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Acidogenic fermentation of organic residual solids from municipal solid waste

by

Maria Ramos Suarez

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ABSTRACT

FACULTY OF ENGINEERING AND PHYSICAL SCIENCES

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Acidogenic fermentation of organic residual solids from municipal solid waste

Maria Ramos Suarez

Solid waste represents a threat to the environment and human health. Organic residues can be converted to valuable products through biological processes, reducing the volume and risks associated to the degradability of the waste. Acidogenic fermentation (AF) is the mixed-culture microbial conversion of organics to volatile fatty acids (VFA), such as acetic, propionic, and butyric acids. VFA are chemical platforms used for the synthesis of high value-added products. There is currently a lack of understanding on mechanisms to control pathways to avoid unwanted by-products; and maximise VFA yields.

In this thesis, organic residual solids (ORS) have been used as substrate to study the effect of different fermentation conditions such as pH, hydraulic retention time, carbon to nitrogen ratio, $CaCO_3$ addition, urea addition, feeding mode, inoculum pretreatment, and enzymatic pretreatment. It was found that fermentation conditions highly influence the type of VFA generated and the VFA yield. Fed-batch feeding mode resulted in increased VFA concentrations and yields; and longer chain fatty acids were produced.

A large-scale process was also proposed followed by an economic assessment of the AF process using ORS as substrate, further demonstrating the economic potential of biological waste conversion to high-value products. The assessment concluded that most of the variable operational cost is due to feedstock pretreatment and product recovery, due to the high cost of enzymes and the energy required for VFA recovery.

Keywords: Acidogenic fermentation; volatile fatty acids; municipal solid waste; anaerobic digestion; organic waste; renewable chemicals; acetic acid; propionic acid; butyric acid.

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List of Accompanying Materials

Data supporting this thesis is available from:

https://doi.org/10.5258/SOTON/D2526

Declaration of Authorship

I, Maria Ramos Suarez, declare that this report and the work presented in it are my own and has been generated by me as the result of my own original research.

Acidogenic fermentation of organic residual solids from municipal solid waste

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this report has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the report is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. Part of this work has been published in https://doi.org/10.1007/ s11157-021-09566-0.

Signed: .....

Date:

Preface

Early 2000s, my mother is walking me to my catholic school in the humble neighbourhood where we live in Cadiz, Spain. I open the "Conocimiento del Medio" (Knowledge of the Environment) book which I already scanned through during the summer, before the academic year began. *Wachi-whachi*, our teacher, is smiling as always. He is an unusual man. He has long hair, pierced ears and often wears colourful psychedelic t-shirts. Most people describe him as a hippie. *Wachi-wachi* does not mind if we do not call him "mister" like the other teachers. He is the one who introduced me to the famous quote "we do not inherit the earth from our ancestors; we borrow it from our children" and the motto "reduce, reuse, recycle". I look outside our classroom window and the sight of the tall green bushes reminds me of what my mother said about why she chose this school. It apparently is the only school in the vicinity with a pretty garden. Green spaces are rare in the city of Cadiz where buildings occupy all the land available from one sea line to another.

Twenty years later I am writing my thesis, this thesis, with the desire of being awarded a PhD in Environmental Engineering. It is hard to focus on writing in the lockdown imposed by the UK government to tackle the COVID-19 pandemic. I clearly am more of a 'lab rat' than a writer. Scientists believe climate change could be a major factor influencing the spread of infectious diseases. Who would have thought? More information can be found on "Impact of climate change on human infectious diseases: Empirical evidence and human adaptation" by Wu et al. (2016). I am not saying that COVID19 is a direct consequence of global warming, since there is no scientific evidence of it, but it certainly is something to ponder on. What we know for sure is that climate change is not a hoax, as some politicians suggest, and if humankind continues its activity as 'normal' we will face catastrophic consequences.

This work is my small contribution to humankind. For the sake of future generations.

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Dedicado a mi madre, padre y hermana. Espero que mi trabajo les traiga orgullo. [Dedicated to my mom, dad, and sister. I hope this work makes them proud.]

Definitions and Abbreviations

AD	Anaerobic digestion
AeAS	Aerobic activated sludge
AF	Acidogenic fermentation
AFEX	Ammonia fibre explosion
AIC	Akaike information criterion
AIL	Acid insoluble lignin
AnAS	Anaerobic activated sludge
ASL	Acid soluble lignin
BES	2-bromoethanesulfonate
BNR	Biological nutrient removal
BOD	Biological oxygen demand
C/N	Carbon to nitrogen ratio
CEPC	Chemical Engineering plant cost index
CHF	Consecutive hydrolysis and fermentation
CHF	Consecutive hydrolysis and fermentation
COD	Chemical oxygen demand
CSTR	Continuous stirred tank reactor
DMDO	Dimethyldioxinane
DoA	Degree of acidification
DoE	Design of experiments
EBITDA	Earnings before interests, taxes, depreciation, and amortisation
GC	Gas chromatography
HAc	Acetic acid
HBu	Butyric acid
HHe	Hexanoic acid
HPr	Propionic acid
HRT	Hydraulic retention time(s)
HVa	Valeric acid
IC	Ion chromatography
iHBu	Iso-butyric acid
iHVa	Iso-valeric acid
MFC	Microbial fuel cell(s)
MS	Mixed sludge
MSW	Municipal solid waste
NMMO	N-methylmorpholine-N-oxide
NPV	Net present value
OF	Organic fraction
OFMSW	Organic fraction of municipal solid waste
OLR	Organic loading rate(s)
ORP	Oxidation-reduction potential
ORS	Organic residual solids
PBBR	Packed bed bioreactor
PEBA	Polyether block amide

PFR	Plug flow reactor
POMS	Peroxymonosulphate
PPCO	Polypropylene copolymer
PS	Primary sludge
PTFE	Polytetrafluoroethylene
RMSE	Root mean square error
S/I	Substrate to inoculum ratio
sCOD	Soluble chemical oxygen demand
SDBS	Sodium dodecylbenzene sulfonate
SHF	Simultaneous hydrolysis and fermentation
SHF	Simultaneous hydrolysis and fermentation
TAN	Total ammonia nitrogen
TDCC	Total direct capital costs
TDDA	Tridodecylamine
TICC	Total indirect capital costs
TKN	Total Kjeldahl nitrogen
TOC	Total organic carbon
TOPO	Trioctylphosphine oxide
TPEC	Total purchased equipment cost
TS	Total solids
TSS	Total suspended solids
UASB	Upflow anaerobic sludge blanket reactor
VFA	Volatile fatty acid(s)
VS	Volatile solids
VSS	Volatile suspended solids
WAO	Wet air oxidation
WAS	Waste activated sludge

Chapter 1 Introduction

This thesis studies the acidogenic fermentation (AF) of a waste stream from the Fiberight process for volatile fatty acid (VFA) production. In this chapter, the research problem and motivations are defined by giving context on global waste production, the different waste management practices currently available and an overview of the Fiberight process. Finally, the research aims and objectives as well as the scope of this thesis are described.

1.1 Research problem

1.1.1 Global waste problems

Global production of municipal solid waste (MSW) is currently approximately 2,010 million tonnes per year. Due to population growth, increasing urbanisation and global wealth, it is estimated that MSW generation will rise to 3,400 million tonnes a year by 2050 [1]. High-income countries (defined as countries with a gross national income per capita of international-$12,476 or higher in 2015) are the main contributors to MSW generation (34% of total generation). The North America region (USA, Bermuda and Canada), of all regions, had the highest MSW generation rate of 2.21 kg per capita per day [1].

MSW is a biological hazard which needs to be managed and disposed away from residential areas in order to avoid the spread of diseases, pests and parasites. Generation, management and disposal of MSW has a significant impact on the environment. Current global waste generation accounts for about 5% of global greenhouse gas emissions as a result of transportation, processing and, mostly, decomposition of organic waste after disposal [1]. Waste management also has a significant impact on the public economy: waste management expenditure can vary from 4% to 20% of total municipal budget, for high and low income countries respectively [1].

The composition of MSW varies across countries and cities, depending mainly on number of inhabitants and consumption habits [2]. Generally, MSW contains food waste, plastic packaging, garden waste, cans, glass, paper, cardboard and other household and commercial waste, including fabrics. Approximately 44% of MSW across the world is comprised of biodegradable matter containing food and green wastes, and 61% if paper and cardboard are included [1]. Currently, most MSW is sent to landfills or dumped in the environment or on designated land, while only 30% is recycled, composted or incinerated (see Figure 1). Most of the open dumping and uncontrolled burning of MSW happen in developing countries, which causes pollution and increased risks of human diseases. In developed countries, open dumping is usually prohibited,

but landfilling remains a common practice in many places due to the lack of economic drivers for recycling MSW.

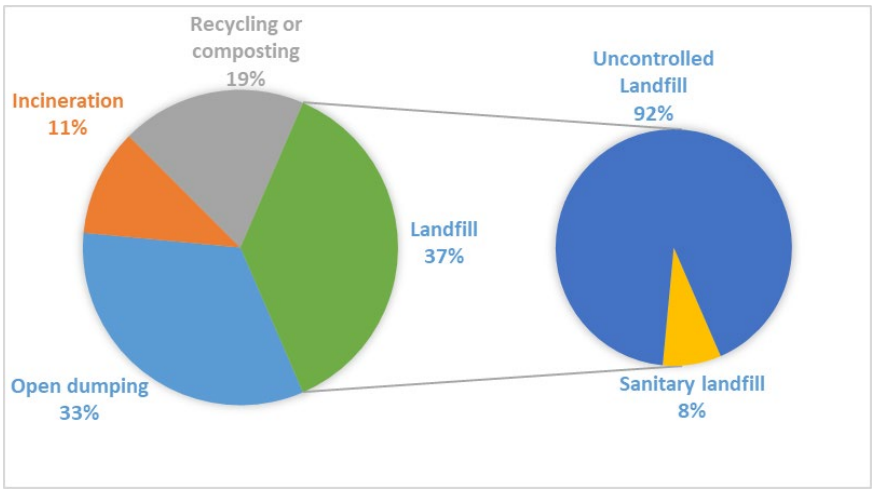

Figure 1. Global MSW management practices. Data obtained from [1]

Numerous environmental problems are associated with uncontrolled landfilling, including methane and carbon dioxide emissions, pollution of water bodies and land use. Engineered landfills with biogas and leachate recovery diminish these problems in addition to generating bioenergy, but leakages are unavoidable, representing a risk to public health and the environment [3]. Landfills can have high operating costs (up to 120 USD or 90 GBP per tonne) [4], and after closure, the surface cannot be used for civil constructions due to structural soil instability [3].

Incineration has the advantage of reducing waste volumes [5], sanitising the waste, and facilitating energy recovery in the form of heat or electricity. In developed countries, incinerators generally run under strict emissions regulations established by local or national governments or other regulatory bodies such as EPSA in the USA, and recent technology allows relatively low emissions of NO_x, SO_x, dioxins, particulate matter and heavy metals [6]. Even in a well-operated modern system, some problems are still associated with incineration: strict requirements in composition and moisture content (calorific value) of the waste [5], ash generation, high capital cost (up to 1000 USD/ annual tonne) [4], and loss of resources (recyclables) and nutrients such as nitrogen.

In Europe, public opinion and the Zero Waste public policy [7] are pushing the agenda towards more environmentally friendly alternatives to landfill and incineration. The Landfill Directive 1999/31/EC was implemented across the European Union and EEA countries in order to achieve zero recyclable waste sent to landfill by 2025 [8], including food waste and other organic wastes. The UK still sends more than 7 million tonnes of biodegradable MSW to landfills and recycling rates are lower than 50% [9], therefore significant improvements are needed to achieve this.

1.1.2 Alternatives to landfill and incineration

With the exception of landfill, most waste management treatments require a mechanical sort to separate materials of different composition. Ideally, MSW should be source segregated, allowing a more selective separation of different fractions for more efficient recycling. This is often not the case, as it requires high consumer awareness, a varied recycling collection system and minimisation of mixed materials in packaging. MSW can be mechanically separated into different fractions to recover recyclables (plastics bags, cans, glass, fabrics, bottles, etc.) and obtain an organic fraction (OFMSW) that can be converted chemically or biologically into valuable products.

Apart from incineration, other thermal treatments available are gasification and pyrolysis. Gasification is a thermochemical conversion with limited O_2 (i.e. partial combustion) and pyrolysis is carried out in complete absence of O_2. Both processes generate a gaseous mixture known as syngas (H_2, CO_2, CO, CH_4 and C_xH_x) which can be combusted for energy production, a solid char, and a liquid bio-oil (tar) [10]. These technologies have some impediments such as the need for homogeneity, low moisture content and high caloric value [11], which are rarely qualities of OFMWS.

Biological processes also play an important role in the management of the organic wastes. For highly heterogeneous wastes, e.g. OFMSW, composting and anaerobic digestion (AD) are the best options. Both processes involve the use of mixed cultures and produce a soil amendment; however, AD has the added benefit of producing biogas (a mixture of CH_4 and CO_2) [2].

Other bioenergy processes include production of bioethanol using yeasts (ethanol fermentation) or lipids for biodiesel using heterotrophic microalgae. Both technologies need a high level of solubilisation, therefore the use of solid wastes, including OFMSW, for lipid production has not been widely studied [12]. These processes require a sourced segregated waste and they are generally more appropriate for agricultural wastes with a more homogeneous composition. The complex composition of OFMSW as well as the technology readiness, make AD the best option available for energy conversion of mixed food waste or the OFMSW. AD conditions can be varied to target H_2 production. This process is known as dark fermentation and it is an emerging technology.

Other biological processes (fermentations) using yeast, bacteria or fungus can be applied to produce chemicals such as solvents and acids [13, 14]. Acidogenic fermentation (AD with inhibited methanogenesis) is the microbial production of volatile fatty acids (C2-C6 fatty acids) and OFMSW is a suitable substrate for this process [15–18]. Some of the advantages of AD apply to acidogenic fermentation (AF).

1.1.3 Fiberight process overview and challenges

Fiberight is a company which developed a novel process to separate different MSW fractions, such recyclables (plastic, cans and glass), and recovers the organic biodegradable fraction for conversion to biofuels and other value-added products using chemical and biological technologies. More information can be found at fiberight.com. The main advantage of this process is that the waste does not need to be source-segregated.

Figure 2 shows a schematic overview of the Fiberight process. The MSW is subjected to pre-sorting to remove textile and large objects, and subsequently to a thermal treatment known as "pulping". This partially sterilises the waste and breaks down the cellulose of paper creating a soft and wet pulp. This treatment also helps with the second sorting to remove recyclables. Afterwards, the organic fraction is sent to a washing unit where water removes impurities to generate a clean fibre. This fibre can be hydrolysed into sugars using enzymes, and these sugars can be utilised in other biological applications to generate high value products or biofuels. The dirty water resulting from the washing stage is sent to an anaerobic digester where soluble organics are metabolised to biogas, which can be used as an energy source to power the plant. The resulting liquid stream can be reutilised in the washing process to minimise the demand for fresh water. The dirty water must be previously clarified (i.e. cleared of suspended solids) before digestion to avoid solids accumulation inside the liquid-only AD system. This clarification step generates a waste stream of organic residual solids (ORS).

The ORS has an organic content measured as volatile solids (VS) of 65-80% (w/w), depending on the waste source. The ORS contain pulp (lignocellulosic material) and other impurities, including organic (lipids and proteins) and inorganic substances (mostly grit). Further treatment of this ORS could help reduce the volumes and the biodegradability of waste sent to landfill by the Fiberight process, complying with the circular economy principle and the zero waste philosophies that the company is driven by. Lower biodegradability of the final ORS is desired as it will lead to lower emissions if sent to landfill or it might increase its potential for energy recovery.

