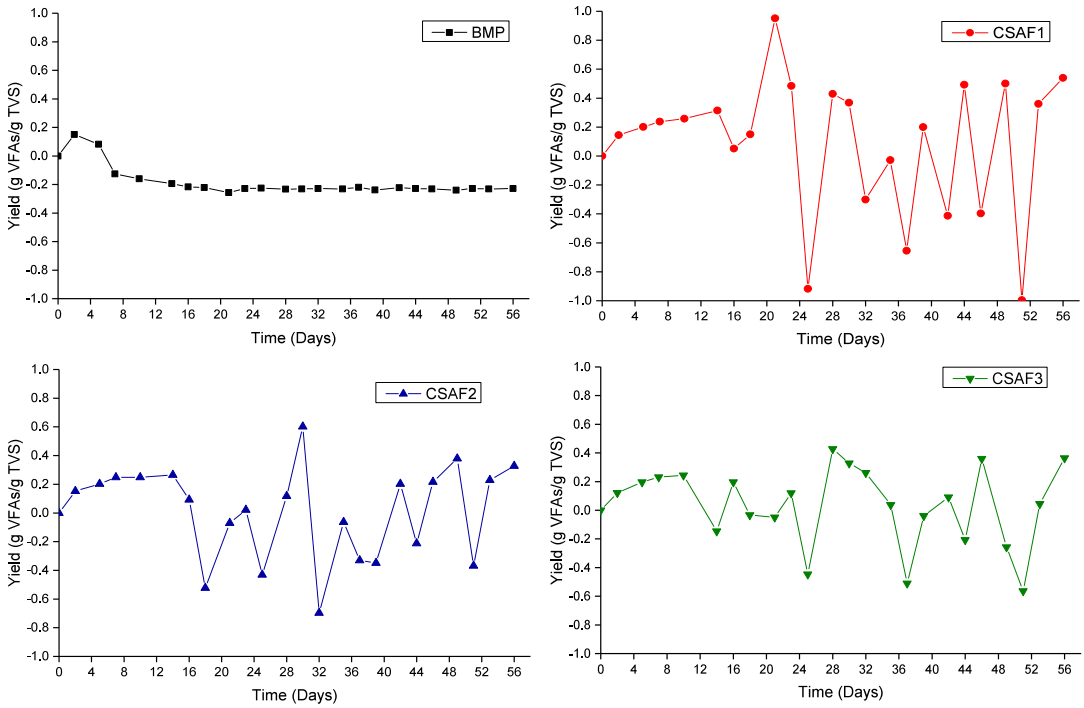

Figure 7.8. Average VFAs yields from acidogenic anaerobic fermentation of HPTH-WAS in semi-CSTR mode at pH 9.

(*Key: BMP: Biomethane potential test; CSAF1: Continuous Acidogenic fermentation 0.3 g TVS_{WAS}/L·d; CSAF2: Continuous Acidogenic Fermentation 0.6 g TVS_{WAS}/L·d; CSAF3: Continuous Acidogenic fermentation 1 g TVS_{WAS}/L·d).*

In concordance with the behaviour from TVS and COD, the reactor BMP showed its highest yield on day 2 (0.15 g VFAs/g TVS) and then decreasing because of the biogas production which is reflected on the high TVS removal from day 25 to 56, caused by the lack of pH adjustment or the addition of any methanogenic inhibitory agent. In contrast, all the acidogenic fermentations in semi-continuous process with HPTH-WAS and pH 9, showed similar trend on yields during the batch stage, with average values of 0.29 g VFAs/g TVS, which is almost a third of the entire concentration of TVS inside the fermentor.

Batch operations conducted by Wang *et al.* (2016), found yields of about 0.19 g VFAs as COD/g VSS, accumulated in the broth, and 0.01 g VFAs/g VSS, converted into biogas, in experiments with WAS at pH 9, 21ºC for 24 days for the production of biomethane. This behaviour can be endorsed to the low amount of SCOD in the WAS used in the fermentations, being about 0.19% with regards to the initial TCOD. Similar experiments were conducted

by Liu *et al.* (2015) with thermal-alkaline pre-treated WAS in batch anaerobic acidification and 10 days of HRT, reporting yields of 0.571 g COD/g TVS added with pH 10; those high yields can be attributed to the usage of only the soluble portion of the treated WAS. Studies carried out by Ma *et al.* (2016), found also low VFAs yields of 0.152 g VFAs as COD/g TVS in fermentations with alkaline-treated WAS and mixed acidogenic process with pH 10 after 9 days of operation. Mentioned study concurs with the reports from Yuan *et al.* (2006) with yields of 0.250 and 0.173 g VFA (COD)/g VSS) for experiments with pH 10 and pH 9 respectively, after 8 days of HRT. It is clear that although respectable yields were achieved in cited studies, the remaining organic substrate in the broth could be still being converted to VFAs by allowing longer HRT.

Other studies working with fermentations of WAS in mono-substrate report VFAs yields, such as, 0.420 g COD/g VSS in fermentation with thermophilic conditions, 7 days HRT, pH 7 (Cagnetta *et al.* 2016), 0.129 g VFAs/g TVS at pH 10, 4 days of HRT (Huang *et al.* 2014), 0.355 g COD/g VSS in fermentations at 24.6ºC, 6 days HRT, 0.258 g TOC/g VSS in fermentations with pH 10, 60ºC and 7 days of SRT (Mengmeng *et al.* 2009), 0.404 g COD/ g TVS in co-fermentation of WAS+ henna plants biomass, HRT 6 days (Huang *et al.* 2016), and 0.250 f COD/g VSS in fermentations at pH 10 and 8 days of HRT Wu *et al.* (2009). One important outcome to point is that many of these studies carried out fermentations in alkaline conditions, corroborating the high impact that high pH levels have on the production and accumulation of VFAs.

After the batch phase, the average yields presented erratic behaviour in all fermentors as the VFAs concentrations could vary in a substantive way by the time the sample was collected. In view of this intricacy, it is reported only the final yields on day 56 which were 0.539, 0.328 and 0.364 g VFAs/g TVS for fermentors CSAF1, CSAF2 and CSAF3, respectively, which suggests a respectable attainment in terms of VFAs production from WAS. Another significant outcome is that fermentors working with OLRs of 0.6 and 1 g TVS/L·d achieved similar yields in terms of VFAs which could suggest that even high OLR could lead to decent VFAs production. It is important to mention that as fermentors CSAF2 and CSAF3 did not reach a steady-state by the time the process was interrupted; further investigation could be done to find a stable value of yields in terms of VFAs per gram of substrate when the reactors finally reach a steady-state.

These findings concur with the results reported by Lafitte-Trouqué and Forster (2000) who found concentrations above 9 g/L VFAs when working with WAS in a dual digestion and low OLR (0.333-0.631 g/L·d), concurring that the system could be benefited with a longer HRT for the higher production of biogas; also, mentioned study presented an erratic production of VFAs during the 125 days that the experiment was run.

On the other side, Yuan *et al.* (2009) found a higher VFAs yields with long HRT (10 days) and low OLR (4.8 g/L), reaching a final value of 0.14 g VFAs as COD/g TCOD, whereas Morgan-Sagastume *et al.* (2011) reports averages yields of 0.2 g VFAs as COD/g TCOD on fermentations with HPTH-WAS in mesophilic conditions and HRT of 2, 4 or 6 days, confirming that longer HRT could benefit the production of carboxylic acids in the broth. In contrast, studies from Bouzas *et al.* (2002) reports low yields of about 0.195 g VFAs/g TVS for fermentations at 30ºC, 8 days HRT and 3.44% of OLR, corroborating the hypothesis that high temperature and low OLR affect the overall VFAs production. Also, Ji *et al.* (2010) report a value of 0.118 g COD/g VSS when fermenting a mixture of primary sludge and WAS for 10 days with no pH adjustment. As can be seen, the production of VFAs have a strong dependence on operational conditions, such as, HRT, OLR, types of substrate and pH; all of which could lead to a wide range of yields. Cited studies working with semi-continuous process reported yields that fall under 0.3 g VFAs/g TVS/VSS/TCOD, mostly due to the short HRT employed in the majority of the cases. Lastly, the average VFAs yields obtained in this study represent a respectable production of VFAs probably because of its long HRT which played a positive role on the adaptation of the inoculum and the conversion of organic material into carboxylic acids. Calculations of productivity in terms of g VFAs/L·d were made without finding any trend on the production as the generation of carboxylic acids was erratic.

7.5.2 VFAs composition on the acidogenic fermentation of HPTH-WAS in a single stage semi-CSTR

Mixed acidogenic fermentation involves the conversion of organic material into a diverse number of organic products, such as lactic, acetic, propionic and butyric acids, which depends on the fermentative pathways that are favoured by the operational conditions in the reactor (Bastidas-Oyanedel *et al.* 2015). The operation in semi-continuous process is one of the conditions that can affect the speciation of the VFAs in the of acidogenic fermentations as some arrangements of OLR and HRT could change the dominant

microbial population inside the reactor by changing the enzymatic or metabolic routes, which could cause a shift on the predominant carboxylic acid, with an eventual accumulation or consumption of the VFAs (Bastidas-Oyanedel *et al.* 2015; Yang *et al.* 2014). In this segment, is discussed the effect of OLR in a continuous system with alkaline pH, on the speciation of the VFAs using WAS in mono-fermentation, results can be seen in Figure 6.7.

Firstly, the BMP reactor presented an inconsistent trend on the VFAs production along the entire process. During the batch cycle, the acetic acid content decreased to values lower than 10% on the mixture of VFAs by day 7, which suggests the consumption of acetic acid for the transformation and production of biogas, as the conversion of acetate to biogas is more favourable energetically than the conversion of propionate or other medium-chain fatty acids (Schiel-Bengelsdorf and Dürre 2012; Thauer *et al.* 1977; Zeikus *et al.* 1975) (Equation 7.5).

$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$ …..($\Delta G^{o'}$=-311 kJ/reaction) ……Equation 7.5

Whereas the conversion of propionate implies more steps and energetically unfavourable reactions (Fukuzaki *et al.* 1990) (Equation 7.6 to 7.8):

$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2$…($\Delta G^{o'}$=+76.1 kJ) …… ……Equation 7.6

$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$ …….($\Delta G^{o'}$=-135.6 kJ) ……Equation 7.7

$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$ …..($\Delta G^{o'}$=-311 kJ/reaction) ……Equation 7.8

After batch period, acetic acid was the most predominant in the acid blend as it is produced through an energetically favourable reaction on the mixed acidogenic fermentation process. Surprisingly, the acid with the second highest concentration was the isobutyric acid, which can be endorsed to the catabolism of proteins and its complexity of further conversion to acetate and a transformation into biomethane (Schink and Thauer 1988):

$2CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COO^- + 2H_2 + H^+ .(\Delta G^{o'} = +48\ kJ/mol)$ ……
……Equation 7.9

For the case of the CSAF1 (0.3 g TVS/L·d), it is clear that the most predominant carboxylic acid was the acetic during the batch and semi-CSTR cycles with percentages ranging between 83.12 and 88.92% with an average value of 85.68%, which agrees with the energetic feasibility of the acetate production by anaerobic bacteria. Despite the acetic acid was also the main product in the carboxylates mixture in reactors CSAF2 and CSAF3, its production presented a slightly different tendency; after a very rapid transformation of acetic acid on the last days of the batch cycle, the acetic acid tend to increase to reach an steady state with average values of 76.32 and 77.91% for CSAF2 and CSAF3, respectively, which could propose that higher OLR could have an slight impact on the VFAs profile, by increasing the amount of medium/large-chain carboxylic acids, specially propionate.