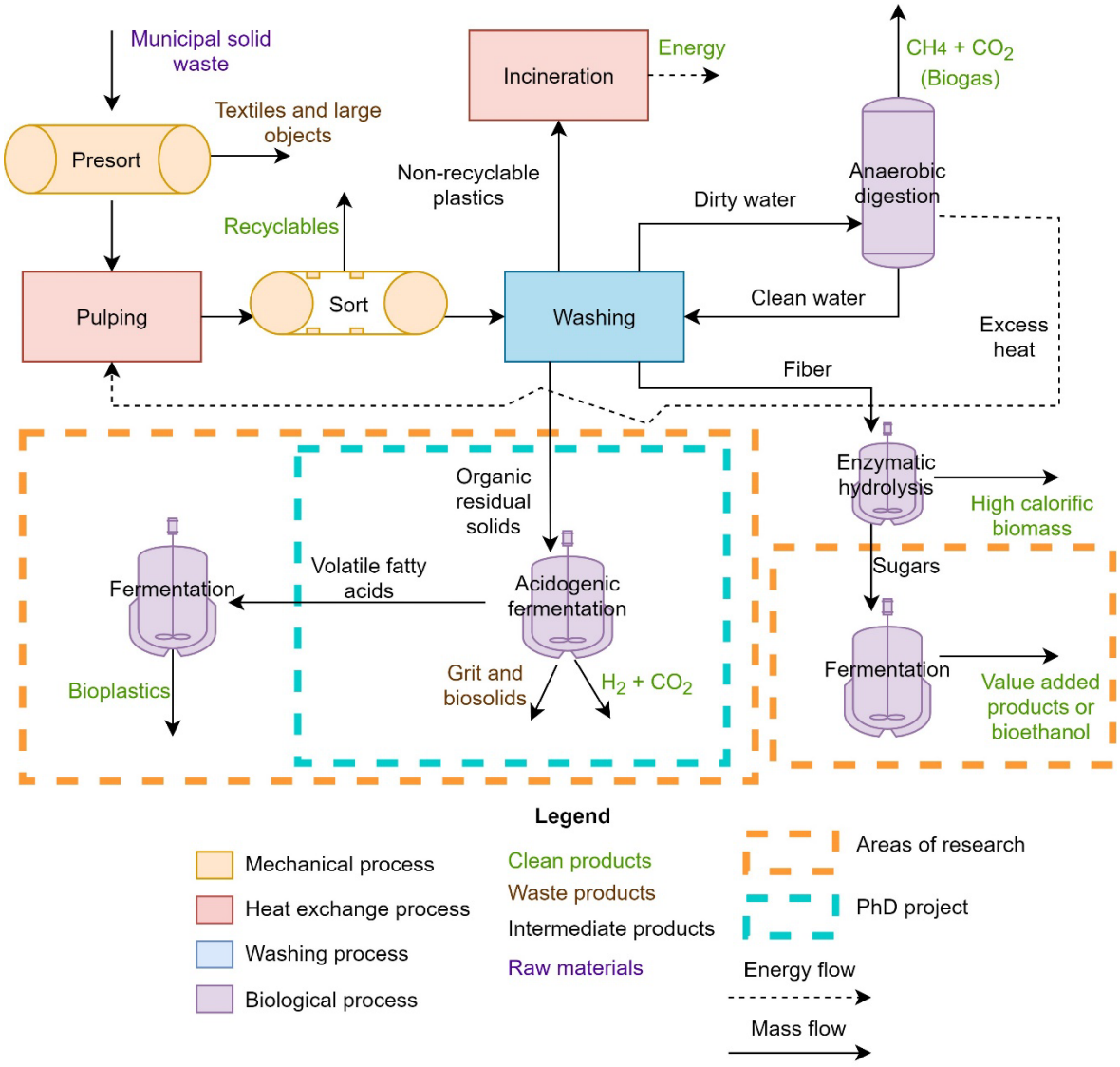

Figure 2. Block diagram of the Fiberight process

As of March 2020, Fiberight Ltd possessed a demonstration facility at Lawrenceville, Virginia (USA) and the company had sold its technology to Coastal Resources of Maine, which commissioned a plant in Hampden (Maine, USA). Fiberight has been planning to expand commercial activity to UK, where most of its research is carried out [19, 20].

1.1.4 Preliminary research

Several approaches are possible in order to increase solubilisation of organic solid wastes, including physical, chemical and biological treatments. Enzymatic treatment is a promising option in terms of energy demand, chemical input and environmental factors [21]. Although the use of enzymes for conversion of MSW was first reported in 1980 [22], these studies are scarce. As enzymes are specific to the substrate (i.e. different type of enzymes are required for each type of

substrate), the characterisation of the waste composition is advantageous to predict what enzymes are suitable.

A study was conducted in summer 2017 with ORS pretreated with enzymes to improve methane production. A manuscript with the results from this study can be found in Appendix B. After a techno-economic analysis it was found that methane uplift due to enzymatic pretreatment did not compensate for the cost of the enzymes due to the relative low value of methane. In addition, it was found that when the ORS were incubated at mild temperatures for 24 hours prior to AD, the acidification step was accelerated, producing an accumulation of volatile fatty acids (VFA) and a consequent negligible methanisation. These results led to the conclusion that the ORS could be used for VFA production. VFA production theory and the literature on VFA productions is discussed in detail in Chapter 2.

1.2 Research aim

The aim of this research was to assess the degree of feasibility of VFA production from the ORS via acidogenic fermentation and identifying the main factors affecting VFA yields. This aim was divided in the following four objectives:

1. Optimising the enzyme pretreatment for enhanced ORS solubility and digestibility by AF bacteria, identifying the right enzyme mixture and operational variables;
2. finding the mechanisms to control product pathways and maximise VFA yields, varying different batch fermentation conditions such as pH, alkalinity, substrate to inoculum ratio, substrate concentration, urea addition, and substrate concentration;
3. studying the effect of various feeding loads and timings in different reactor modes, semi-continuous and fed-batch, on the VFA profile, VFA yields, and by-product formation;
4. proposing a large-scale process configuration and performing a preliminary economic evaluation for the continuous AF of ORS, using experimental data and data found in the literature.

1.3 Knowledge gaps and scope of research

This thesis contains a detailed literature review on volatile fatty acids (VFA) production from organic wastes studies using mixed culture, to establish a baseline for comparison with this research work. Several knowledge gaps on AF have been identified, among which the most important have been listed:

- Lack of understanding on fermentation parameters interactions;

- insufficient evidence of optimum parameters for VFA generation and the effect of these parameters on VFA profile, particularly for OFMSW or similar substrates;
- scarce data on pretreatment effect on AF, particularly enzymatic pretreatment;
- limited information on VFA production from OFMSW or similar substrates using stirred tank reactors in continuous or semi-continuous feeding mode;
- absence of studies on AF of OFMSW or similar substrates in fed-batch feeding mode;
- and limited economic data on VFA production from wastes.

Microbial VFA production using pure culture and/or sugars as substrate are outside the scope of this work. Information on VFA recovery methods and uses has been included in the literature review to provide an understanding of the context of the proposed process.

The experimental work reported in this thesis, is entirely focused on improving the fermentation stage using ORS as substrate and understanding the underlying mechanisms to inhibit methanogenic activity, avoid by-product formation and maximise VFA yields in batch, fed-batch and semi-continuous feeding modes. Finally, an economic assessment is presented, using experimental data from this work and that reported in the literature, on the continuous large-scale AF of ORS for VFA production.

Chapter 2 Literature review

Chapter 2 critically discusses the literature on acidogenic fermentation technology. The results from published acidogenic fermentation studies are qualitatively and quantitatively compared. Most of the content of this chapter has been published in the journal *Reviews in Environmental Science and Bio/Technology* (Ramos-Suarez et al. 2021) [23].

2.1 Introduction

The term volatile fatty acid (VFA) generally refers to fatty acids containing from two to six carbon atoms, also named short-chain fatty acids in some publications. These are: acetic (CH_3COOH), propionic (CH_3CH_2COOH), butyric ($CH_3CH_2CH_2COOH$), iso-butyric ($(CH_3)_2CHCOOH$), valeric ($CH_3CH_2CH_2CH_2COOH$), iso-valeric ($(CH_3)_2CHCH_2COOH$), and caproic ($CH_3CH_2CH_2CH_2CH_2COOH$) acids. Some authors also include heptanoic acid ($CH_3CH_2CH_2CH_2CH_2CH_2COOH$) in this group, although it is almost a consensus that fatty acids with seven or more carbons should be classified as medium or long chain fatty acids. High purity VFA are mostly manufactured through chemical synthesis from fossil fuels. Microbes can also produce VFA metabolising 'natural' carbon sources such as sugars or CO_2, therefore, microbially produced VFA can be considered renewable chemicals. One example of microbial VFA processes is vinegar production. Microbial VFA production can be carried out by pure culture (single strain) or by mixed culture (multiple strains). Acidogenic fermentation generally refers to mixed culture VFA production. While pure culture fermentation provides higher product selectivity (i.e. high yield for one single product), mixed culture fermentation does have some advantages: The ability to metabolise substrates with a more complex composition, e.g. MSW and food waste, and to operate under non-sterile conditions, which may enable a simpler and less energy intensive process.

2.2 Acidogenic fermentation vs. anaerobic digestion

2.2.1 Technical aspects

Anaerobic digestion (AD) is process where the substrate (carbon source) is metabolised/digested by a mixed culture towards biogas, a mixture of methane and carbon dioxide. The term 'anaerobic' is given as the process works best in the absence of free oxygen. There are three main stages in AD; hydrolysis, acidogenesis/acetogenesis and methanogenesis [24, 25]. Figure 3 shows a simplified diagram of the AD pathways. During hydrolysis, insoluble polymers are broken down into soluble oligomers and monomers by hydrolytic bacteria. Hydrolytic bacteria carry out their

activity by generating enzymes, which are proteinaceous molecules that catalyse reactions, including the breakdown of large molecules (hydrolysis). There are three main bacterial groups involved in the hydrolysis step: *Cellulomonas*, *Bacillus* and *Mycobacterium* [25]. The second step known as acidogenesis or acidogenic fermentation involves the metabolisation of soluble organics such as sugars, amino acids and fatty acids into volatile fatty acids, alcohols and other carboxylic acids (lactic, succinic, etc.). In parallel, hydrogen and carbon dioxide are also generated. These metabolic conversions can be carried out by *Clostridia* and E*scherichia coli* [25]. Acidogenic fermentation is therefore a combination of multiple fermentations carried out by different bacteria. Acetogens then produce acetic acid (acetogenesis) using the soluble products from the acidogenic fermentation and the hydrolysis or the H_2 and CO_2 gas. Acetogens are in the genera *Acetobacterium*, *Clostridium* and *Sporomusa* [25]. Methanogenesis is the final step when acetic acid, H_2 and CO_2 are transformed into CH_4 by methanogenic Archaea [25]. All microorganisms involved in AD can be found in nature: in manure, the depths of water bodies, degraded waste, etc. For commissioning a new AD process, it is best to use a 'healthy' inoculum from an existing AD system and allow the culture to adapt to the new substrate for a period of time.

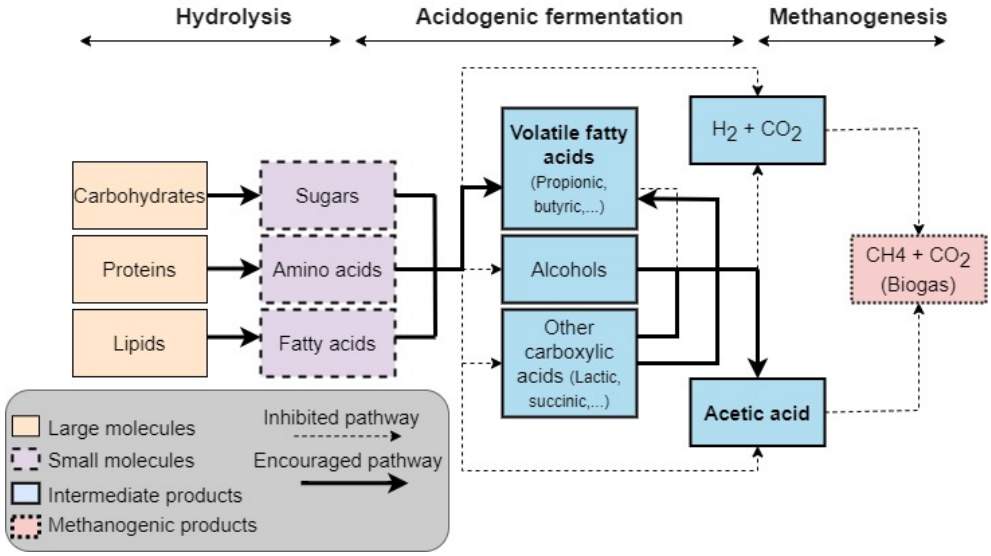

Figure 3. Anaerobic digestion and acidogenic fermentation pathways. Differentiation between inhibited and encouraged pathways apply only to acidogenic fermentation.

AD is a mature technology and widely used for waste valorisation to biogas. AD technology possesses many advantages [26]: single main product (as opposed to a mixed VFA product), easy in situ product recovery (ISPR) as methane has poor solubility in water, mild operational conditions (neutral pH and mild temperatures), thermodynamically favourable process [26], no aeration required, low cell growth (hence low waste biomass production) [25] and absence of product inhibition. The relative low value of methane, however, make it economically unattractive for waste processing companies in the absence of subsidies [27–29]. In addition, as methane

global warming potential is twenty-three times that of carbon dioxide [26], handling requirements are very strict in order to minimise emissions. Additionally, capital expense for AD might be high due to the extra volume required for longer hydraulic retention time (HRT), or lower organic loading rates (OLR).

Although AD continues to be a suitable waste treatment technology, there is a growing research interest in acidogenic fermentation (AF), sometimes known as dark fermentation, for VFA production. The literature usually refers to AF when the process aims to produce mostly VFA with H_2 as by-product, and dark fermentation when H_2 is the end product. AF is equivalent to the AD process without the final methanogenic step. Thus, some advantages of AD apply to AF, including the ability to use complex feedstocks and work under non-sterile conditions.

In comparison, the main operational advantage of AF over AD resides in the fact that acidogenic bacteria can work in wider range of operational conditions (pH, HRT, OLR, temperatures, redox potential, etc) and, therefore, it is more resilient to change than AD. For example, acidogenic bacteria are active in pH ranges between 5 and 6 as well as alkaline conditions (above pH 8). AF, however, is limited by product inhibition at high concentrations. Another advantage is that VFA forming bacteria grow faster than methanogens, meaning shorter retention times can be used. This, however, also means that larger quantities of biomass are produced (0.15 kg VSS kg^{-1} COD compared to 0.03 for methanogens) [25]. Another limitation is that inhibition of methanogenic species in the mixed culture is required to avoid product consumption. Based on the literature, there is a lack of consensus on the optimum ranges for the different operational variables [17] as well as the best strategies to inhibit methanogenic activity. Lastly, separation technology to recover VFAs from fermentation broths is still undeveloped due mainly to two physiochemical aspects: the low VFA concentrations achieved in the fermentation, and VFA being in the carboxylate form when pH>pKa (general case), hence forming strong interactions with water (hydrophilic).

2.2.2 Economic aspects

One recurrent argument to switch the focus from biogas to VFA is the economic drive:

Bastidas-Oyanedel and Schmidt [30] considered a food waste biorefinery for VFA production with a focus on acetic and butyric acids. They found that AF provided a net income of 296 USD $tonne^{-1}$ VS, compared to AD with 19 USD $tonne^{-1}$ VS for gas to grid. AF, however, resulted in increased operating costs due to the complexity of separating the acids.

Bonk et al. studied the economic feasibility of VFA production from the organic fraction of municipal solid waste (OFMSW) [31]. It was suggested that for a plant with a capacity of 350,000 tonnes of OFMSW per year, revenues of $21.5 \cdot 10^6$ USD $year^{-1}$ would be obtained from VFA alone or $25 \cdot 10^6$ USD $year^{-1}$ if hydrogen and compost revenues are included. The calculations were based on the price of individual VFA rather than a mixture of VFA, for which market data are not yet available. Revenues were therefore based on a process with a VFA recovery stage, which has not yet been developed. They calculated, however, the purification cost should not exceed 15 USD $m^{-3}_{effluent}$ to achieve profitability.

Fasahati and Liu estimated that the minimum selling price of mixed VFA from brown algae should be equal or greater than 384 USD $tonne^{-1}$ [32], which is lower than the current market price for (petrochemical) acetic acid, therefore proving potential profitability. This study did not account for the acidification cost required to reduce pH to acceptable values for recovery or for the membrane distillation process to recover the VFA which they suggest.

Liu et al. studied the economics of the use of VFA for biological nutrient removal (BNR) in waste water treatment, and found that VFA production is 2.5 times more profitable than biogas production [33]. The authors claimed the main economic advantage resided on shorter residence time which results in lower capital cost as well as higher product value.

An alternative use for mixed VFA could be polyhydroxyalkanoate (PHA) production. Kleerebezem et al. proposed that PHA revenues are 4.6 times higher than that of biomethane [26], although the cost of a PHA recovery stage was not included in the calculations.

The literature strongly indicates that effective VFA production process could be more profitable than traditional AD towards biomethane. For more accurate assessments, future economic analyses will need to include all aspects of the AF process including product recovery and final product use/market.

2.3 Present and future of volatile fatty acids

Volatile fatty acids (VFA) are used in wide range of industries such as pharmaceutical, food, chemical and agriculture [15]. The major use of acetic acid is the fabrication of vinyl acetate monomer (VAM), followed by purified terephthalic acid (PTA), acetate esters and acetic anhydride [34]. Propionic acid is mostly applied as a food additive and animal feed preservative as an antifungal agent [35], but it is also an intermediate in the production of chemicals, including polymers such as vinyl-propionate and pesticides [36]. Butyric acid is widely used in the food industry for flavouring and its ester derivatives are used in perfumes [15]. The main application of

valeric acid is in the manufacture of flavours, perfumes, pesticides and lubricants [37]. Table 1 shows VFA market data. All sources predict that the demand for VFA will continue growing over the next few years.

Table 1. Volatile fatty acids market data; average price, global market size and global production.

Acid	**Average Price (USD per kg)[1]**	**Global market size (billion USD)**	**Global production (kilotons per year)**
Acetic (HAc)	0.89	12.48 in 2018 [37]	17,900 in 2018 [38]
Propionic (HPr)	2.20	1.2 in 2018 [39]	400 in 2013 [40]
Isobutyric (iHBu)	2.75	-	-
Butyric (HBu)	2.55	0.19 in 2017 [41]	74 in 2020 (estimated) [40]
Valeric (HVa)	4.63	-	-
Hexanoic (HHe)	5.13	-	-

[1]Price data gathered throughout April 2018 [42]

The major VFA producers are currently petrochemical companies, which depend on finite non-renewable raw material. Most VFA are synthesised from the petroleum and natural gas chemical routes, which involve high pressure and temperature processes [15]. These processes generate heavy metals and other harmful by-products due to the use of catalysts [15]. Acetic acid is predominantly produced via methanol carbonylation [43], whereas propionic and butyric acids are synthesised by carboxylation of ethylene [44] and by oxidation of butyraldehyde [44] respectively. VFA production via biological processes would be beneficial to overcome oil price instability, finite fossil fuel reserves and climate change.

Pure individual VFA have multiple applications as demonstrated, either in acid or salt forms (e.g. sodium acetate). There is, however, no current market (or at least none of significant size) for a mixture of VFA, whether pure or diluted in water, and the development of separation technologies should therefore be prioritised.

Mixed VFA uses are being investigated by multiple researchers. The primary use of mixed VFAs considered in the scientific literature is as a feedstock for biological conversion, including the

production of polyhydroxyalkanoates (PHA); medium-chain fatty acids (MCFAs); bioenergy and electricity through microbial fuel cells; or for biological nutrient removal (BNR) in wastewater treatment to produce a fertilizer [33, 45–52]. The VFA could be converted to biogas, through methanogenesis. This is the equivalent of a two-stage AD process with the addition of a separation stage to capture the VFA which is fed to the methanogensis stage [46]. Alternatively, VFAs could also transformed into hydrogen through photo-fermentation or microbial electrolysis [53]. Whilst there is scope for the conversion of VFA into energy sources, this does not appear to be the most value-adding use of bio-VFAs as there are already proven and well-established technologies for biogas, electricity and hydrogen production from renewable sources. Chemical routes for the production of esters have also been researched [54–56].

At present, there are no commercial mixed-culture AF processes. A few companies have achieved demonstration stage for the microbial production of VFA: Metabolic Explorer SA [57], from second-generation biomass; ZeaChem Inc. [58], from lignocellulosic biomass; LanzaTech Inc. [59], from waste gases; and reNEW Technologies, from organic wastes [60, 61]. The latter extracts the VFA which can be used as cleaning agent. The popularity of research on VFA production within industry and research institutions is increasing with projects like URBIOFIN [62] funded by the European Union's Horizon 2020 programme [63]. The aim of URBIOFIB is to valorise municipal solid waste (MSW) following biorefinery models, using VFAs as building blocks for production of polyhydroxyalkanoate (PHA), a biodegradable plastic.

Figure 4 shows the number of patents on acidogenic fermentation under the key words ‘volatile fatty acids waste acidogenic fermentation’. An upward trend can be observed in the last decade.

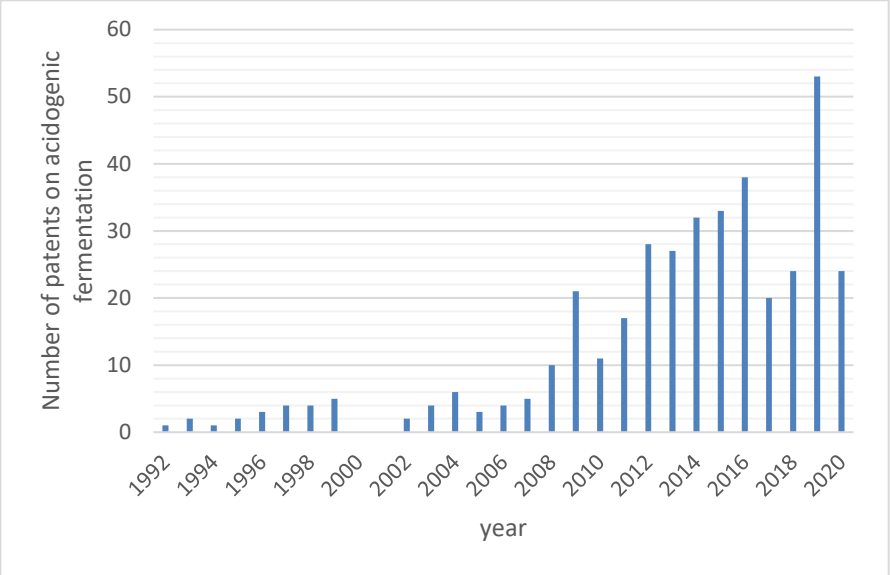

Figure 4. Number of patents related to ‘volatile fatty acids; waste; acidogenic fermentation’ keywords on Google patents since 1992 (last updated: 17/02/2020).

2.4 Feedstock types

Most biodegradable feedstocks can be used as substrate for AF. The use of food crops as (first generation) biomass for energy or chemical production is increasingly regarded as socially unacceptable due the negative consequences that this may have in the food supply market [64]. Waste biomass can be an alternative feedstock to avoid this problem, in addition to reducing pollution that results from waste degradation. Therefore, this is generally the focus of AF processes.

The most abundant solid wastes used in AF research include animal manure, agricultural residues, livestock residues, sewage sludge, organic fraction of municipal solid waste (OFMSW) and food waste. For liquid wastes: sewage, agroindustrial, chemical industry, food processing and pharmaceutical wastewaters have all been studied [26, 46, 65–67]. Novel substrates such as algae and microalgae biomass are recently gaining research interest [68–70].

All substrates contain proteins, fats and carbohydrates among other substances. The majority of liquid waste compounds are solubilized organics easier to metabolise than solid wastes, but they can also contain suspended solids. An ideal feedstock has low human pathogen content and contain a balanced C/N ratio (30/1 to 15/1 recommended for AD). Animal manures generally have good buffering capacity and contain anaerobic microorganisms suitable for AD, reducing or eliminating the need for additional inoculum [71]. Most solid wastes comprise a proportion of lignocellulosic material. The lignin in the lignocellulosic structure provides resistance to microbial degradation, affecting AD efficiencies [72]. The degree of cellulose crystallinity also affects biodegradability of the substrate. In this regard, amorphous cellulose is more accessible to the hydrolytic enzymes [65]. Generally, wastes with high lignocellulosic content require pretreatment to promote the breakdown of the lignocellulose structure into soluble carbohydrates [73]. This is the case with OFMSW and agricultural wastes. In addition to a relatively high lignin content, OFMSW is deficient in nutrients and contains inorganics [65], such as broken glass, grit, plastics and metals. The attractiveness of using algae as biomass for AD resides in the high growth rates compared to terrestrial biomass, the CO_2 uptake during growth and removal of nutrients from wastewaters [65]. But this substrate does have drawbacks such as an unbalanced C/N ratio, compounds toxic to microorganisms, and relatively low biodegradability [65, 74]. Additionally, as algae are not waste, the production and supply cost have to be factored in.

2.5 Pretreatments

Some recalcitrant substrates, particularly lignocellulosic feedstocks, benefit from pretreatment prior to AF or AD [75]. Pretreatments can be classified as mechanical, chemical, physical, physiochemical, biological and others. The aim is to increase biodegradability by removing the lignin from the lignocellulosic matrix, decreasing the cellulose crystallinity, fragmenting the lignin and/or hydrolysing the carbohydrates and proteins into simpler molecules [76]. This results in an acceleration of the hydrolysis stage and improved conversion yields.

Pretreatment studies focusing on mixed VFA production are scarce. As VFA are intermediate products of AD, studies of pretreatments for enhanced AD can work as examples, although these are not optimised for VFA production. Pretreatment methods for AD have been extensively reviewed by Cesaro and Belgiorno [77], Ariunbaatar et al. [78], Romero-Cedillo et al [79] and Bharathiraja et al. [65]. This section provides an overview of the advantages and drawbacks of each treatment with a focus on AF for VFA production. Examples of biogas or hydrogen production were given for those pretreatments with no existing VFA production studies. The results are summarised in Table 2.

Table 2. Summary of results from pretreatment techniques with a focus on AF application, based on available literature

Technique		**VFA/H_2 increases demonstrated**	**Energy intensive**	**Most suited feedstock**	**Additional Chemicals required**	**Accelerates hydrolysis**	**Toxic compounds introduced or made**	**Degrades fermentable sugars**	**Degrades Lignin**	**Recovery/ neutralization treatment required**	**Expensive**	**Ref.**
Mechanical		Up to 30% increased solubility	No	Dry feedstock	No	-	No	No	No	No	No	[80]
Chemical	Acid Treatment	Up to 370% increase in VFA yield	No	High protein wastes	Yes	Yes	Yes	Yes	No	Yes	Yes	[81, 82]
	Alkali Treatment	Up to 400% increase in VFA yield	No	Primary sludge and lignocellulosic waste	Yes	-	Yes	-	Yes	Yes	Yes	[83]
	Peroxidation	Not tested	No	-	Yes	-	Yes	-	Yes	Not for H_2O_2	Yes	-
	Ozonation	Up to 158% increase in H_2 yield	Yes	Lignocellulosic waste	No	-	Yes	Yes	Yes	No	Yes	[84]
	Organic Solvents	Not tested	Yes	-	Yes	-	Low	-	-	Yes	Yes	-

Technique		VFA/H_2 increases demonstrated	Energy intensive	Most suited feedstock	Additional Chemicals required	Accelerates hydrolysis	Toxic compounds introduced or made	Degrades fermentable sugars	Degrades Lignin	Recovery/ neutralization treatment required	Expensive	Ref.
Physical	Thermal treatment	Up to 380% increase in VFA yield	Yes	WAS	No	Yes	Yes	-	-	No	Yes	[85, 86]
	Microwave	Small increases on VFA yields (<30%)	Yes	Sewage sludge	No	-	Yes	-	-	No	-	[87]
	Ultrasound	57% increased solubility	Yes	Only tested on WAS	No	-	Yes	-	-	No	-	[88]
	Focused Pulsed	Not tested	Yes	MSW, WAS	No	-	-	-	-	No	-	-
Physiochemical	Thermo-chemical	Up to 470% increased solubility	Yes	-	Yes	-	Yes	Yes	-	No	Yes	[89]
	Wet air oxidation	Not tested	-	Liquid wastes	No	-	-	-	-	-	Yes	-
	Ionic Liquids	Not tested	Yes	-	Yes	-	Yes	-	Yes	Yes	Yes	-

	Ammonia fibre explosion	Small increases on VFA yields (<30%)	-	Low lignin content	Yes	-	Low	-	Yes	-	Yes	[90]
Technique		**VFA/H_2 increases demonstrated**	**Energy intensive**	**Most suited feedstock**	**Additional Chemicals required**	**Accelerates hydrolysis**	**Toxic compounds introduced or made**	**Degrades fermentable sugars**	**Degrades Lignin**	**Recovery/ neutralization treatment required**	**Expensive**	**Ref.**
Biological	Fungi	Not tested	No	-	No	-	No	Yes		No	No	-
	Extracellular Enzymes	Improved solubility/ digestibility	No	-	No	Yes	No	-	Not if performed under sterile conditions	No	Yes	[91]

2.5.1 Mechanical

Milling, grinding and chipping increases the surface area, improving biodegradability. It is reported that excessive particle size reduction, however, can hinder biogas production in AD by acceleration of VFA accumulation [77, 79]. Therefore, this is a good pretreatment candidate for VFA production. For food waste, smaller particle sizes (0.4 mm) trigger acetic acid production, whereas larger particle sizes (0.9 mm) favour butyric acid [92]. Milling requires relatively low energy and is simple to implement, although it can be more energy intensive for moist waste, garden waste and OFMSW [77]. In addition, simple mechanical treatment may improve dewaterability of the waste and does not normally generate inhibitory compounds. Drawbacks of this type of treatment consist of lack of removal of lignin or pathogens [79] and high equipment maintenance [77].

2.5.2 Chemical

The most common chemical pretreatments are alkali/acid treatment, peroxidation, ozonation and use of organic solvents.

Acid treatment:

Acid treatment hydrolyses the hemicellulose, but it is not effective in dissolving the lignin [79]. This treatment has the disadvantage of degrading monosaccharides into furfurals, which are generally considered toxic to the microorganisms [79]. Acid substances are also toxic and corrosive. Production of furans and phenolic compounds, which are toxic for the microorganisms, increases with increasing acid concentration during pretreatment. Additionally, substrate pH decreases, negatively affecting AD/AF processes. Acid treatment has been proven more effective than other pretreatment options for protein rich wastes [77]. For waste activated sludge (WAS), a 153% and 370% VFA yield increase was observed using hydrochloric acid [81] and free nitrous acid [82], respectively.

Alkali treatment:

Alkali treatment breaks down lignocellulosic structures by dissolving the lignin [93]. In addition it neutralises uronic acid, which is often present in feedstocks and is inhibitory for hydrolytic bacteria [79]. Alkali treatment has shown better results than acid treatment for most AD processes, due to the buffering capacity addition which prevents pH drops during acidogenesis [94]. In the case of AD, wash out of the alkaline substance is sometimes required as methanogens are inhibited at high pH [77]; however, high pH can be advantageous in the case of VFA production. Alkali addition can be used for the saponification of greasy substrates to achieve

emulsification and improve biodegradability; however, the formation of toxic compounds can inhibit VFA production [95]. Alkali treatment has shown improvements in methane and hydrogen production [79], but little attention was put to VFA production. For lignocellulosic feedstocks, a solubilisation rate of 19% was achieved after alkaline pretreatment but this resulted in a more than 40% increase in H_2 production [96]. Guo et al. [97] achieved a 6 times increase in acetic and butyric acid yield with NaOH pretreatment, although maximum VFA concentrations were low (<2 g/L). A study using primary sludge pretreated with different alkali substances found Na_2CO_3 to give a VFA yield 4 times that of untreated sludge. The success was attributed to the increased starting pH (10) which is beneficial for the fermentation, and the breakdown of the sludge flocs by the alkali [83].

Peroxidation:

Peroxidation involves the use of peroxides such as H_2O_2, peroxymonosulphate (POMS) and dimethyldiozirane (DMDO) which help with the delignification of the substrate through oxidative reactions. The main advantage of using H_2O_2 resides in the fact that it decomposes into water and oxygen. Studies on peroxidation as pretreatment in the AD context are scarce [98, 99]. Pretreatment with POMS and DMDO showed a 2 and 2.5 increase in biogas production respectively [100]. To the author's knowledge peroxidation pretreated feedstock has not yet been used for AF.

Ozonation:

Ozone treatment helps remove recalcitrant compounds and break down large molecules through oxidation, achieving effective delignification of substrates [101, 102]. Ozone is regarded as environmentally friendly since it quickly decomposes to O_2. The oxidative reactions cause the cell wall of microorganisms to break (oxidative burst), therefore acting as sterilisation process [103]. The disadvantages of this method include: the possible formation of toxic by-products, such as formic acid and formaldehyde [104]; the degradation of fermentable sugars at high ozone concentrations [105]; and the high energy demand required for ozone production, approximately 12 kWh kg^{-1} O_3 [101, 106]. For lignocellulosic wastes, hydrogen production was reportedly increased by 158% [84], however it negatively affected the dark fermentation of food wastes due to the degradation of proteins and carbohydrates [107].