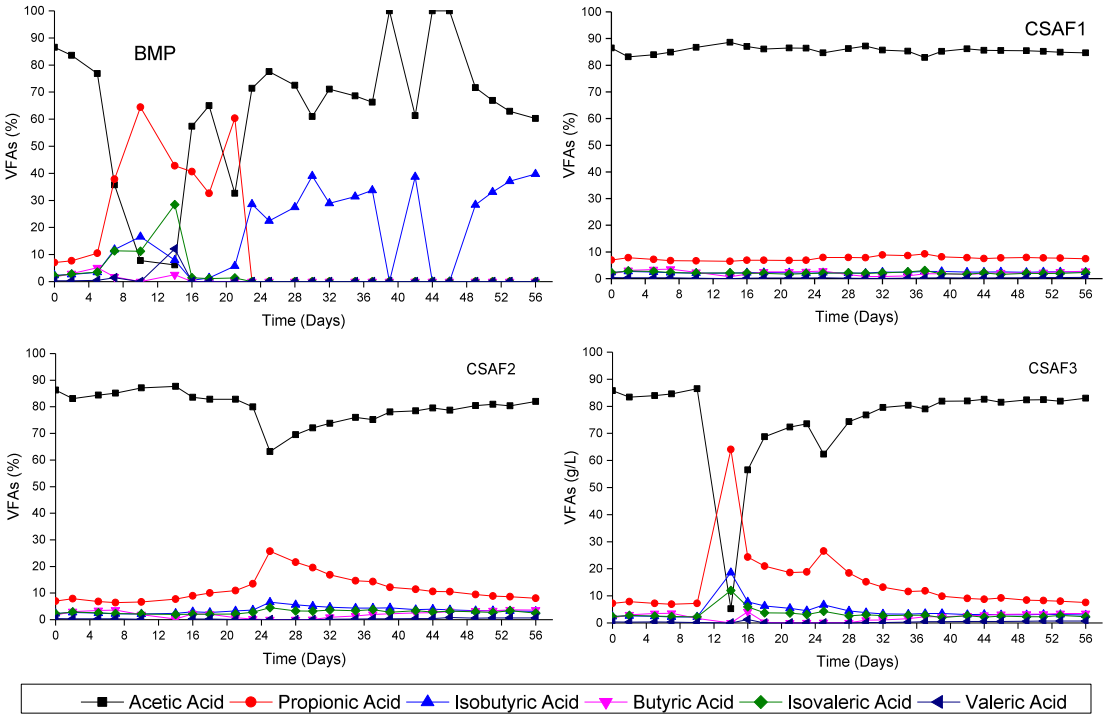

Figure 7.9. VFA profiles from acidogenic anaerobic fermentation of HPTH-WAS in semi-CSTR mode at pH 9.

(*Key: BMP: Biomethane potential test; CSAF1: Continuous Acidogenic fermentation 0.3 g TVS_{WAS}/L·d; CSAF2: Continuous Acidogenic Fermentation 0.6 g TVS_{WAS}/L·d; CSAF3: Continuous Acidogenic fermentation 1 g TVS_{WAS}/L·d).*

These findings are similar to the values reported by Yang *et al.* (2014) with acetic (51.8%) and propionic acids (16.7%) as the main products in the carboxylates blend in continuous fermentations of hydrolysed WAS at pH 12, with a SRT of 8 h; Wan *et al.* (2016) in mesophilic experiments at pH 10 with WAS from the secondary sedimentation tank and acetic acid with 54.1%; Yu *et al.* (2008) when working with WAS at pH 10 and mesophilic/thermophilic temperatures with a content between 72 to 100%; and Maspolim *et al.* (2015) who reports acetic acid contents above 50% in semi-CSTR experiments at pH 9 using sewage sludge in mono-substrate fermentation which confirms the effect of the alkaline pH on the production of short-chain carboxylic acids (acetic acid) as stated in previous chapters. Other studies working with a broad range of operational conditions can corroborate the viability on the production of acetic acid as it was presented as the main component in the acid blends, such as, Banerjee *et al.* (1999) (61-76%), Bouzas *et al.* (2002) (50.2-89%), Ghosh (1991) (44.46%), Hao and Wang (2015) (43.5-50.5%), Ji *et al.* (2010) (37%), Kumi *et al.* (2016) (35-40%), Maharaj and Elefsiniotis (2001) (56-74%), Morgan-Sagastume *et al.* (2011) (37-50%), Zhuo *et al.* (2012) (46-48%) and Zhou *et al.* (2014) (45%). It is also importance to notice that even in fermentations at low pH, acetic acid is the most predominant which is evidenced on the works from Elefsiniotis and Oldham (1994) who worked with pH levels below 6.2 and found concentrations of acetic acid above 42.4% of the total acid mixture in the fermentor. All this data supports the hypothesis of the high concentration of carbohydrates in the WAS as the products profile were mostly acetic acid which comes directly from the fermentation of simple carbohydrates to acetate by acetyl-CoA as intermediate (Shanmugam and Horan 2009; Temudo *et al.* 2007; Wang *et al.* 2014) and opens the possibility of the extraction and recovery of a more homogeneous product by the preference of a fermentation type (Yang *et al.* 2015). No long chain fatty acids (butyric or valeric) were detected in concentrations higher than 10% of the total VFAs mixture which also advises a low content and/or conversion of proteins into carboxylic acids (McInerney 1988). Overall there was a small or null effect of the OLR on the VFAs speciation in mixed semi-CSTR acidogenic fermentation as acetic acid presented concentrations higher than 80% of the content of carboxylic acids mixture.

Is also important to bear in mind the proportion on the conversion of the soluble organic material into VFAs, otherwise named as the degree of

acidification VFAs/SCOD as it represents activity of the microbial consortia in the fermentor; those results are presented in Figure 7.10.