Organic solvents:

Organic solvents such as N-methylmorpholine-N-oxide (NMMO) have been investigated as a feedstock pretreatment for the production of ethanol and biogas [79]. NMMO dissolves cellulose and reduces its crystallinity. Recovery of NMMO is an environmentally friendly and cost effective

option [79]. Treatment with organic solvents can require the addition of catalysts such as sulphuric or hydrochloric acid, and can involve high temperatures [79]. Other organic solvents used for this purpose include methanol, ethanol, acetic acid, formic acid, amines and ketones. Ethanol pretreatment can improve methane production by 32-84% for lignocellulosic substrates [108]. Pretreatment with NMMO has shown up to 100% improvement in methane yields [109]. Treatment with organic solvents also produces fewer inhibitory compounds compared to acid treatment [79]. Removal of the organic solvent prior to fermentation is necessary, making this process energy intensive [110].

2.5.3 Physical

Physical pretreatments include thermal treatment, microwave irradiation, ultrasound and focused-pulse technology.

Thermal treatment:

Thermal pre-treatment can be classified depending on temperature and pressure range as well as the presence of water (hydrothermal) [111]. Thermal treatment can accelerate hydrolysis by altering the structure of the insoluble fraction, reduce viscosity and increase the soluble chemical oxygen demand (sCOD). This treatment is energy intensive due to the use of high temperatures [77]. Thermal treatments can also lead to the formation of melanoidin, which is a polymer formed by the combination of sugars and amino acids that is toxic for the AD microorganisms [112]. Thermal pretreatment accelerates the hydrolysis and acidogenesis steps [113], which sometimes may lead to inhibition of methanogenesis, and therefore, it is a suitable pretreatment for VFA production. Despite this advantage, little research has focused on the use of thermal pre-treatments for the production of VFA [114–117]. A 680% increase in VFA production has been observed after thermal treatment (100°C, 60 min) for waste activated sludge when the fermentation pH is kept at 9 [118]. For neutral pH, the increase was lower (~300%). It should be noted that the authors do not mention the pH of the control experiment (untreated), and therefore it cannot be concluded that the increase is due solely to pretreatment, as pH adjustment clearly had a significant effect. Lower VFA yield increases (~55%) were observed for food waste [119]. Thermal treatment in combination with other treatments such as enzymatic treatment or pre-fermentation with hydrolytic microbes could increase VFA yields from food waste by 380% [85] and 200% [86] respectively.

Steam explosion is a common thermal treatment used in the context of AD for lignocellulosic substrates [120–123] and waste activated sludge [124, 125], which involves high temperatures (~200°C) and pressure. In addition to the high energy demand, steam explosion can form inhibitory compounds such as furans and furfurals.

Microwave irradiation:

This treatment combines thermal and non-thermal effects since the electromagnetic field destroys the crystalline structures and heats the aqueous environment simultaneously [21]. The treatment is highly energy demanding [21] and, therefore, expensive. Previous studies have suggested that the methane yield does not improve significantly as the solubilised compounds were less biodegradable [21] and melanoidin was also produced [21]. In contrast, this treatment showed yield improvement in dark fermentation processes. For the pretreatment of sewage sludge a 66% H_2 increase was observed [87]. Microwave irradiation combined with alkaline addition showed a 30% increase in solubilisation and a 400% increase VFA/H_2 production from lignocellulosic waste [96].

Ultrasound:

Several investigations have suggested that ultrasound (US) is the most efficient physical pretreatment technique [126–128]. The ultrasound waves combine physical and chemical substrate degradation by the collapse of cavitational bubbles and the generation of free radicals. US promotes the production of different enzymes or improves the activities of the existing ones, depending on the case. Its main limitations, however, are high energy consumption and maintenance costs [77]. US pretreatment improved digestibility of WAS 28 times in terms of sCOD, consequently enhancing acidification [129]. US was also tested on food waste, with a disintegration degree of 57% and a maximum VFA production of 0.98 g COD g^{-1} VS [88].

Focused-pulsed technologies:

Focused-pulsed technologies consist of cell disruption by short, high voltage pulses (20-30 kV, 2000-3000 Hz). This method was successful in improving AD yields of OFMSW at lab scale and raw sludge at full-scale [77, 130]. One disadvantage is the strict safety requirements associated with high voltages. This technology is still at an early stage of development and needs further research [77].

2.5.4 Physiochemical

Physiochemical pretreatments are a combination of physical and chemical approaches. The most common physiochemical pretreatments are thermochemical, wet air oxidation, use of ionic liquids and ammonia fibre explosion.

Thermochemical:

Thermochemical treatment involves the simultaneous use of a chemical agent and heat. This combination can help to increase the solubilisation of the material. Alkaline hydrothermal

treatment was reported to be the most useful thermochemical method to solubilize lignocellulosic material [79]. Thermal treatment can include high pressure, which can lead to solubilisation of oxygen from the air and consequently the degradation of the organic matter [77]. This problem may be solved by using an oxygen-free atmosphere. Another drawback of this method is the production of furans and phenolic compounds which inhibit the fermentation [79]. Kumar and Mohan [89] observed a 4.7 times improvement in the solubilisation of vegetable waste using 1% H_2SO_4 and autoclaving at 121°C for 15 min. This resulted in an AF yield of 0.62 g_{VFA} per g of reducing sugars (under controlled pH 6). However, a comparison of VFA yield between treated and untreated substrate was not provided. Zhang et al. [131] used diluted HNO_3 for the pretreatment of lignocellulosic waste (corn stover) and concluded that the pretreatment was successful despite only acidifying less than 10% of the soluble sugars.

Wet air oxidation:

Wet air oxidation (WAO) is the contact of oxygen with liquid substrates using high temperatures (150-320°C) and high pressures (20-150 bar) during 15-120 min [132], to oxidize and degrade toxic substances such as melanoidins and polyphenolic compounds [133]. Due to the operating conditions, this treatment can be costly [77]. A yield increase of 280% in methane production from distillery effluent was observed after WAO pretreatment [133]. Padoley et al. [134] claim that WAO can improve the biodegradability index (BOD/COD) of distillery effluent by up to 4 times and part of the COD is chemically transformed into VFA. Despite this advantage, no AF studies using WAO pretreatment were found.

Ionic liquids:

Ionic liquids (IL) are salts with low melting point (usually below room temperature). IL as a pretreatment for lignocellulose microbial conversion is very novel [79]. Pretreatment with IL typically involves heating in temperature ranges of 80-180°C. The ILs have the ability to dissolve cellulose, which can be recovered by addition of water or ethanol. Some ILs also dissolve lignin, therefore, choosing the type of IL must be linked to the carbohydrates recovery method. IL also help reduce cellulose crystallinity [79]. One advantage of this pretreatment is the thermostability of the IL. Pretreatment with 1-N-ethyl-, 1-N-butyl and 1-N-hexyl-3-methylimidazolium chlorine increased biogas production by 64-140% from different lignocellulosic substrates [135]. In a study using 1-ethyl-3-methylimidazolium acetate with tomato pomace as substrate, despite the improved digestibility, pretreated feedstock did not show improved biogas yields, presumably due to the formation of inhibitory compounds such as melanoidins and N-derivative amides [136]. The main drawback is the high cost of IL. Recycling of IL could be a solution, but further investigation is

necessary [79]. Most IL pretreatment studies are based on ethanol fermentations and further efforts should be applied to determine its effectiveness on AD/AF.

Ammonia fibre explosion:

Ammonia fibre expansion/explosion (AFEX) consist of the use of ammonia, high temperature (60-100°C) and high pressure [137], which provokes decrystallisation of cellulose [79], hemicellulose hydrolysis and disruption of lignin linkage to carbohydrates [138]. Production of inhibitory compounds is low with this method [138]. This pretreatment, however, can be costly due to the large amounts of ammonia required [139]. Although studies of AFEX pretreated materials for AD/AF are scarce, it has been demonstrated to improve VFA yields by 21% from lignocellulosic substrates [90].

2.5.5 Biological

Biological pretreatment can be performed by the addition of different microorganisms, usually fungal, which are better at hydrolysing the substrate than those typically found in AD communities. Specific extracellular enzymes can also be used. The main advantages of biological treatments are their non-polluting nature and low energy demand [77, 79]. Additionally, there is no need for expensive corrosion-resistant or pressurized reactors [79] and no separate waste stream is generated [77], as the enzymes or biological agents will eventually degrade in AD. Another benefit is that biological agents can be added during AD or AF, simplifying the process. Also, biological treatment does not produce inhibitory compounds. Pre-aeration of the substrate can also be classified as biological treatment since it activates aerobic microorganisms present in the substrate that could help with hydrolysis [21]. In the case of VFA production, aeration is also suitable as it inhibits methanogens.

Fungi:

Microbiological agents researched for the pretreatment of lignocellulosic material include brown, white, and soft rot fungi [79]. White fungi, for example, hydrolyses the cellulose and metabolizes phenolic compounds [79]. Although the use of microorganisms is advantageous in terms of cost, energy demand and formation of inhibitory compounds, it may require long hydrolysis times from several weeks to months [77]. Other drawbacks of microbiological treatments are the need for careful control of growth as other unwanted microbiological species may proliferate [79] and part of the carbohydrate fraction is degraded during the pretreatment. A 21%, 43% and 154% increase in methane yield was reported after fungal pre-treatment of corn stover silage, wheat straw and yard trimmings, respectively [140].

Extracellular enzymes:

Enzymes are protein molecules that catalyse metabolic reactions. Pretreatment with extracellular enzymes can take from a few hours to as much as three days; however, this pretreatment is quite complex since the optimum mixture of enzymes depends on the composition of the substrate [77]. Enzymatic pretreatment can be performed in a separate step prior to or during AD. In the second case, however, the integrity of the enzymes can be affected due to the action of endogenous proteases released by AD microorganisms [141]. Choosing the correct type of enzyme is problematic since it affects both the hydrolysis and the AD step. For example, proteases such as trypsin are ideal to hydrolyse protein rich wastes, but this enzyme type can negatively affect acidogenic bacteria by degrading bacterial proteins [142, 143], and therefore it is not recommended for VFA production. The use of exogenous commercial enzymes is also expensive. The most typical enzyme used, cellulase, costs 6.27 USD kg^{-1} (2010) of protein [144]. Studies have therefore considered using endogenous enzymes of hydrolytic species extracted from the AD digestate [91]. Using the extracted amylase/protease enzymes biogas production from sludge increased by 23%. An alternative to this could be *in situ* production of enzymes from species not normally found in AD.

2.5.6 Other

Additives can adsorb inhibitory compounds such as ammonia and promote hydrolysis [21]. Ammonia removal might not be a desired effect in AF since it provides alkalinity. The most common additives used are micro-porous crystalline solids called zeolites [21] . This pretreatment might not be suitable for VFA production as additives such as porphyritic andesite can adsorb VFA [21].

Surfactants can be used to promote solubilisation and hydrolysis by decreasing surface tension and, in some cases, can inhibit methanogenesis [145]. Addition of chemical surfactants such as sodium dodecylbenzene sulfonate (SDBS) proved to enhance VFA production from activated sludge [146, 147]. Bio-surfactants such as rhamnolipid, surfactin and saponin have the advantage of being degradable, which makes them a low risk of harm to the environment and act as extra COD for the process [145].

2.6 Acidogenic fermentation

It is generally accepted that many aspects of acidogenic fermentation for VFA production are still not well understood [16, 17, 26]. These include microbial populations; substrate composition; fermentation variables (pH, temperature, feeding system, retention times, etc.); and the interactions between them. These factors affect both the product yield and VFA range. The majority of experimental studies have generated short chain fatty acids (C2-C5) as the main

products and therefore, this is the focus of this section. De Groof et al. reviewed some of these key aspects for production of medium chains fatty acids (C6-C12) from wastes [148]. One thing that is clear is that for best AF performance, methanogenesis needs to inhibited to avoid conversion of VFA to biogas. This can be achieved by addition of inhibitors such as 2-bromoethanosulfophate [149] or by operating under unfavourable conditions for methanogens (e.g. pH<6).

When using mixed culture, the metabolic pathways of AF are complex. Figure 5 shows a simplified diagram with the different metabolic pathways from glucose to VFA. Some undesirable by-products can be formed such as formate, lactate, ethanol, butanol, isopropanol and succinate. If these pathways are not carefully controlled, the fermentation broth could be highly 'contaminated' by these by-products which could hinder the downstream process or even the secondary fermentation process.

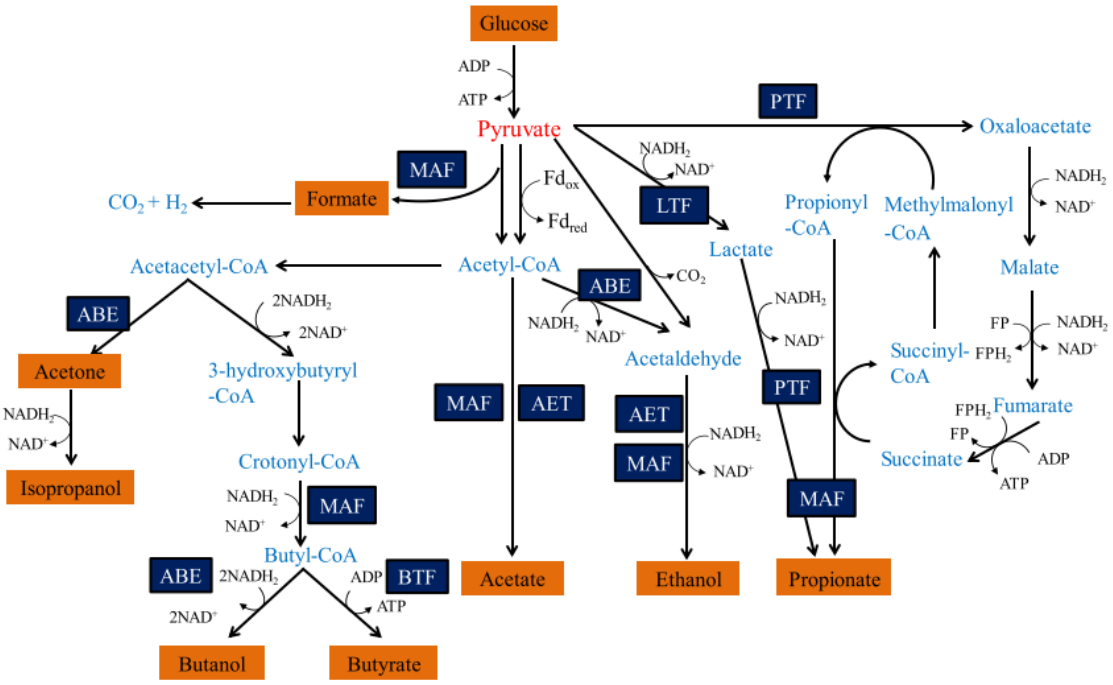

Figure 5. Metabolic pathways of acidogenic fermentation from glucose [150]

2.6.1 AF operating and performance parameters

The main difficulty when reviewing VFA production studies is the inconsistency in the performance parameter definitions and units reported by different authors. Important operating parameters in AD, such as organic loading rate (OLR), are often not included in the methodology of AF studies. Each feedstock has a specific composition; therefore, reporting OLR in terms of chemical oxygen demand (COD) or volatile solids (VS) is more suitable than using units of mass or volume as it can then allow direct comparison between different feedstock types.

VS percentage is commonly used for solid waste and is useful for characterizing substrate consumption in the context of AD. In AF, however, a smaller percentage of VS is converted to CO_2 and it is not a straightforward indicator of performance as VFA also account for a proportion of VS. COD units are better suited to define VFA concentrations and yields in order to allow comparison of values with other soluble materials present in the fermentation broth such as alcohols and monomers released in the hydrolysis. Standardizing product concentrations in terms of COD allows comparison across a range of feedstocks. The ratios of each VFA present should also be provided as this will provide with an understanding of product distribution.

The 'degree of acidification' (DoA) is defined as the amount of soluble COD present as VFA over total soluble COD of the fermented broth [151, 152]. Instead, many authors use the term DoA to refer to what hereinafter is referred to as VFA yield: VFA produced per unit of substrate fed (g COD_{VFA} g^{-1} VS or g COD_{VFA} g^{-1} COD) [153–156]. This lack of consensus on the term DoA can lead to misinterpretation of results. The DoA, as defined in this thesis, is an indicator of the fermentation performance, as it measures the product 'purity' within the aqueous solution. Until significant developments in product recovery and purification are achieved, the fermentation step should aim to reach the maximum DoA possible. Several studies report VFA concentrations (g COD_{VFA} L^{-1} or g VFA L^{-1}) and do not comment on yields. Reporting of VFA concentrations is valuable as this parameter is important in downstream processing, however, this is dependent on substrate concentration, and therefore it is not an indicator of the fermentation performance in its own right.

Table 32 (appendix B) summarizes the data found on AF literature with the most relevant parameters such as HRT, temperature, pH, VFA yield and effluent VFA concentration. For comparison purposes, literature data from AF from waste studies has been standardised and presented in the same units. The values were obtained from the reported results (both graphical and textual) or calculated from the data provided in their methodology using the equations and unit conversions shown in appendix C.

2.6.2 Hydraulic retention time

Hydraulic retention times (HRT) plays an important role in AF. Short HRTs (<10 days) are preferred to wash out methanogenic microorganisms [25, 157]. If the substrate is a solid waste, however, short retention times will result in lower yields as hydrolysis is generally the rate limiting step [158]. To achieve maximum conversion in the fermentation step at least 3 days HRT is required [159]. In batch mode, relatively short retention times of 4-9 days are needed to reach maximum VFA concentrations. In continuous systems, it was observed that a minimum fermentation time of

4 to 5 HRT was needed to achieve steady state for different substrates [160–162]. In some cases, however, instability was still observed after that time [163, 164].

2.6.3 Organic loading rate and substrate concentration

The organic loading rate (OLR) can be defined as the amount of organic material fed (VS, COD or TOC) per volumetric unit of the reactor per unit of time. The recommended values for AD range from 2 to 7 g VS L^{-1} day^{-1} [25, 157]. Using higher values of OLR can help inhibit methanogenesis and promote acidogenesis. Once past the AD threshold in typical CSTR systems (7 g VS L^{-1} day^{-1}), increasing OLR results in higher VFA concentrations but lower yields [165–167] under submerged conditions (TS<20%). In practice, the aim should be to optimise yield and concentration with regards to overall process efficiency. Moisture content also plays an important role. Dry conditions (TS~20%) are preferred to reduce methanogenic activity and maximize VFA production [168], although VS destruction is lower compared to submerged fermentation.

2.6.4 Reactor mode and product inhibition

Most AF-from-waste research has been carried out using bench scale batch reactors (<5 L). Batch fermentations can achieve higher VFA concentrations than continuous processes, however it can have a negative impact on the yield, potentially due product inhibition. VFA can be inhibitory to a number of biological processes (including AF), although inhibitory concentrations are generally reported in hydrogen and methane production studies, with propionate reportedly being the most toxic VFA. In pure culture acidogenic fermentations, propionate can be inhibitory at concentrations of 10 g L^{-1} [169]. Veeken et al. found that VFA at 30 g COD L^{-1} and low pH (5) inhibited hydrolysis rate [170]. Establishing exact VFA concentration thresholds is difficult as it depends on operational variables, microorganism consortium and type of VFA produced. The maximum reported VFA concentration achieved from a batch fermentation is 58 g COD_{VFA} L^{-1} using kitchen wastes at a high substrate concentration (125 g VS L^{-1}) [171]. However, the VFA yield was not maximized (<0.5 g COD_{VFA} g^{-1} VS). Within the same study, a continuous system with solid recirculation (i.e. removal of liquid broth to uncouple the solid and liquid retention times) to increase solid retention time (SRT) resulted in a yield increase of 15% compared to batch due to reduced product inhibition (25 g COD_{VFA} L^{-1}). Increasing SRT can also help with the degradation of more recalcitrant substances that might require higher hydrolysis time. Some studies included solid waste recirculation in their experimental set-up [171, 172], but do not show a comparison data without solid recirculation, and therefore could not demonstrate the advantage of solid recirculation. Other studies of continuous fermentations were, typically, carried out using stirred tank type reactors at bench scale.

Batch studies are useful to indicate the capability for AF, but they provide limited information on the implications of OLR, solid/liquid recirculation, wash out of methanogens, inoculum adaptation and product removal. To understand the full potential of VFA fermentation and achieve industrial-scale application, progression to continuous fermentations is required. The disadvantage of continuous fermentations is that they are difficult to rapidly optimize and gain understanding of the parameter interactions.

A limited amount of work has been carried out on the transition of an optimized batch AF to continuous AF. One example is Yu and Fang [173] who studied batch and continuous (upflow reactor) AF of dairy wastewaters at different strengths. From the data provided, it can be seen that the product range is significantly different at low strengths (2 g COD L^{-1}): acetic is the predominant acid in continuous (0.27 g COD L^{-1}), and acetic and propionic in batch (0.2 and 0.25 g COD L^{-1} respectively). At high strengths (20 g COD L^{-1}), however, the behaviour of both operating modes was similar with propionic as the predominant acid (0.6 g COD L^{-1} in continuous), followed by acetic (0.45 g COD L^{-1}). This indicates that, in certain cases, optimised batch processes can provide information to design a continuous process.

Due to its dry nature, OFMSW is generally treated in AD plug flow reactors (PFR). There are some examples of CSTR studies using OFMSW towards biogas production [174]. Only one study on AF of OFMSW using a semi-CSTR was found [175]. In the aforementioned study, it was found that the increase in soluble COD by alkaline pH (~10) did not result in improved VFA production, probably due to inhibition caused by an increase in free ammonia concentration [175]. Therefore, acidic pH (~6) is preferred in order to avoid the overuse of chemicals to increase pH. Some studies have focused on AF of food waste using semi-CSTR. Highest VFA yields were observed at mesophilic (35 °C) temperatures compared to 25°C and thermophilic (45°C) [176]. In a pH range of 5 to 6, pH 6 resulted in highest VFA yield [176]. At neutral pH, the hydrolysis of vegetable and salad waste was improved with respect to pH 6, but VFA yields were lower due to methanogenic activity [177]. With respect to OLR and HRT, it was found that at the same OLR (5 g TS L^{-1} day^{-1}), increasing HRT (4-12 days) led to higher VFA yields, and the same effect was observed with increasing OLR (5-13 g TS L^{-1} day^{-1}) at constant HRT (8 days) [176]. In order to keep a constant HRT, OLR needs to be modified by adjusting feed concentration. In continuous AF of food wastes, the HRT and OLR have a significant impact on VFA profile and formation of by-products [176, 178]. In these studies, increasing OLR led to an increase in concentration of by-products such as succinic and lactic acid [176] and a decrease in the degree of acidification (DoA) from 93 to 69% [179]. The effect of HRT and OLR at constant feed concentration was also studied [159]. Higher HRT (i.e. lower OLR) led to higher VFA yields at pH 5.5 when thermophilic compost from kitchen waste was used as inoculum. Typically, continuous reactors need a form of pH control such as NaOH or KOH. Wu et

al. opted for an alternative approach by introducing waste activated sludge (WAS) inoculum as part of the feed [179], and optimised the process for maximum DoA with a S/I of 5 g VS g^{-1} VS in the feedstock. This method, however, falls within the co-fermentation category as the WAS-inoculum also contains fresh nutrients and carbon. Although the transportation of WAS to a MSW/food waste treatment plant would require complex logistics, co-fermentation provides an alternative to WAS treatment/disposal derived from small treatments (typically transported to and treated in AD of large wastewater treatment plants). Further studies are necessary to fully understand the effect of OLR and HRT on the continuous acidogenic fermentation of wastes such as food waste and OFMSW.

To the author's knowledge, there are no studies on fed-batch AF using OFMSW or food waste. One argument against using fed-batch is the pH decrease, which could lead to inhibition. This could be overcome with alkaline or organic nitrogen addition. Placido and Zhang investigated acidogenic fermentation of slaughterhouse blood in fed-batch mode with 1 L reactor [143]. They started the experiment using half the volume with 7 days of batch fermentation at 122 g VS L^{-1} and fed the reactor every two days after that. They achieve VFA concentrations of 100 g L^{-1}, however this concentration was reached after 5 days and maintained until day 45. The flat concentration curve could be explained by the fact that blood is added in liquid form, hence increasing total working volume. They did not use any alkaline addition, because the substrate itself has high nitrogen content which acts as buffer, however there was no mention about pH changes in this study for fed-batch conditions.

2.6.5 Inoculum and microbial community

VFA distributions and yields are effectively the result of the microbial activity. The taxonomic identification of microbial communities, which is typically carried out by 16S rDNA gene sequencing analysis, can help understand the performance of AF systems [180]. The acidogenic species can be introduced by an inoculum and/or the substrate.

Most AF studies have used anaerobic digestate as inoculum as it contains a greater population of acidogens compared to other inoculums such as aerobic activated sludge [181, 182]. Digestate, however, also contain methanogens which degrade VFA. In batch fermentations, optimizing the substrate to inoculum ratio (S/I, in g VS g^{-1} VS) can help prevent methanogenesis [183]. A S/I higher than 3-4 was found to inhibit methanogens successfully [184, 185]. Inoculum acclimation can be another strategy to improve AF fermentation. Plácido and Zhang [163] observed a 43% increase in VFA when inoculum was acclimated prior to batch fermentation. Acclimation can be achieved by operating a continuous reactor for at least three HRT. Some authors have suggested

subjecting the inoculum to a thermal treatment in order to kill methanogens. There is little information, however, on optimal conditions for heat shock treatment [186] or on its effect on AF. Often, the conditions used seem arbitrary or based on H_2 production [187, 188]. In some cases, inoculum is not needed as an acidogenic population is provided by the substrate. External substances, such as 2-bromoethanesulfonate (BES), can also be used to inhibit methanogens too. Despite BES used commonly to inhibit methanogens, it has shown it can affect acidogens [182], but its effect is not well understood.

In acidogenesis, different types of bacteria carry out different metabolic reactions. High abundance of *Firmicutes* has been associated with high acidification yields associated to both their hydrolytic and acidogenic capabilities [180, 182, 189], particularly *Clostridium* genus [180]. *Firmicutes* has shown to proliferate when substrate has high carbohydrate content [189, 190]. *Pseudomonas* (*Proteobacteria*) are the genus with the highest number of genes related to the metabolism of carbohydrates and amino acids [190]. The anaerobic digestates derived from wastewater treatment plants typically contain microbes of the phyla belonging to *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria, Chloroflexi* and *Euryarchaeota* [189, 191]. *Euryarchaeota* phylum encompass methanogens. In AF of food waste and sewage sludge, the majority of phyla found belongs to the first three aforementioned phyla [182, 190, 192]. Major class within *Firmicutes* in food waste AF is *Clostridia* [182] and *Bacilli* [192]. For these substrates, and when anaerobic conditions are strict, conditions class *Bacteroidia* within *Bacteroidetes* has shown to dominate [182, 192]. In AF of sewage sludge, *Proteobacteria* is mostly comprised of *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacterial* [190].

In AD, microbes can be found in slurry or granular form. Both forms have been investigated for their effect on AF performance. The use of 'large' granular sludge (~3.5mm) resulted in higher DoA and yields compared to 'small' granular (~1.5mm) sludge or typical slurry form using glucose as substrate at pH 10 [180]. Large granules were also more effective in the AF of cheese industry wastewater [193]. Despite the differences in microbial relative abundance found in the glucose study, the type of sludge did not result in different VFA distributions. This suggests fermentation conditions (for example, pH) and substrate have a larger influence than microbial community over final product distribution [180]. Differences in microbial relative abundance were seen when using the same inoculum with two different substrates, either milk or cheese industry wastewater, indicating that microbial relative abundance is influenced on substrate type [193].

In AF of food waste under mesophilic temperature, pH 7 and pH 5 led to high abundance (>50%) of classes Clostridia and Bacilli, respectively. At pH 7, thermophilic temperature led to higher relative abundance of Bacteroidia compared to mesophilic temperature. In contrast, the change

in relative abundance due to different temperatures was less significant at pH 5 [192]. High abundance (43%) of Lactobacillus-bacilli (responsible for lactic acid production) was found in mesophilic temperature and pH 5, despite it being found in low abundance in the inoculum. It is likely that Lactobacillus-bacilli was introduced by the substrate [192]. At mesophilic temperatures, pH changes also had an effect on microbial relative abundance of potato waste fermentation [191]; at pH 6 major phyla were *Proteobacteria* and *Firmicutes*, whereas at pH 7 and 8 (highest VFA yields), *Bacteroidetes* and *Firmicutes* dominated. The highest *Euryarchaeota* (methanogens) relative abundance was observed at pH 7 [191]. When using sewage sludge as substrate: pH 7-8 resulted in high levels of *Euryarchaeota* (60%) and *Firmicutes* (20%), followed by *Actinobacteria*. At pH 9 (highest VFA yield) dominating phyla were *Firmicutes* (60%, mostly *Clostridia*) and *Actinobacteria* (24%). pH 10 led to 35% *Firmicutes*, 39% *Actinobacteria* and 16% *Proteobacteria* [194]. Another study found that different substrates, such as cucumber, tomato or lettuce; influenced the relative abundance, especially at a genus level [189]. All three substrates led to high levels of class *Clostridiales*, but *Ruminicocus* were only significant in cucumber and tomato fermentations. AF of lettuce resulted in significant levels of *Acidaminococcus* [189]. Apart from pH, temperature and substrate composition, strong correlations of organic loading rates and oxidation-reduction potential with microbial consortia were found [195].

Genomic studies are also used to quantify microbial diversity. Microbial diversity, especially for complex substrates such as food waste or OFMSW, is strongly linked to high VFA yields [192] and performance stability [195]. The use of a secondary substrate (co-fermentation) often results in improved microbial diversity and consequently improved VFA yields. Xin et al. showed that by co-fermenting WAS with corn stalk or pig manure, diversity indicators improved and VFA yield increased by two fold [195].

It has been discussed throughout this chapter that AF is a mixed culture fermentation, ideal to metabolise complex substrates. However, AF is likely to result in a multiple product solution which currently does not have a commercial application. Recent research, therefore, focuses in targeting one single product via bioaugmentation techniques. Atasoy and Cetecioglu studied bioaugmentation technique with the introduction of *Clostridium butyricum* monoculture to target butyric acid production from cheese industry wastewater using a sequencing batch reactor [196]. This was achieved by injecting a small volume of medium containing the target culture. A 4.6 and 2.3 times increase in BuH and total VFA average yield, respectively, was observed after bioaugmentation, respectively, which resulted in a rise of BuH selectivity from 21% to 61% [196].

2.6.6 pH and alkalinity

In AF, pH is a key parameter for control of VFA yields and product distribution. pH can be controlled by acid/base addition. Optimum pH for methanogens is around 7.0, although they can operate at slightly alkaline pH (pH 8). Acidogenic bacteria can handle wider pH ranges and, therefore, pH has a relevant role in AF to minimize VFA degradation. Slightly acidic pH (5-6) improves hydrolysis due to higher hydrolytic bacteria activity [178], and inhibits methanogens, but pH lower than 5 can be inhibitory for acidogens [197, 198]. This is explained by the high toxicity of VFA in their free acid form [16], which is predominant when the pH is lower than the pKa. Alkaline pH (8-11) can also be beneficial [199] as it improves substrate digestibility by dissolving lignin (abiotic effect), inhibits methanogens and the substance added to increase pH may offer buffering capacity [200]. At pH 11 or higher, the metabolic activity of acidogens slows down due to the toxic effects of strong alkaline conditions [49]. Alkali addition can add substantially to operational costs. In contrast, acidic pH can be achieved by accumulation of VFA, depending on the buffering capacity of the substrate. As a general rule, alkaline pH will favour acetic acid production, while acidic pH will favour propionic and butyric acids [201–203]. In some cases, neutral pH (7) showed better VFA yields. For example, gelatin degradation efficiency was 98% at neutral pH compared to 85% at pH 5, resulting in higher VFA concentrations [204]. In the case of kitchen wastes, pH 7 also gave the highest solubilisation rate of 82% in terms of COD, which coincided with the highest VFA concentration, compared to less than 70% for acidic and alkaline pH values [205]. This could be explained by the differences in substrate composition, as hydrolysis of proteins and lipids is optimum at neutral pH [16]. This further supports the view that the optimum pH is substrate dependent.

In wastewater AD systems, alkalinity is mostly a result of ammonia release from the digestion of amino acids and other natural sources of nitrogen present in raw sludge. Alkalinity and pH can also be affected by CO_2 production, which leads to bicarbonate formation in equilibrium with carbonic acid. Ammonia gas has high solubility in water, and when it dissolves, it forms an ammonium cation and a hydroxide anion, thus increasing pH [25]. Nitrogen content in the substrate can consequently have an effect on the performance of AD microbes, and there is abundant literature available on this topic focusing on biogas production. For example, it is known that ammonia/ammonium concentrations of 1.5 to 3 g L^{-1} can be inhibitory for methanogens at pH>7 and at any pH for concentrations above 3 g L^{-1} [25]. This is advantageous in AF, since methanogenic activity is not desired as it leads to VFA consumption. Urea and NH_4Cl have been used as ammonia sources to study the inhibition of methanogenic activity. It was found that urea has a double toxic effect on methanogens due to the release of free ammonia gas (which is more

toxic than ammonium) and to the pH increase to alkaline levels, whereas no increased pH was observed when using NH_4Cl as inhibitor [206].