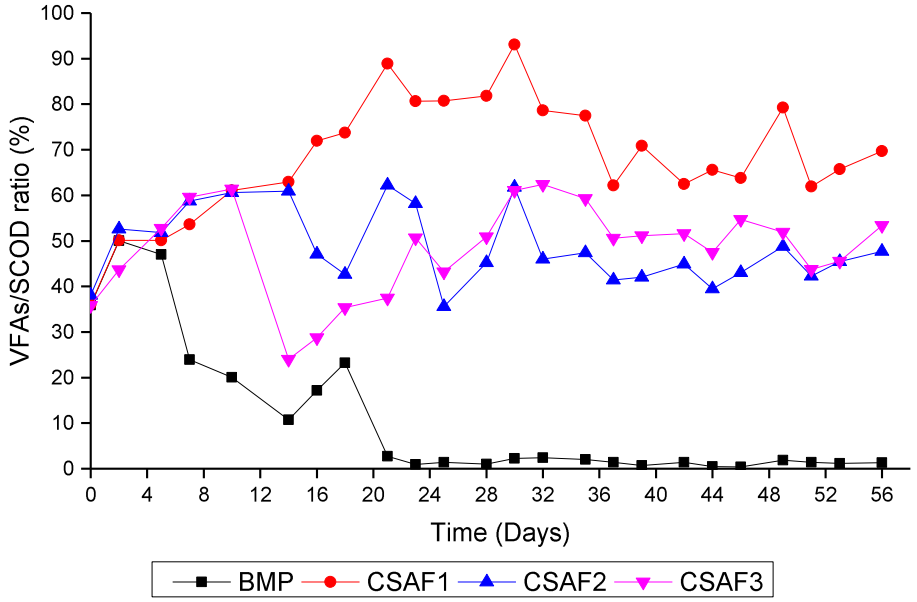

Figure 7.10. VFAs/SCOD ratio from acidogenic anaerobic fermentation of HPTH-WAS in semi-CSTR mode at pH 9.

(*Key: BMP: Biomethane potential test; CSAF1: Continuous Acidogenic fermentation 0.3 g TVS_{WAS}/L·d; CSAF2: Continuous Acidogenic Fermentation 0.6 g TVS_{WAS}/L·d; CSAF3: Continuous Acidogenic fermentation 1 g TVS_{WAS}/L·d).*

Degree of acidification from all the fermentors followed similar trend to the results of the degree of hydrolysis (SCOD/TCOD); for example, BMP reactors presented a quick increase on the degree of acidification on the first 2 days, with an augment of 30% with regards to the initial value, to reach a figure of 0.50 g VFAs as COD/g SCOD, but then dropped to a value of 10.73% on day 14 where most of the soluble COD were consumed and converted into biogas. During the semi-continuous cycle, there was a slight increase of the SCOD/TCOD on day 18 (23.24%) which can be endorsed to the lack of adaptability of the microbial consortia to a continuous feeding. Following that, there was a decrement on day 21 to a value of 2.69% and maintaining similar ratios until the end of the process on day 56, with an overall VFAs/SCOD value of 0.014, which suggest the rapid consumption of the VFAs from the WAS fed, into biogas and also,to the stability of the anaerobic digestion system.

VFAs/SCOD value in fermentor CSAF1 concur with the SCOD/TCOD progress reported previously, by increasing the degree of acidification steadily during the batch cycle and reaching a final value of 0.62 on day 14. When operated in continuous conditions, there was an evident increase on the degree of acidification ratio until reaching its highest value on day 30 (0.93 VFAs/SCOD) followed by a drop and an apparent semi-steady state until the end of the fermentation with a final value of 0.69 VFAs/SCOD, which signifies the adapted microbial consortia in the system was able to convert more than two-thirds of the soluble organic material in the fermentor into VFAs. On the other side, reactors CSAF2 and CSAF3 presented similar behaviour to the CSAF1 during the batch phase but with dramatic drops due to VFAs consumption at the end of the batch cycle which agrees with the values of the SCOD of mentioned fermentors. The continuous stage of CSAF2 and CSAF3 as well as CSAF1 reached a semi-steady state from day 30 in terms of the degree of acidification, with final valued of 47.7 and 53.4%, which shows a respectable degree of conversion of the soluble organic material from the HPTH-WAS into biochemicals.

Batch studies working with heat-alkaline pre-treated WAS at pH 10, using 2-bromoethanesulfonic acid sodium (BES) and HRT of 9 days conducted by Ma *et al.* (2016) report degrees of acidification of 19.63% that were smaller than same experiments ran at pH 7 (30.98%); on the other side, Zhou *et al.* (2013) reports degrees of acidification of below 4% when using ultrasonic pre-treated WAS, initial pH 10, and 10 days of fermentation. The performance in both studies could be attributed to the high alkaline pH that represent an extreme condition for the acidogenic bacteria as studied in this project, fermentations at pH 10 reached low production of VFAs.

Experiments ran in continuous stage with HPTH-WAS were conducted by Morgan-Sagastume *et al.* (2011) finding values between 0.5-0.6 g VFAs as COD/g SCOD in thermophilic conditions and 2,4 and 6 days of SRT. In contrast, investigations working with WAS in alkaline pH showed mixed results, such as, Li *et al.* (2011) with a VFAs/SCOD of 0.569 at pH 10 and 8 days HRT, 0.125 at pH 9 in semi-CSTR and mesophilic conditions, and, Yang *et al.* (2014) with 0.06 in CSTR, pH 12, 8 days of SRT and 43 days of fermentation. These results confirms that the experiments carried out in this project achieved respectable degrees of acidification possibly due to the long HRT used in the experiments and the slight alkaline conditions.

7.6 Effect of OLR in the biogas production on the acidogenic fermentation of HPTH-WAS in a single stage semi-CSTR.

Experiments in semi-CSTR in this project were carried out in equipment AMPTS II fitted out with a biogas measurement device, which passed through a solution of NaOH 10N to remove the CO_2 and hence, it was assumed that only CH_4 was quantified by the gas counter. Results are displayed below (Figure 7.11):

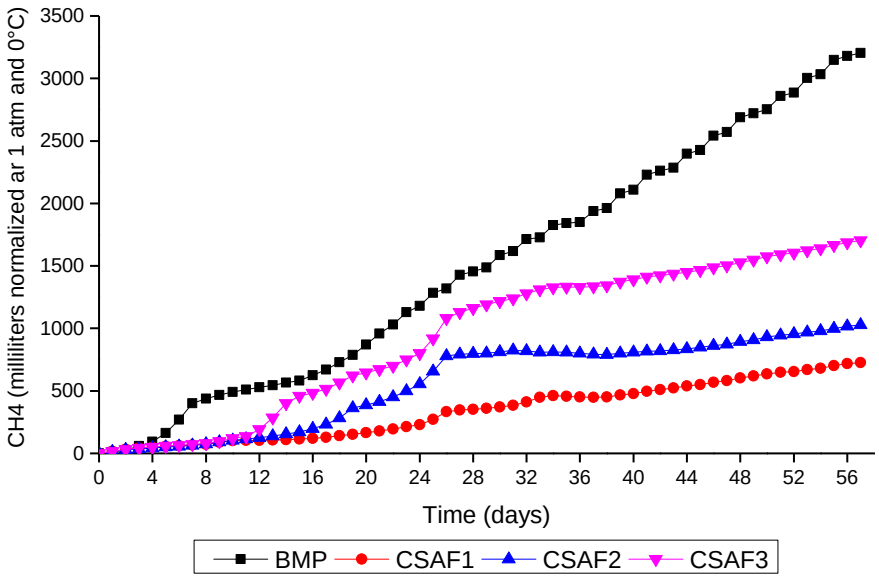

Figure 7.11. Biogas production results from acidogenic anaerobic fermentation of HPTH-WAS in semi-CSTR mode at pH 9.

(*Key: BMP: Biomethane potential test; CSAF1: Continuous Acidogenic fermentation 0.3 g TVS_{WAS}/L·d; CSAF2: Continuous Acidogenic Fermentation 0.6 g TVS_{WAS}/L·d; CSAF3: Continuous Acidogenic fermentation 1 g TVS_{WAS}/L·d).*

The results of BMP reactor show a typical tendency of a methane production in two stages, the first one by digesting soluble and easy degradable compounds with a delay from day 0 to day 5 and a quick first increase until day 8; and the second stage, the conversion of slowly degradable materials (Rodríguez 2012).

Although operated using high pH to avoid the production of methane by the methanogenic bacteria, the CSAF reactors presented similar trend to the BMP fermentor which suggest the ability for the production of VFAs and also

biomethane, making the biogas an opportunity of resource recovery. The lag phase in all CSAF fermentors was longer than the lag phase in BMP as the alkaline pH affected the methanogenic bacteria for a period of around 12 days when minimal biogas was produced. Apparently, OLR has an impact on the production of biogas as higher OLR generated higher amount of methane, with final results of 1702.6, 1028.8 and 725.2 mL of CH_4 on CSAF3, CSAF2 and CSAF1 respectively.

A non-linear regression, the Gompertz equation (Lay *et al.* 1997), was used to understand the production of biomethane during the experiments carried out (Equation 7.10):

$$M=P\cdot\exp\left(-\exp\left(\frac{R\cdot e}{P}\cdot(\lambda-t)+1\right)\right) \quad \text{.....Equation 7.10}$$

where M is the cumulative methane production (mL), t is the incubation time (days); λ is the lag-phase-time (d); P is the methane production potential (mL); R is the methane production rate (mL/day) and e is exp(1). The Gomperzt fitting was done using OriginPro 9.1 (OriginLab, Northampton, MA), for BMP, CSAF2 and CSAF3 and a linear regression for CSAF1, using the linear equation y=a+bx; results are showed in Figure 7.12.

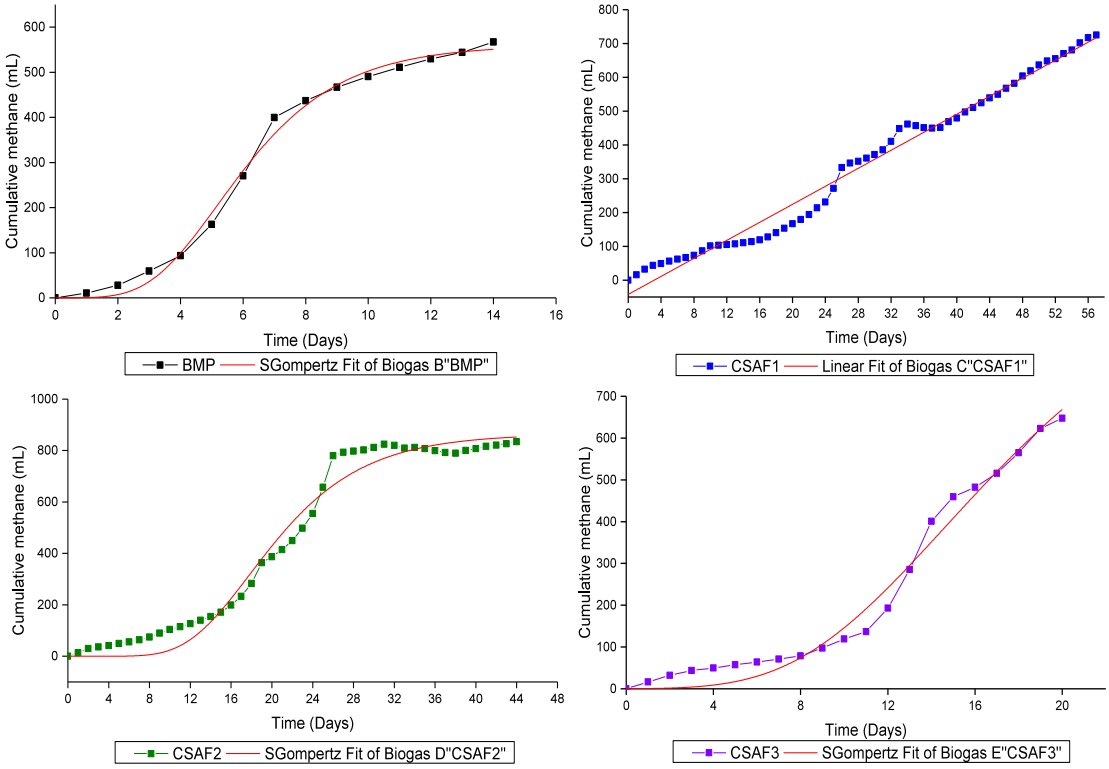

Figure 7.12. Cumulative methane results and linear/Gompertz model fitting from acidogenic anaerobic fermentation of HPTH-WAS in semi-CSTR mode at pH 9.