Consequently, some AF studies have utilised urea as a nitrogen source for ammonia release and increased alkalinity. Nitrogen/urea addition proved to have a beneficial effect on VFA production from kitchen wastes [205]. However, careful consideration must be taken when designing the AF process, as when the ammonia concentration is not high enough to inhibit methanogens, it can still increase pH from acidic to neutral and promote methanogenesis [207]. The ammonia release from urea is a biological process by the action of urease enzymes generated by the hydrolytic bacteria and it follows chemical equations 2.1 and 2.2:

$$(NH_2)_2CO + H_2O \xrightarrow{\text{urease}} NH_3 + H_2NCOOH \rightarrow 2NH_3(gas) + CO_2(gas) \qquad \text{Equation 2.1}$$

$$NH_3(gas) + H_2O \leftrightarrow NH_4^+ + OH^- \qquad \text{Equation 2.2}$$

The mechanisms of this conversion might be affected by the fermentation conditions as well. The addition of urea to the substrate-water mixture results in an inevitable change in C/N ratio. There is limited information on the effect of C/N ratio on AF, but one study found that increasing C/N ratio from 9 to 20 adding co-substrates was beneficial for the AF of WAS [208].

It has been demonstrated that the addition of buffering substances (e.g. $CaCO_3$) can help stabilise pH and consequently improve anaerobic digestion processes [209]. Two previous studies on acidogenic fermentation utilised $CaCO_3$ and $(NH_4)HCO_3$ as buffering agents to maintain stable pH [210, 211]. Another utilised $NaHCO_3$ as a buffer agent [175], however, NaOH was also used in order to increase pH, making it unclear how $NaHCO_3$ was affecting the pH control. A study with vegetable and salad waste as substrate used 0.6 M $NaHCO_3$ to prepare the feed, which resulted in neutral pH during the continuous fermentation [177].

2.6.7 Temperature

Temperature has a significant effect on the yield and type of VFA produced. Under thermophilic conditions (~50°C), VFA accumulation is higher than under mesophilic (~35°C) in most cases [152, 202, 204], due to improved substrate digestibility. Kinetics of acidogens under thermophilic conditions, however, are slow, and longer retention times are required to reach maximum product concentrations [152, 202]. Thermophilic temperatures can help to improve the hydrolysis of solid wastes, but pH is reported to a greater influence over this compared to temperature [152]. In the case of food waste, it was found that, despite improved substrate solubility,

thermophilic temperature hindered AF [178]. Due to the higher operational cost of maintaining reactors at high temperatures, it may thus be preferrable to operate at mesophilic instead of thermophilic conditions for VFA production. VFA production at psychrophilic temperatures (10°C) is feasible but not competitive with mesophilic conditions due to lower VFA concentrations and yields [172]. Temperature can influence the type of VFA produced, but results are not consistent [202], probably due to the lack of systematic studies on parameters interactions. For example, temperature can have an effect on ammonia release [178], making it difficult to study the effect of these variables independently. Garcia-Aguirre et al. [152] found that temperature had no significant effect on product distribution for slaughterhouse wastewater and paper mill wastewater. The same observation was made for protein rich wastewater [204]. In the case of OFMW and winery wastewater, butyric acid was predominant (>70% of COD) at acidic pH (5.5) and thermophilic temperature, compared to propionic and acetic predominance under any other conditions [152]. Jiang et al. also found that thermophilic temperatures promote butyric acid production from food waste at pH 6 [178].

2.6.8 Feedstock composition

The type of feedstock affects the output of the fermentation. Comparison of AF performance using different feedstock compositions is difficult due to differences in the reported parameters definitions and methods. Compositions for feedstocks of the same type can also vary depending on location or season, for example, resulting in high variability in the reported data. There are numerous studies comparing AD performance for different feedstock types [212], but similar studies on AF for VFA production are scarce. Although AD studies can serve as a reference, optimum substrate compositions might be different for AF. Cheese whey, molasses and OFMSW showed higher VFA potentials over wastes such as glycerol, slurry, winery wastewater, olive mill effluent and landfill leachate [213]. This was further supported by Garcia-Aguirre et al. [203] who reported that OFMSW had the highest VFA potential compared to slaughterhouse wastewater, paper mill wastewater, winery wastewater, crude glycerol, sewage sludge, and meat and bone meal. Further investigation is necessary to identify optimum composition ranges and substrate characteristics for AF with respects to the required outputs (e.g. yields, VFA profile, etc).

Feedstock composition can be modified by mixing different substrates, in which case the process is known as co-digestion or co-fermentation. Co-fed substrates can improve yields in some cases due to synergistic effects [214]. Co-fermentation can also dilute toxic compounds present in the feedstocks and improve the nutrients ratio, e.g. adding protein substrates improves N content. Protein content also affects buffering capacity as ammonia is released, and high ammonia concentrations can be inhibitory to methanogens [163]. Hong and Haiyun [215] optimized the

co-fermentation of synthetic food waste and excess sludge, obtaining a maximum VFA concentration of 29 g L^{-1} for 88% (VSS basis) food waste. The synthetic food waste used in this study did not contain animal products, indicating that a certain amount of protein derived from the excess sludge was beneficial. In a different study using WAS, potato peel waste and food waste, it was found that carbon-rich substrates promoted butyrate production, whereas more proteinaceous feedstocks led towards propionate and valerate, with acetate being the predominant VFA in all cases [216]. The highest VFA yield observed in this study, 344 mg COD g^{-1} VS, was achieved at a ratio of WAS to potato peel of 1:3 (on a VS basis).

2.6.9 Substrate to inoculum ratio

Substrate to inoculum ratio (S/I) has been optimised for AD batch systems towards biogas. For BMP tests, where maximum methane yields are desired, a maximum S/I of 0.5 g VS g^{-1} VS is recommended [217]. S/I can be increased to inhibit methanogenic activity and maximise VFA yields/concentration [218]. Iglesias et al. studied the effect of S/I on the acidogenic fermentation of potato solid waste and found that higher concentrations of VFA were achieved at higher S/I ratios of 4.2 g VS g^{-1} VS [219]. VFA profile was also affected by S/I with lower acetic percentages at higher S/I, replaced with higher percentages of propionic, butyric and valeric. A study using food waste demonstrated that increasing S/I from 2 to 4-6 g VS g^{-1} VS significantly inhibits methanogenic activity and increases VFA yields [220]. However, higher S/I of 8-10 resulted in decreased VFA yields.

2.6.10 Other variables

Other parameters can also play important roles in AF. Salt and oil content can be a problem in AF systems: substrates should have salt and oil contents lower than 6 and 5 g/L respectively [221]. The oxidation-reduction potential (ORP) has a significant effect on cellular activity. Methanogenesis occurs under ORP below -300 mV, whereas acidogenic fermentation happens below -100 mV [25]. The presence of sulphate can hinder methanogenic conversion of acetate [25].

2.6.11 Acidogenic fermentation of OFMSW

OFMSW usually undergoes natural AF during collection and storage [75], accumulating VFA before reaching treatment plant. For this reason, OFMSW has attracted attention as substrate for VFA or H_2 production:

D'Addario et al. [222] produced VFAs for the production of methyl esters from OFMSW. This study was particularly valuable as it attempted both the production and recovery of VFA. They used a batch fermenter with subsequent screen and settlers to separate the solid residues from the VFA aqueous solution. This configuration showed a 37% TS reduction during acidogenesis, and 85% yield for VFA extraction with organic phases. This process, however, generated large amounts of chemical wastes due to the extraction method (0.7g of $Ca(OH)_2$ per g of VFA) using TOPO as extractant and ammonium salts as the recovery solution. Sans et al. [223] studied the acidogenic fermentation of OFMSW in a plug flow reactor under mesophilic and thermophilic conditions using a semi-solid mixture (20-25% TS). In this study, VFA concentrations of 19.6 g L^{-1} were obtained but no details of solids destruction were provided. Bolzonella et al. [224] also investigated the production of VFA using wet anaerobic fermentation under batch operation and psychrophilic conditions (14-22°C). Although the system did produce VFA (15 g COD_{VFA} L^{-1}), the DoA was only 15%. In addition, according to the mass balance shown in this study, the TS destruction in the fermenter was less than 15%. Yesil et al. [225] studied the formation of VFA from OFMSW using a leach-bed reactor. After 30 days of incubation, the reactor achieved a maximum VFA formation rate of approximately 27 g COD/kg TS. No values were presented for TS destruction. Zahedi et al. [226] studied the evolution of microbial population in dark fermentation of OFMSW using continuous stirred tank reactors (CSTR) with 20% TS. Reactors achieved relatively good hydrolysis and acidification yields (approximately 57 and 60% respectively) for an OLR of 40 g VS L^{-1} day^{-1} and HRT of 2 days. Gameiro et al. [227] studied the effect of total solids and alkalinity on the wet anaerobic fermentation of OFMSW using batch reactors. Results show that maximum yields (78% g COD_{VFA} g^{-1} COD_{TOTAL}) were achieved for lowest TS concentration (5%) and highest alkalinity (50 g $CaCO_3$ L^{-1}). Low TS was detrimental to final VFA concentrations.

OFMSW usually has a nutrient (N and P) deficit, especially after pretreatment [75]. For this reason, co-fermentation of OFMSW with protein rich wastes is often chosen: Kumar Tyagi et al. [228] investigated the co-digestion of OFMSW with primary sludge (PS), waste activated sludge (WAS) or mixed sludge (MS). Although the main objective of this work was to maximise H_2 production, the best co-substrate for both H_2 and VFA production was MS. The experiments were carried out in batch operation.

Bonk et al. [31] assessed the economics of VFA production from OFMSW using literature data for hydrogen and VFA yields with SuperPro designer default values for equipment cost. This study reported that the major source of revenues was VFA, whereas revenues from H_2 were negligible. Results showed that a minimum selling price of 550 USD/tonne VFA is necessary to make the process profitable, which is significantly lower than the average price of different VFA (Table 1). This suggests that economic feasibility of AF from OFMSW is reachable.

Numerous studies on AF use food waste as substrate and can serve as reference points for the design of AF from OFMSW processes [155, 159, 165, 166, 176, 178, 179, 181, 221, 229–231]. Composition of OFMSW, however, differs from the composition of food waste due to the greater proportion of inorganic materials and, generally, higher lignocellulosic content. Further research of the AF of OFMSW is therefore necessary to achieve optimum conversion yields.

2.6.12 Conclusion and suggestions

This literature review was carried out to identify knowledge gaps in AF processes. While there is a wealth of studies, the data has been reported unsystematically. The influence of AF parameters on AF performance is highly dependent on feedstock type. Food waste is the most widely studied feedstock, followed by primary and waste activated sludges. The optimum parameters of AF for VFA production for each substrate have not been established. Although the microbial production of VFA has proven feasible, the process is not well understood especially compared to AD. To move forward, more systematic studies using both batch and continuous systems are necessary. Additionally, further efforts should be made towards the understanding of product inhibition and how reactor operating modes with solid retention/recirculation systems can help overcome this problem.

2.7 VFA recovery

VFA recovery can be incorporated to the acidogenic fermentation process in order to manufacture a rich concentrated product with higher value. When recovery is performed ‘in situ’, product inhibition can be relieved [225]. Although techniques to recover a primary acid from pure culture fermentations are well established [232], little research has been carried out using mixed VFA broths.

Several separation techniques have been tested for VFA recovery including adsorption, distillation, gas stripping, pervaporation, filtration and liquid-liquid extraction. Usually, a solid removal step is required prior to the VFA recovery as solids can hinder the mass transfer or contaminate the recovery units. Solid removal can be achieved by filtration, centrifugation, freeze-thawing, electrocoagulation or a combination of these.

Selection of the separation method also depends on the chemical and physical properties of the compound to be isolated, such as hydrophobicity, or octanol-water partition coefficient (P_{OW}), molecular weight, boiling point, etc. Table 3 and Table 4 display the main chemical and physical properties of VFA and fermentation by-products respectively. The main difference observed is

that VFA, except for acetic acid, are more hydrophobic (more positive Log P_{OW}) than the other organic acids.

Table 3. Chemical and physical properties of VFA [35]

Volatile Fatty Acid	Log P_{OW}	Molecular weight (g/mol)	Boiling point (°C)	pK_a at 25°C
Acetic acid	-0.17	60.05	118	4.76
Propionic acid	0.33	74.08	141	4.88
Butyric acid	0.79	88.11	164	4.82
Isobutyric acid	0.94	88.11	154	4.84
Pentanoic (valeric) acid	1.39	102.13	185	4.84
Isopentanoic (isovaleric) acid	1.16	102.13	177	4.77
Hexanoic acid	1.92	116.16	205	4.88
Heptanoic acid	2.42	130.19	223	4.89

Table 4. Chemical and physical properties of fermentation by-products [35]

Fermentation by-products	Log P_{OW}	Molecular weight (g/mol)	Boiling point (°C)	pK_a at 25°C
Lactic acid	-0.72	90.08	122	3.86
Succinic acid	-0.59	118.09	235	4.21
Itaconic acid	-	130.10	268	-
Citric acid	-1.64	192.12	310	2.79
Ethanol	-0.31	46.07	78	-
Butanol	0.88	74.12	118	-

The properties of the fermentation broth play a key role in designing a product recovery process. AF broths will generally contain suspended solids including cells; and VFA and other soluble compounds. VFA are extracellular products and, therefore, can be found in the liquid fraction without the need for cell disruption. Many studies achieved VFA concentrations of 10-60 g COD L^{-1}

(Table 32). Recovery from broths below that concentration range is likely to be ineffective for technical reasons in the downstream processing, and concentrations above are desirable. The concentration and composition of soluble compounds is highly dependent on the substrate used, substrate concentration, chemical addition to control pH, and the fermentation performance in terms of by-products formation. Properties of the fermentation broth, excluding VFA concentrations, are often unreported. Most AF studies were carried out at neutral or slightly acidic pH. Suspended solids are generally at high concentrations when solid wastes are used as a substrate. For example, VSS from AF of primary sludge varied between 6-9 g L^{-1}, depending on fermentation conditions [233]. 200 mg NH_4^+-N L^{-1} was reported in AF broth from WAS [147]. Nitrogen and phosphorus concentrations fell in the ranges of 50-300 mg NH_4^+-N L^{-1} and 20-50 mg PO_4^{3-}P L^{-1} respectively. 100-2000 mg NH_4^+-N L^{-1} were observed in broths from food waste and kitchen wastes [167, 198, 234]. As these concentrations are low, they have low probability of affecting the recovery process. Other ions such as Na^+, which are present as a result of NaOH addition for pH regulation, are more likely to have an impact as they can be found at concentrations of up to 25 g L^{-1} [234].

2.7.1 Adsorption

For the recovery of organic acids from aqueous solutions, ion exchange resins and macroporous adsorption resins have been used [169]. This technique requires solid removal. Drawbacks of this technology are that the presence of anions in the mixture can affect the process efficiency [169], it can only be operated in batch mode and high product purities can never be achieved [26]. Optimal pH is dependent on the type of resin and its interactions with the VFA; i.e. hydrophobic or ionic properties [235–238]. Hence, this technology offers the opportunity to work at different pH values by selecting the appropriate resin. This technique also needs a second stage for desorption which involves the use of solvent such as ethanol or NaOH to separate the VFA from the resin. Ethanol is more promising option for desorption as it can easily be recovered via distillation without generating residues [236]. Recovery yields of up to 74% have been achieved using adsorption technology [238].

2.7.2 Gas stripping

Injecting gas through the fermentation broth, known as gas stripping, can successfully recover VFA [239], although recovery rates have yet to be determined. Acidification of the broth to achieve pH< average pKa is necessary [239]. This technique also requires a ‘desorption’ step, where the saturated gas is injected in an alkali solution, which will solubilize the VFAs. This results

in the formation of acid salts. The requirement to use of acid/alkali substances is a disadvantage and the potential of the technique for VFA recovery has not been widely explored.

2.7.3 Precipitation

Precipitation involves the addition of chemicals such as $Ca(OH)_2$ or $CaCO_3$. Calcium forms salts with organic acids which will precipitate. The reactions of acetic acid with $Ca(OH)_2$ and $CaCO_3$ are as follows:

$$CaCO_3(s) + 2CH_3COOH(aq) \rightarrow Ca(CH_3COO)_2(aq) + H_2O(l) + CO_2(g) \quad \text{Equation 2.3}$$

$$Ca(OH)_2(s) + 2CH_3COOH(aq) \rightarrow Ca(CH_3COO)_2(aq) + 2H_2O(l) \quad \text{Equation 2.4}$$

This process requires solid removal. The solubility of calcium acetate in water is high (370 g L^{-1} at 0°C [35]). Therefore, in order to precipitate the salt, the fermentation broth must be concentrated or extra salt must be added to decrease the solubility, adding to the operational cost. To reverse the calcium acetate to acetic acid, a strong acid like sulphuric can be used. This process generates $CaSO_4$, which has low solubility in water (2 g L^{-1} at 0°C [35]), generating a solid waste [169]. The addition of chemicals also increases the process cost considerably. Cylcodextrin is another substance used to precipitate butyric acid with 100% selectivity [169].

2.7.4 Liquid-liquid extraction

Dichloromethane and methylteret-butyl-ether can be use as extractants to recover VFA [169]. Non-reactive extractants can also be used such as alcohols, aliphatic hydrocarbons, ethers, ketones and organophosphates [18]. The main disadvantages of this method are the solvent toxicity, hence the inability to be used for 'in situ' recovery, and the energy consumption of the subsequent distillation step to recover the extractant [169]. This technique requires the acids to be in the free form, therefore pH to be below the pka [240]. Reactive and non-reactive solvents have been investigated for extraction of VFA. 75%-85% of VFA can be recovered using solvents such as 2-pentanol [241, 242] and tri-n-octylphosphine in kerosene [241]. The use of salts can facilitate phase separation for more effective extraction. This technique is known as salting-out. Ammonium sulphate [243] and monosodium phosphate [244] have been used for the extraction of VFA with alcohols. Up to 94% recovery has been achieved using this technique, but high concentrations of salt were required (23%) [243, 244].

2.7.5 Membrane separation

Membrane separation involves the use of a thin sheet that is only permeable to certain components of the solution, which are driven to the other side by a differential pressure or concentration gradient [18]. Examples of membrane separation technologies are microfiltration, nanofiltration, reverse osmosis, pervaporation, membrane distillation, zeolite membranes and electrodialysis (ED).

The main advantage of this technology is considered to be the easy scalability [26]. Another benefit is the flexibility in range of feedstock composition suitable for a fixed product purity and the product specificity [18]. Membrane fouling is unavoidable, however, and it makes this method expensive and unattractive. VFA recovery rates using membrane technologies are still low [225].

Microfiltration (pore size = 0.1 – 10 μm) can be used to separate solids and impurities as well as to sterilise the effluent. Nanofiltration (pore size = 1-10 nm) can retain VFA based on molecular size and electrical interactions. pH can alter membrane charge and, therefore, needs to be monitored. At alkaline pH the membrane becomes more negatively charged, which helps retain dissociated VFA [245]. This represents an advantage with respect to other recovery techniques as fermentation broth does not need to be acidified. Up to 85% of VFA were recovered in the retentate using nanofiltration [246]. If pressure is applied (reverse osmosis) VFA containing solutions can be concentrated by removing the water collected as permeate [247], although this limits the use of reverse osmosis for 'in situ' recovery.

Electrodialysis is another technique that does not require acidification of the broth since it works better when VFA are in their charged form. This technique has some disadvantages: the need for clarification to avoid membrane fouling and electrical resistance; and higher selectivity for other anions such as Cl^{-1} generally added as NaCl to improve mass transfer [248, 249]. With this technique, up to 95% recovery rate has been achieved [250], however, VFA concentrations in the recovered solutions are still low (maximum 20 g/L) [239, 251].

Membrane extraction has also been investigated for VFA recovery. It follows the same principles as liquid-liquid extraction, but a membrane is placed between the feed and the extractant. For VFA, typically a hydrophobic membrane is used [252, 253]. In this case, either an organic solvent or an alkaline solution can be used as extractant [254, 255], with the former having the advantage of not needing an extra separation step. In the case of porous membranes, although high recovery rates are achieved (~80%) [55], membrane fouling seems to be the main obstacle in applicability of this method. Non-porous membranes have the advantage of not needing organic or alkaline solvents, but larger membrane areas are required [256].

Pervaporation involves the use of a membrane subjected to vacuum on the permeate side. Pervaporation works best for volatile substances such as alcohols [257], therefore, acidification of the medium is required to turn VFA into free acid form. This technique was more recently successful in the separation of VFA from AF using membranes made of PTFE, PTFE filled with TDDA, silicone-PTFE composite [258] and PEBA-graphene composite [259], with the latter achieving a 19 times more concentrated permeate in butyric (final concentration of 114 g L^{-1}).

2.8 VFA for biotechnological applications

VFA are building blocks in the synthesis of chemicals, fuels, plastics and solvents. VFA can be converted into their final products via chemical or biological routes. Chemical routes require the VFA to be separated from the other fermentation products and concentrated. As noted above, VFA recovery and separation technologies are not fully developed and can be expensive. AF fermentation broths containing VFA are more likely to have an imminent application in biotechnology processes as product specifications are less strict. This section discusses the main biological uses for VFA investigated so far. Table 5 summarises the requirements for each application. As can be seen, required VFA concentrations are low (10 g L^{-1} or lower) which fits well with the results of most AF studies.

Table 5. VFA concentration requirement and type of VFA preferred for different biological applications

VFA application	**approximate VFA concentration required**	**Type of VFA preferred**	**Other conditions required**	**References**
Bioplastics (PHA)	10 g L^{-1}	HPr, HBu	Low nutrient content	[260, 261]
Lipids for biodiesel	6 g COD L^{-1}	HAc	Low nutrient content	[262]
Hydrogen	2 g L^{-1}	HAc, HPr	-	[53]
Methane	1 g L^{-1}	HAc	Neutral pH	-
Microbial fuel cells	< 4 g L^{-1}	HAc, HPr	Solids free	[263]
Nutrient removal	1 g L^{-1}	HAc	Solids free, neutral pH	[264]

The limited oil reserves and climate change have driven research towards VFA transformation into bioenergy (hydrogen, methane, biodiesel and microbial fuel cells). Despite the environmental benefits, bioenergy processes from VFA are not competitive, due to the low cost of fossil fuels and the development of other reliable renewable energy technologies. Based on the literature, VFA conversion to bioplastics and VFA as a carbon source for nutrient removal seem the most promising options.

2.8.1 Bioplastics

Polyhydroxyalkanoates (PHA) can be used as substitute for petrochemical derived plastics, as a building block to produce other chemicals or as biofuel. Currently, PHA cannot commercially compete with petrochemical plastics [265] due to high production costs associated with the substrate [17], but its high biodegradability and environmental benefits make it an attractive product. VFA derived from wastes can serve as an alternative to other more expensive carbon sources. The advantage of using VFA for PHA production is that PHA can be easily recovered, hence skipping the VFA purification step. Ideally, ammonium and phosphorus content should be low in the fermentation broth to avoid microbial growth over PHA synthesis [17]. For this application, the VFA species plays an important role as it will determine the type of PHA produced. Acetic and butyric acids favour poly-3-hydroxybutyrate (P3HB) production while propionic and valeric mostly lead to poly-3-hydroxyvalerate synthesis (P3HV) [17, 169]. P3HV has better properties such as flexibility and oxygen impermeability, which make it suitable for a wider range of applications [17].

2.8.2 Lipids for biodiesel

VFA could be a cheap carbon source as an alternative to glucose for microbial lipid production [18], as the production cost of biodiesel is mainly attributed to the raw material [17]. Subsequently, lipids can be used to produce biodiesel through transesterification. Yeast can accumulate lipids, but microalgae has attracted much attention for this purpose, due to its capacity for CO_2 uptake. Acetic, propionic and butyric are suitable VFA for microalgae assimilation [18], but higher acetic concentrations are more favourable [17]. In addition, high nitrogen content can negatively affect lipid production [17].

2.8.3 Hydrogen

In addition to the H_2 produced as by-product during the AF, VFA could be used as substrate to produce extra H_2 through photofermentation [17, 18]. Acetic, propionic and butyric are the VFA

preferred by photosynthetic bacteria [18], with butyric showing the lowest yields [17]. In addition, low ammonium content is required [17]. Currently, H_2 production rates are low (0.1-1.5 L H_2 L^{-1} h^{-1}) [266] and, therefore, improvements in efficiency would be needed to make this a feasible technology and competitive against electrolytic H_2 production from renewables. Dark/acidogenic fermentation and photofermentation can be carried out simultaneously. The benefits are that alkalinity is regulated as VFA are consumed and there is no need for dilution step prior to photofermentation [266].

2.8.4 Methane

VFA are intermediate products in the AD process. Instead, VFA can be produced in a first step, acidogenesis and acetogenesis, and transferred to a second one, methanogenesis, which sometimes results in improved efficiency [17]. One potential advantage of two step AD is the individual recovery of H_2 and CH_4. Although AD is a mature technology, the production and recovery of VFA might be economically advantageous due to higher VFA prices compared to methane. Propionic acid is strongly inhibitory to methanogens, and therefore, acetic and butyric are preferred for this application [17].

2.8.5 Microbial fuel cells

In a microbial fuel cell (MCF), microorganisms convert organic compounds into electricity under anaerobic conditions [17, 18]. In this system, VFA act as electron donors. This application is particularly attractive because, in principle, the fermentation broth can be used directly without any treatment [17]; however, solids removal is recommended to avoid electrode fouling. For this technology, acetic and propionic are suitable VFA [18], although acetic achieves significantly higher efficiencies [17].

2.8.6 Nutrient recovery

VFA can be used as carbon source for phosphorous and nitrogen removal [17, 18]. Carbon available in wastewater is usually insufficient for nutrient removal, and the provision of alternative carbon sources, such as synthetic VFA, represents a cost, therefore, the use of waste-derived VFA can be a cost-effective solution [17]. Most of the research to date was carried out using synthetic VFA solutions [18]. Acetate and butyrate are the preferred VFA for denitrification, followed by propionate [17, 18]. In contrast, there is evidence that propionate might lead to higher efficiency in phosphorus removal [17].

2.9 Conclusions

Recent publications suggest that acidogenic fermentation for VFA production could become a more cost-effective solution to the treatment of wastes than anaerobic digestion for biogas. Several studies also evidence the higher potential of OFMSW and food waste as substrate for AF over other organic wastes. Knowledge on optimum operational conditions of AF of wastes using mixed culture is still limited, however, particularly in terms of parameter interaction and product pathways. Recovery methods or downstream treatments represent the bottleneck of the process and the major operational cost. Therefore, improving and integrating both stages of the process, production and recovery, should be a key area for research.

In addition to VFA recovery, fermentation broths containing VFA could be directly used as substrate to produce other products easier to recover such as biopolymers. Given the current technology available, this is the most promising path. This, however, limits the potential uses for VFA. Most of the recovery technologies have been explored using synthetic VFA aqueous solutions rather than fermentation broths. Further investigation is therefore necessary to assess the performance of separation methods when other organic molecules apart from VFA are present. Similarly, for VFA biological applications, the majority of studies were performed using synthetic VFA solutions.

Whichever the application, the type of VFA produced should be optimised accordingly as it will affect the VFA conversion yields. An optimisation of the waste conversion and the VFA conversion should be achieved using process optimisation tools.

Chapter 3 Methodology

This chapter describes the main methodology: Materials, experimental setup and analytical methods. Specific conditions for each experiment are described in the following chapters discussing results.

3.1 General

3.1.1 Reagents

All reagents used were of laboratory grade and obtained from Fisher Scientific (Loughborough, UK) or Sigma-Aldrich (Darmstadt, Germany). Enzymes were provided by a private third party for testing purposes. These enzymes were classified based on their functionality: Complex A was a mixture of cellulase/beta-glucosidase/hemicellulase, complex B was arabinase/cellulase/beta-glucanase/hemicellulase/xylanase. Amylase, lignase and lipase solutions were also used.

3.1.2 Water

Unless stated otherwise, solutions and standards were prepared with ultra-pure deionised (DI) water obtained from two different ultrapure water purification systems set to give a resistivity of 18 MΩ cm; either Barnstead Nanopure (Thermo Scientific, UK) or Arium mini (Sartorius, Germany).

3.1.3 Laboratory practice

Good laboratory practice was followed in all experiments and analyses. For each activity, an individual risk assessment and COSHH was submitted and approved by safety staff. All equipment was operated in accordance with the instructions provided by the manufacturers. All glassware was washed using detergent and rinsed with tap water followed by DI water.

3.2 Substrate and inoculum

The organic residual solids (ORS) used as substrate in this thesis were collected from the industrial plant in Maine, USA, or from the Fiberight R&D pilot plant in Southampton, UK. In Southampton, the municipal solid waste (MSW) collected from a waste transfer station is first pulped in a third-party facility. Once at the pilot plant, the recyclables are removed from the MSW (processing conditions commercially confidential). The pulped material is washed to remove soluble organics

and fine particulate or ORS. The ORS is then collected by filtering the washwater through nylon filter cloth and then stored at -18°C until used. Image 1 shows the ORS formation steps in the pilot plant.

Image 1. ORS formation steps. Top left: pulped OFMSW. Top right: washing process. Bottom left: filtering wash water. Bottom right: drained ORS

The industrial plant in Maine follows a similar process at large scale (explained in section 1.1.3 When more ORS was needed than could be supplied by the Southampton pilot plant, ORS was shipped from Maine to allow for completion of the work.

Digestate for inoculation was obtained from a mesophilic anaerobic digester treating wastewater raw sludge in Millbrook wastewater treatment plant, Southampton (UK). Inoculum was sieved and incubated for 24 hours at mesophilic temperatures (35±2°C) prior to any experiment.

3.3 Digesters/fermenters setup

3.3.1 Equipment description

Continuous and fed-batch fermentations were carried out in 1.0 L stirred reactors with 0.5-0.8 L working volume (see Image 2 and 3), provided by CJC labs (CJC-056 Bio-reactors) [267]. The vessels were 9.8 cm in diameter and 13.3 cm in height. The reactors were fitted with an asymmetrical anchor agitator and immersed in a water bath at 37°C with the temperature controlled by Anova precision cooker (Anova Applied Electronics, Inc., California, USA). Feeding and sampling of solid-liquid mixture was carried out by removing top lid with stirrer.