(*Key: BMP: Biomethane potential test; CSAF1: Continuous Acidogenic fermentation 0.3 g TVS_{WAS}/L·d; CSAF2: Continuous Acidogenic Fermentation 0.6 g TVS_{WAS}/L·d; CSAF3: Continuous Acidogenic fermentation 1 g TVS_{WAS}/L·d).*

Table 7.1 reveals that the maximum biomethane production potential (P) from the Gompertz model, are similar to the real values of BMP on day 14 (567.1 mL), CSAF2 on day 44 (834.8 mL) and CSAF3 on day 20 (647.3) which suggests the good fitting for the two first reactors and a poor fitting for the CSAF3, possibly due to the diauxic production of biomethane in mentioned reactor. Among all fermentors, BMP showed the shortest lag phase time with about 5.18 days, which could be explained because of the lack of control of the pH for the inhibition of methanogenic bacteria and the accumulation of VFAs. This outcome contrasts with the longer lag phase encountered for the CSAF2 and CSAF3 reactors, which is beneficial to the acidogenic fermentation purposes as the aim is the production and accumulation of VFAs and the avoidance of the generation of biomethane at alkaline pH, in semi-continuous mode. For the case of the CSAF1 (0.3 g

TVS/L·d) methane production did not adjust to the Gompertz model but a linear regression could model its behaviour, showing outcomes, such as, that the generation of methane was slow and steady, the VFAs production presented also a steady-state, most of the organic material was converted into VFAs and not into biogas, and, the concentration of VFAs is a direct function of time.

Table 7.1. Estimated regression parameters from the linear regression and the non-linear modified Gompertz equation.

Reactor	P (or a) [mL]	λ (or b) [d]	R [d]	R^2	Experimental [mL]
BMP	560.36	5.18	0.46	0.993	567.1
CSAF1	-42.32	13.32	12.72 mL/d	0.981	-
CSAF2	874.65	17.82	0.163	0.975	834.8
CSAF3	1053.60	14.63	0.1468	0.978	647.3

7.7 Stoichiometric methane potential (SMP) and biochemical methane potential (BMP) of the acidogenic fermentation of HPTH-WAS in a single stage semi-CSTR

In order to compare the potential advantage of the production of VFAs plus methane against the current anaerobic digestion for the production of only biomethane, theoretical biochemical methane was calculated considering the VFAs concentration and the biogas production on day 56 for the CSAF fermentors versus the BMP reactor using the formula proposed by Buswell and Mueller (1952), in order to determine the viability of the introduction of the carboxylate platform for the development of a biorefinery from WAS in the current WWTPs in the UK.

As the fermentations in this part of the project were carried out in semi-continuous configuration, the BMP and SMP were calculated in terms of millilitres produced during the experiment and also, obtaining a yield of methane per gram of TVS added. The biomethane calculation was according to the equation below:

Biomethane Potential=(Biogas in the reactor-biogas in the reactor blank)

+theoretical biogas from VFAs …….....Equation 7.11

With this information, the final amount of biomethane from the BMP reactor (OLR=0.3 g TVS/L·d) was 3214.6 mL, for CSAF1 (OLR=0.3 g TVS/L·d) was 4236.0 mL, CSAF2 (OLR=0.6 g TVS/L·d) with a value of 3745.7 mL and reactor CSAF3 with 6085.6 mL (OLR=1.0 g TVS/L·d). This is showing that fermentor CSAF1 with the same OLR as the BMP reactor, showed a theoretical improvement on the biogas over 31%. On the other side, the reactor CSAF2 only presented and increase of 16.5% even when the OLR was double than the BMP reactor which can be correlated with the non-stability on the system. Finally, the fermentor CSAF3 presented the highest concentration of VFAs on day 56 which caused an overall increase of 89.3% of the theoretical methane production, despite of not reaching the steady-state on the production of VFAs, which is endorsed to the 3.3-fold the OLR of the BMP reactor. As the steady state was not reached in reactors CSAF2 and CSAF3, it can be envisaged a further increase on the theoretical methane potential caused by the further accumulation of VFAs in the broth.

With the results calculated for the potential biomethane, a further analysis of the biogas yields was determined based on the amount of CH_4 (mL) and the theoretical TVS inlet in all the fermentors as follows:

$$\text{Methane Yield}=\frac{(\text{methane produced}+\text{methane calculated from VFAs})}{\text{Theoretical TVS added}}\ldots\text{Equation 7.12}$$

Methane yields of the four semi-continuous reactors carried out in this project were 173.76 mL CH_4/g TVS for the BMP reactor, 228.97 mL CH_4/g TVS for CSAF1, 140.81 mL CH_4/g TVS for CSAF2 and 148.42 for CSAF3 reactor. This results make clear that the fermentor with alkaline pH for the production of VFAs with an OLR of 0.3 g TVS/L·d showed again an increase of 31% of the biomethane potential with regards to the BMP reactor, whereas that fermentations with higher OLR presented only 81 and 85% of the biogas reached by the BMP reactor which implies that neither CSAF2 or CSAF3 reached steady conditions. When compared with the stoichiometric methane potential of the HPTH-WAS used in this study (461 mL CH_4/g

TVS), the fermentation with OLR of 0.3 g TVS/L·d could achieved 49.6% of the theoretical methane potential which is promising in the recovery of resources from HPTH-WAS.

Studies from Cano *et al.* (2014) report a CH_4 yield of 278.0 mL CH_4/g TVS, Bougrier *et al.* (2007) with a value of 256 mL CH_4/g TVS and Qiao *et al.* (2011) with a 257.3 mL biogas/g TVS when using similar conditions, HPTH-WAS (170ºC, 30 min) in mono-substrate digestion, in CSTR for 18-20 days. In contrast, Ferrer *et al.* (2008) report a lower 180 mL CH_4/g TVS in fermentations in CSTR, HRT 20 days of mixed sewage sludge treated at 70ºC for 9 hours. The results from these studies showed that fermentations with 0.3 g TVS/L·d achieved good methane yields but with the advantage of the recovery of VFAs which could have a higher value than the biomethane.

Further economic and energetic analysis is proposed to be done with regards to the addition of NaOH for the control of pH and the potential extraction of liquid biofuels instead of energy recovery based only on the production of biogas.

7.8 Summary

The single-stage semi-continuous acidogenic fermentation of HPTH-WAS using an OLR of 0.3 g TVS/L·d presented the highest efficiency on the COD solubilisation at pH 9 (39.4%), suggesting a respectable hydrolytic microbial activity and showing the potential for its use in pilot or full-scale reactors.

In terms of VFAs production, fermentor with OLR of 0.3 g TVS WAS/L·d showed VFAs concentration on day 56 of 6.059 g VFAs/L, whereas fermentations with OLR=0.6 g TVS WAS/L·d a maxima of 5.449 g VFAs/L, and finally, fermentations using OLR of 1 g TVS WAS/L·d, a final VFAs content of 8.905 g VFAs/L. Fermentations with 0.6 and 1 g TVS WAS/L·d did not reach a steady-state in terms of VFAs concentration which can be attributed to the short acclimation period for the microbial population, suggesting an potential further increase on yields and production of VFAs when stability is reached.

The final yields on day 56 were 0.539, 0.328 and 0.364 g VFAs/g TVS for fermentors with OLRs of 0.3, 0.6 and 1 g TVS WAS/L·d, respectively, which suggests a respectable production of VFAs from HPTH-WAS and that increasing OLR might not exhibit an effect on the improvement of the overall VFAs yields. As the HRT in these experiments was 14 days, it is advisable to work on the optimisation to diminish the fermentation period which can at the same time provide a high conversion of organic material into carboxylic acids.

Acetic acid was the most predominant in the acid blend in all reactors as it is produced through an energetically favourable reaction, but higher OLR could increase the amount of medium/large-chain carboxylic acids, specially propionate.

Degree of solubilisation reached values 0.69, 0.47 and 0.53 in fermentations with OLRs of 0.3, 0.6 and 1 g TVS WAS/L·d, respectively, which shows a respectable degree of conversion of the soluble organic material from the HPTH-WAS into VFAs, which can be endorsed to the long HRT (14 days) used and the alkaline conditions.

Acidogenic fermentation with OLR=0.3 g TVS WAS/L·d, showed a theoretical improvement on the biogas over 31%, when compared with the BMP reactor, whereas fermentor with an OLR of 1 g TVS WAS/L·d CSAF3

presented an overall increase of 89.3% of the SMP, which is endorsed to the 3.3-fold the OLR of the BMP reactor.

Although acidogenic fermentations in semi-continuous reactors have showed respectable production of VFAs and biomethane, more research is needed to determine the production of other compounds such as hydrogen, lactic, succinic and caproic acids, which were not analysed in these experiments. As this trials aimed to present an approach to the acidogenic fermentation, one of the main advantages on the resource recovery from the carboxylate platform is the additional potential conversion of the remaining organic material to other products but economical and operational analyses are recommended to be run to determine if the energy input in the HPTH process could be recovered after the carboxylate platform process in wastewater treatment works and if can potentially a previous step to the current anaerobic digestion for energy recovery from WAS as part of the biorefinery concept.

Chapter 8. CONCLUSIONS

This project addresses the research on the effect of different parameters, such as methanogenic bacteria inhibitor ratio, pH, feedstock pre-treatment and co-fermentation and C/N ratios, on the mixed acidogenic fermentation of HPTH-WAS from the UK WWTP.

The first part of the project focused on finding the best methane producer bacteria inhibitor ratio using iodoform as an inhibition agent for the production and accumulation of VFAS from the mixed acid fermentation of HPTH-WAS, achieving yields of 0.238 g TVFAs/g TVS_{WAS} with inhibition ratio of 6 mg CHI_3/g VSS, OLR of 5 g TVS_{WAS}/L) and 21 days of HRT. A small or null effect of the iodoform dosage was found on the VFAs speciation with acetic and propionic acid as the main products in the VFAs mixture regardless the CHI_3 dosage applied and, a positive effect on the VFAs production/accumulation; its conversion into methane was poor. The main outcome was the finding of a ratio, which relates to the amount of inhibitor with the $VSS_{Inoculum}$ for the mixed acidogenic fermentation.

The second part of the project focused on testing the effects of different pH levels on the acidogenic fermentation of HPTH-WAS. The main outcomes were: fermentations operating with pH 9 achieved yields of 0.415 VFAs/g TVS_{WAS}, high organic material hydrolysis (0.46 SCOD/TCOD), inhibition of methanogenic bacteria, and the neutralisation of VFAs to avoid the inhibition by the products. It was also found that highly acidic pH (4-5) presented a positive impact on the inhibition of the production of methane, a negative influence on the production of VFAs, and a positive effect for the production of long-chain fatty acids. In contrast, alkaline pH (8-10) presented a positive impact on the solubilisation of organic material which denotes the constant activity of bacteria in the generation of soluble products such as VFAs and, a positive effect on the specific production of short-chain fatty acids, confirmed by the sustained generation/accumulation of acetate and propionate.

The third stage of the project was assessing the impact of the hydrothermal pre-treatment and co-fermentation on mixed acidogenic fermentations of mixtures of HPTH-WAS and food waste or microalgae at pH 9. It was discovered that treatment with HPTH improved the solubilisation of the

organic material with increments from 21.8% to 44.1% for food waste and 8.1% to 39.8% for microalgae.

It was evident that food waste pre-treatment had a positive effect on the final production of VFAs despite the C/N ratio used, as co-fermentations with HPTH-WAS with similar C/N ratios, showed yields of 0.370, 0.391 and 0.496 g VFAs/g TVS for raw, autoclaved and HPTH-food waste. Agreeing with these findings were co-fermentations of microalgae with HPTH-WAS which achieved 0.230, 0.312 and 0.378 g VFAs/g TVS for raw, autoclaved and HPTH-microalgae, which showed the positive impact of the substrate pre-treatment and the co-fermentation on the generation of VFAs regardless of the operation with low C/N ratios. Overall, there was no impact of the co-fermentation of HPTH-WAS with food waste or microalgae or the feedstock pre-treatment on the speciation of the VFAs produced in the mixed acidogenic fermentors.

Finally, after making a comparison of all yields obtained in the tests carried out in this project to find the best operational conditions, the last part of the research focused on the operation of a semi-CSTR using HPTH-WAS as mono-substrate at pH 9 to assess the effect of different OLRs. The main results obtained were yields of 0.539, 0.328 and 0.364 g VFAs/g TVS for fermentors with OLRs of 0.3, 0.6 and 1 g TVS WAS/L·d, respectively, which suggests that increasing OLR did not exhibit an improvement on the final conversion of organic substrate to VFAs and, the small or null effect of the OLR on the VFAs speciation as acetic acid was present in concentrations above 80% of the carboxylic acids content in all experiments tested. In conclusion, fermentations working with 0.3 g TVS WAS/L·d presented an overall VFAs production which stoichiometrically exceeded in 31% the CH_4 production from AD experiments for the exclusive generation of methane carried out in this project.

Having these conclusions in mind, several areas need further investigation in order to optimise the carboxylate platform applied to HPTH-WAS for the full scale use in wastewater treatment plants.

a) As the HRT in these experiments were 14 or 21 days, it is advisable to work on the optimisation to diminish the fermentation period to reach a high conversion of organic material into carboxylic acids in the lowest time frame.

b) Fermentations with medium and high OLR did not reach a steady-state in terms of VFAs concentration or biogas, which opens a gap for the study of fermentations with a number different OLRs and shorter/longer period of fermentation to reach the stability of the system.
c) Different OLR and HRT could be tested simultaneously to develop a biochemical mapping and flux analysis for the optimisation of the acidogenic fermentation.
d) Research is needed to determine the production of other compounds such as lactic, succinic and caproic acids for its possible recovery and/or utilisation or to direct the fermentation via different metabolic routes.
e) To assess the feasibility of the recovery of biohydrogen as it is one of the main by-products of the metabolic routes in the production of VFAs.
f) To calculate economical and operational analyses to determine the operational expenditures of the carboxylate platform process in alkaline conditions, for the potential combination of VFAs production and recovery with the current anaerobic digestion for the production of methane.
g) To investigate the possible extraction and employment of VFAs for conversion to higher valuable products such as ethanol, ketones, alkanes, etc.

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Appendix 1. **Paired samples t-test.**

Table A.1. Paired samples t-test for yields of the acidogenic fermentation of WAS, FW and microalgae and its pre-treatments.

FOOD WASTE	t	p	Best HRT	**MICROALGAE**	t	p	Best HRT
25RFW_d14 vs d7	1.416	0.293	7 d	**25RA_d21** vs d7	2.548	0.126	7 d
25RFW_d14 vs d21	10.267	0.009		**25RA_d21** vs d17	12.124	0.07	
50RFW_d17 vs d7	4.489	0.046	17 d	**50RA_d17** vs d14	4.670	0.043	17 d
50RFW_d17 vs d14	4.832	0.040		**50RA_d17** vs d21	0.609	0.605	
75RFW_d21 vs d14	2.129	0.167	14 d	**75RA_d17** vs d14	12.912	0.006	17 d
75RFW_d21 vs d17	2.621	0.120		**75RA_d17** vs d21	0.402	0.727	
25ACFW_d21 vs d7	2.177	0.161	5 d	**25ACA_d5** vs d7	1.207	0.351	5 d
25ACFW_d21 vs d14	1.732	0.225		**25ACA_d5** vs d10	27.238	0.001	
25ACFW_d21 vs d5	3.677	0.067		**50ACA_d14** vs d10	2.880	0.102	10 d
50ACFW_d14 vs d17	1.876	0.201	14 d	**50ACA_d14** vs d21	1.648	0.241	
50ACFW_d14 vs d21	1.946	0.191		**75ACA_d17** vs d10	15.588	0.004	14 d
50ACFW_d14 vs d10	17.95	0.003		**75ACA_d17** vs d14	0.933	0.449	
75ACFW_d14 vs d17	2.44	0.135	14 d	**75ACA_d17** vs d21	2.868	0.103	
75ACFW_d14 vs d21	2.359	0.142		**25HPTHA_d14** vs d7	16.538	0.004	10 d
75ACFW_d14 vs d7	71.0	0.000		**25HPTHA_d14** vs d10	2.172	0.162	
25HPTHFW_d21 vs d14	1.054	0.402	14 d	**50HPTHA_d14** vs d10	5.512	0.031	14 d
25HPTHFW_d21 vs d17	-2.362	0.142		**50HPTHA_d14** vs d17	0.815	0.501	
25HPTHFW_d14 vs	5.816	0.28		**50HPTHA_d14** vs	0.272	0.811	

d10				d21			
50HPTHFW_d21 vs d14	5.864	0.28	17 d	**75HPTHA_d21** vs d14	19.256	0.003	21 d
50HPTHFW_d21 vs d17	3.605	0.69		**75HPTHA_d21** vs d17	6.381	0.024	
75HPTHFW_d21 vs d14	8.626	0.13	21 d				
75HPTHFW_d21 vs d17	4.547	0.45					

Days with the highest yields are noted in bold letters.

Key: 25RFW_d14 denotes the mixture of 25% raw food waste on day 14, thus, 25HPTHFW_day21 is the mixture of 25% HPTH-FW on day 21.

Table A. 2. Paired samples t-test for yields of the acidogenic fermentation of WAS, FW and microalgae and its pre-treatments.

FOOD WASTE	t	df	p	**MICROALGAE**	t	df	p
25RFW_d7 vs 50RFW_d17	-4.759	4	0.009	25RA vs 50RA	-4.094	4	0.015
25RFW_d7 vs **75RFW_14**	-5.865	4	0.004	25RA vs **75RA**	-15.806	4	0.000
50RFW_d17 vs **75RFW_14**	-2.666	4	0.056	50RAvs **75RA**	-6.234	4	0.003
25ACFW_d5 vs 50ACFW_d14	-4.576	4	0.010	25ACA vs **50ACA**	-13.750	4	0.000
25ACFW_d5 vs **75ACFW_d14**	-10.413	4	0.000	25ACA vs 75ACA	-16.901	4	0.000
50ACFW_d14 vs **75ACFW_d14**	-10.826	4	0.000	**50ACA** vs 75ACA	1.444	4	0.222
25HPTHFW_d14 vs 50HPTHFW_d17	-7.585	4	0.002	25HPTHA vs 50HPTHA	-1.108	4	0.330
25HPTHFW_d14 vs **75HPTHFW_d21**	-11.284	4	0.000	25HPTHA vs **75HPTHA**	-4.667	4	0.009
50HPTHFW_d17 vs **75HPTHFW_d21**	-4.890	4	0.008	50HPTHA vs **75HPTHA**	-4.822	4	0.009

Days with the highest yields are noted in bold letters.

Key: 25RFW_d14 denotes the mixture of 25% raw food waste on day 14, thus, 25HPTHFW_day21 is the mixture of 25% HPTH-FW on day 21.