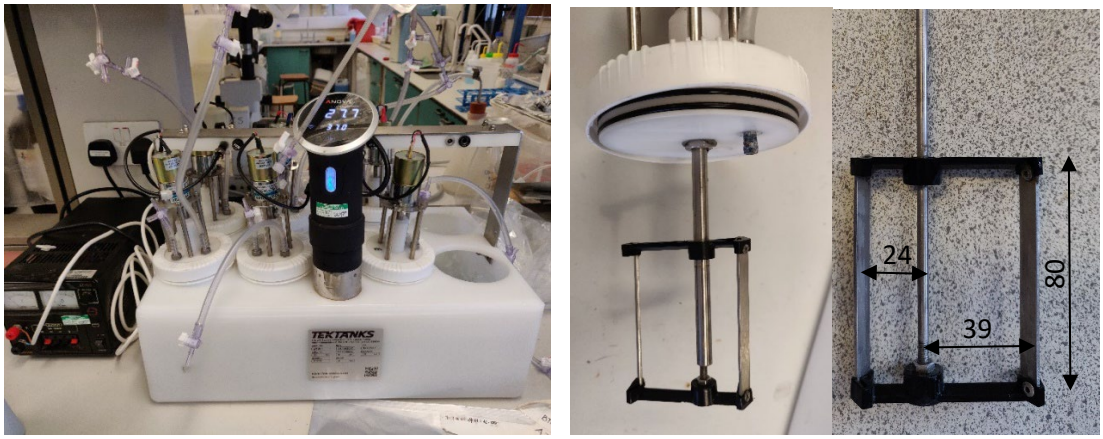

Image 2. Stirred reactors in water bath (left) and stirrer (right). Dimensions are given in mm

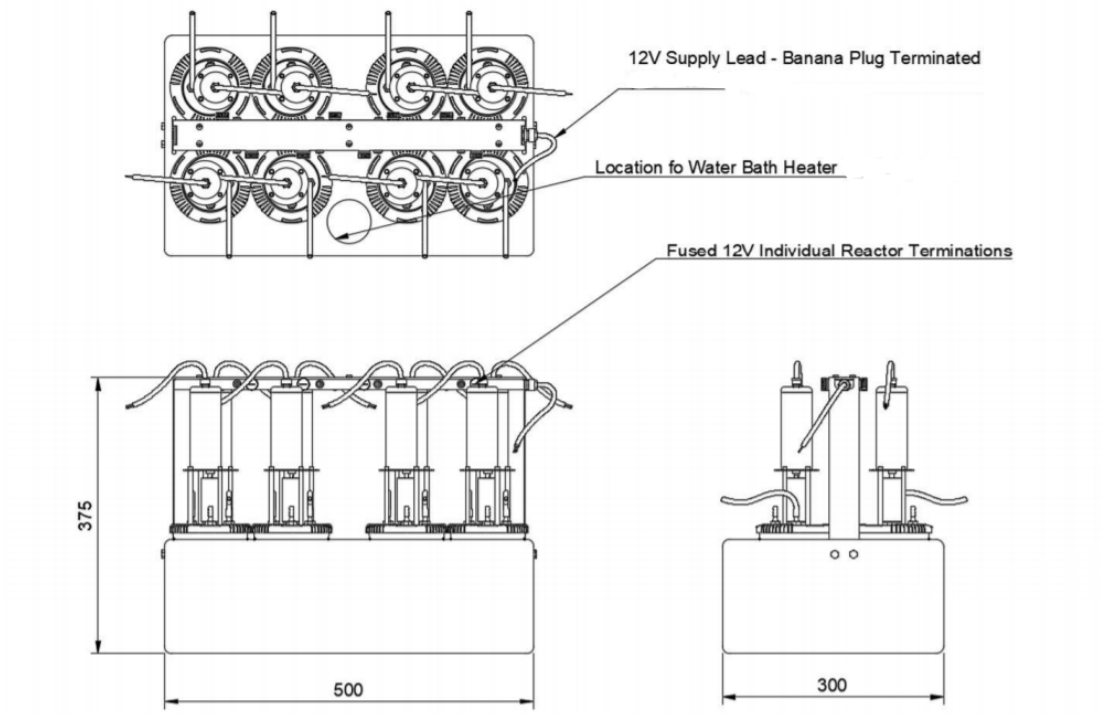

Image 3. Three-view drawing of stirred reactors in water bath [267]. Dimensions are given in mm

The stirrers were powered by 12V XD-37GB520 electromechanical motors (Guang Wan Motor Co., China). The reactors were airtight and connected to 5 L gas-impermeable bags made of multi-layer foil and fitted with a polypropylene valve and septum. The gas bags were connected to the reactors with PVC tubes (Altec Extrusions Ltd, UK).

Batch fermentations were carried out using 250 mL polypropylene copolymer (PPCO) bottles. Bottles were placed in incubator with orbital shaker under mesophilic conditions (35±2°C), either

Orbi-safe TS netwise or Gallenkamp orbital incubator. Depending on the experiment, the bottles were closed by a screw cap or by rubber plug bungs connected to one litre gas-impermeable bags.

3.3.2 Operation and calculations

Batch mode

Batch fermentations involve inoculating the substrate in a closed system, i.e. reactor content is not modified by addition of substrate or removal of broth with exception of samples. The end of batch fermentation experiments were determined when maximum product concentration was achieved (concentration curve flattened out) or when product concentration started to decrease.

Generally, batch experiments were carried out using a mixture of ORS substrate, inoculum, tap water and enzymes. Depending on the experiment, bottles were flushed with N_2 gas to test the effect of strict anaerobic conditions. pH was controlled using a 4M NaOH solution or, in some cases, urea was added to the broth. This is detailed in results chapters for each experiment. Bottles were weighed every few days throughout the experiments for mass balance purposes. Fermentation time was counted from the moment of inoculation and lasted up to 14 days, depending on the experiment.

The performance of the bioreactors was monitored in terms of VFA yield, defined as the chemical oxygen demand (COD) corresponding to VFA divided by the volatile solids (VS) fed, Equation 3.1:

$$\text{VFA yield (g COD g}^{-1}\text{ VS)} = \frac{C_{VFA} \cdot V_{final}}{m_{subs} \cdot VS_{subs} + m_{inoc} \cdot VS_{inoc} + m_{enz} \cdot VS_{enz}} \qquad \text{Equation 3.1}$$

C_{VFA} = VFA concentration (g COD L^{-1})

V_{final} = final working volume (L)

m = wet mass(g)

VS = volatile solid content of substrate (% wet weight)

Subs, inoc, and enz subscripts refer to substrate, inoculum and enzymes respectively.

Semi-continuous mode

Strict continuous mode would be possible in a commercial scale setup. Pumping semi-solid substrate can be challenging at small scale thus it was not attempted. The reactors were operated in a semi continuous mode, i.e. manually fed with a specific amount of feedstock at fixed intervals of time and with part of the reactor contents removed to maintain constant volume. Feeding was carried by disconnecting the reactors from the power supply and removing the screw top with the stirrer attached. It was assumed, as it was measured by water displacement, that 1 g of wet substrate was equivalent to 1 mL. pH was controlled using a 4M NaOH solution. Vessels (without

the stirrer) were weighed every few days throughout the experiment for mass balance purposes. The organic loading rate (OLR) was determined according to equation 3.2:

$$\text{OLR (g VS L}^{-1}\text{ day}^{-1}) = \frac{\text{m. rate}_{\text{subs}} \cdot \text{VS}_{\text{subs}}}{\text{V}_{\text{reactor}}} \quad \text{Equation 3.2}$$

Where:

$m.rate_{subs}$ = mass of substrate added to the reactor on a daily basis (g day^{-1})

$V_{reactor}$ = working volume of reactor (L)

The Hydraulic Retention Time (HRT) is defined by equation 3.3:

$$\text{HRT (day)} = \frac{\text{V}_{\text{reactor}}}{\text{Q}} \quad \text{Equation 3.3}$$

Where:

Q = flow of material (substrate added) through the reactor (L day^{-1})

VFA yield was calculated following Equation 3.4:

$$\text{VFA yield (g COD g}^{-1}\text{ VS)} = \frac{\text{C}_{\text{VFA}} \cdot \text{Q}_{\text{out}}}{\text{m. rate}_{\text{subs}} \cdot \text{VS}_{\text{subs}} + \text{m. rate}_{\text{enz}} \cdot \text{VS}_{\text{enz}}} \quad \text{Equation 3.4}$$

Where:

C_{VFA} = VFA concentration (g COD L^{-1})

Q_{out} = outlet flowrate (L day^{-1}) = Q

m.rate = mass added per day (g day^{-1})

This yield ignores changes in storage; however, it is a close approximation to what would happen in a steady state system.

Specific methane production and VS destruction were calculated using equations 3.5 and 3.6 respectively:

$$\text{Specific CH}_4\text{ production (L g}^{-1}\text{ VS)} = \frac{\text{V}_{\text{CH}_4}}{\text{m. rate}_{\text{subs}} \cdot \text{VS}_{\text{subs}}} \quad \text{Equation 3.5}$$

Where:

V_{CH4} = volume of methane produced daily (L day^{-1})

$$\text{VS}_{\text{reduction}}\ (\%) = \frac{\text{m. rate}_{\text{subs}} \cdot \text{VS}_{\text{subs}} - \text{m. rate}_{\text{dig}} \cdot \text{VS}_{\text{dig}}}{\text{m. rate}_{\text{subs}} \cdot \text{VS}_{\text{subs}}} \quad \text{Equation 3.6}$$

$m.rate_{dig}$ = mass of digestate removed from the reactor on a daily basis (g day^{-1})

Fed batch mode

Fed batch mode involves the continuous addition of substrate without broth removal until a desired volume or product concentration is reached. pH was controlled using a 4M NaOH solution. Vessels (without the stirrer) were weighed every few days throughout the experiment for mass balance purposes. Calculations were carried out as for batch mode.

3.4 Characterisation of substrate and solid fraction

3.4.1 Solids, ash and suspended solids

Total solids (TS) and volatile solids (VS) determination was based on Standard Method 2540 G [268]. Approximately 5 to 10 g of well-mixed sample was transferred into a weighed crucible. Samples were weighed to an accuracy of ± 1 mg using a Sartorius BP 1105 balance (Sartorius AG, Germany) and placed in a convection oven (Heraeus Function Line, LTE Scientific Ltd, UK) for drying overnight at 105 ± 1 °C. After drying the samples were transferred to a desiccator to cool to room temperature. Samples were then weighed again with the same balance, transferred to a muffle furnace (Carbolite Gero 30-3000 °C, Carbolite, UK) and heated to 550 ± 10 °C for two hours. Samples were again cooled in a desiccator for at least one hour before weighing a third time.

After all analyses, crucibles were rinsed with deionised water, dried in an oven overnight and stored in a clean desiccator until required for the next analysis. TS and VS were calculated according to the following equations:

$$\%\ \mathrm{TS}\ = \frac{W_3 - W_1}{W_2 - W_1} \mathrm{x}\ 100 \qquad \text{Equation 3.7}$$

$$\%\ \mathrm{VS\ (on\ a\ wet\ weight\ basis)} = \frac{W_3 - W_4}{W_2 - W_1} \mathrm{x}\ 100 \qquad \text{Equation 3.8}$$

$$\%\ \mathrm{VS\ (on\ a\ TS\ basis)} = \frac{W_3 - W_4}{W_3 - W_1} \mathrm{x}\ 100 \qquad \text{Equation 3.9}$$

Where:

W_1 = weight of empty crucible (g)

W_2 = weight of crucible containing fresh sample (g)

W_3 = weight of crucible and sample after drying at 105 °C (g)

W_4 = weight of crucible and sample after heating to 550 °C (g)

$CaCO_3$ ash was measured by weight difference between material heated at 550°C for 2 hours for VS determination and heated at 900 °C for another 2 hours. The weight difference is associated with the CO_2 produced in the calcination of $CaCO_3$ into CaO [269]:

$$\%\ CaCO_3\ (\text{on a TS basis}) = \frac{W_4 - W_5}{k(W_3 - W_1)} \text{x}\ 100 \qquad \text{Equation 3.10}$$

Where:

W_5 = weight of crucible and sample after calcination at 900 °C (g)

K= 0.44 (CO_2 to $CaCO_3$ molar mass ratio)

Total suspended solids (TSS) and volatile suspended solids (VSS) content was measured according to method 2540 D [270]. A sample of known volume was passed through a 0.4 µm pore size glass fibre filter paper (GF/C, Whatman, UK) of known dry weight (± 0.1 mg). Filtration was performed using a vacuum filtration system with the same diameter as the filter. The sample container was rinsed with DI water to guarantee transfer of all solids to filter surface. After drying at 105°C for 24 hours the paper was again weighed, and the difference determined according to the following equation:

$$TSS\ (\text{mg L}^{-1}) = \frac{(W_7 - W_6)\ \text{x}\ 1000}{V_s} \qquad \text{Equation 3.11}$$

Where:

W_6 = weight of clean filter paper + tin foil tray (mg)

W_7 = weight of filter paper + tin foil tray + dry sample (mg)

V_s = sample volume (mL)

The same sample was then placed in the furnace at 550 °C for two hours and weighed:

$$VSS\ (\text{mg L}^{-1}) = \frac{(W_7 - W_8)\ \text{x}\ 1000}{V_s} \qquad \text{Equation 3.12}$$

Where:

W_8 = weight of filter paper + tin foil tray + burnt sample at 550 °C (mg)

3.4.2 Sample preparation for compositional analyses

ORS samples were freeze-dried using VirTis, benchtop K for 5 days. Moisture content was reduced to 2-3% (weight basis). Freeze-dried samples were milled using an on-site built device to reach a particle size <1mm and stored in air-tight plastic screw top sample pots until analysis. Freeze-dried and milled samples were used in elemental composition, proteins, carbohydrates, lignin, and lipids analyses.

3.4.3 Elemental composition

Freeze-dried and milled ORS was used for this analysis. Samples of approximately 0.4 mg were weighed into standard weight tin disks using a five decimal place analytical scale (Radwig, XA110/X, Poland). Carbon, hydrogen and nitrogen contents of samples were determined using a FlashEA 1112 Elemental Analyser (Thermo Finnigan, Italy). Combustion/reduction reactor was held at 900°C then flash combusted in a gas flow temporarily enriched with oxygen resulting in a temperature greater than 1700 °C and the release of N_xO_x, CO_2, H_2O and SO_2 (depending on the composition of the sample). NxOx was subsequently reduced to N2 in the reduction zone. The gas mixture was passed through a separation column and analysed by GC with the different components are measured by appropriate detectors. The working conditions of the elemental analyser were as described in the manufacturer's technical literature and method sheets. Birch leaf (B2166) with approximately the following composition (%); C=48.33; H=6.36; N=2.09; S=0.16 was used as standard for this method. Results are presented on a dry basis. Oxygen content is calculated as the difference between total sample weight and the sum of S,H,N and S. Oxygen content is therefore an estimated value.

3.4.4 Proteins

The protein content was determined by measuring the total Kjeldahl nitrogen (TKN), which measures the amino-nitrogen that is converted to NH_4^+ in acid digestion and free ammonia. Free ammonia in ORS was assumed to be negligible. TKN is then multiplied by a nitrogen in protein factor of 6.25 as the average nitrogen content in protein is 16 %. TKN was determined according to the standard method 1687 [271]. Freeze-dried and milled ORS was used for this analysis to seek better sample homogeneity. Freeze-dried samples are unlikely to contain free ammonia or any volatile components, as they can are lost in the freeze-drying process. However, this is also the case in TS analysis, therefore, TKN is a close approximation of protein content on a dry basis of ORS.

Approximately 0.2 g (weighed to ± 1 mg) of sample was placed in a glass digestion tube. Two Kjeltab Cu 3.5 catalyst tablets were added to facilitate acid digestion by lowering the activation energy of the reaction. 12 mL of low nitrogen concentrated H_2SO_4 was added carefully to each digestion tube and agitated gently to ensure that the entire sample was completely exposed to acid. The digestion tubes were then placed into the heating block with exhaust system using either a Büchi K-435 Digestion Unit (Büchi, UK) for approximately two hours until the solution colour became a clear blue-green. The heating block was operated at 420 ± 5 °C. Once the reaction was completed the tubes were cooled to around 50°C and 40 mL of DI water was slowly

added to the digestion tube to prevent later crystallisation on further cooling. Glutamic acid was used as standard. Samples, blanks, and standards were then distilled and titrated as for total ammonia nitrogen (see section 3.6.2).

$$\mathrm{TKN} = \frac{(\mathrm{A} - \mathrm{B})\ \mathrm{x}\ \mathrm{MW_N}\ \mathrm{x}\ \mathrm{N}\ \mathrm{x}\ 1000}{\mathrm{W_s}} \qquad \text{Equation 3.13}$$

Where:

TKN = total Kjeldahl nitrogen (mg kg^{-1} dry weight)

A = volume of titrant used to titrate the sample (mL)

B = volume of titrant used to titrate the blank (mL)

MW_N = molecular weight of nitrogen (14 g mol^{-1})

N = normality of the H_2SO_4 titrant, or the theoretical normality multiplied by a correction factor for the specific batch of titrant

W_s = dry weight of sample (kg)

3.4.5 Carbohydrates and lignin

The determination of carbohydrates and lignin was carried out through acid hydrolysis following the National Renewable Energy Laboratory (NREL) method [272] with slight modifications. Freeze-dried and milled ORS was used for this analysis. Approximately 300 mg of sample was accurately weighed in test tube. 3 mL of 72% sulfuric acid was added and the tube was placed in 30°C water bath for one hour of digestion. Samples were manually stirred from time to time using a glass stirrer. Samples were transferred (rinsing with DI water) to PPCO bottles suitable for autoclave. 84 g of DI water was added to dilute sulphuric acid to 4%. Bottles were placed in autoclave for 1 hour at 121°C. Samples were filtered using a pre-weighed filter paper which has been treated in the furnace at 550°C for 2 hours and stored in a desiccator. The solids were washed with DI water, oven dried (105°C for 4 hours) and burnt in the furnace (550°C for 2 hours) for acid insoluble lignin (AIL) and ash determination. Filtrate was used for acid soluble lignin (ASL) and sugar determination. AIL percentage was calculated as follows:

$$\%\mathrm{AIR} = \frac{(\mathrm{W_{crucible+AIR}} - \mathrm{W_{crucible}})}{\mathrm{ODW_{sample}}}\mathrm{x}100 \qquad \text{Equation 3.14}$$

$$\%\mathrm{AIL} = \frac{(\mathrm{W_{crucible+AIR}} - \mathrm{W_{crucible}}) - (\mathrm{W_{crucible+ash}} - \mathrm{W_{crucible}}) - \mathrm{W_{protein}}}{\mathrm{ODW_{sample}}}\mathrm{x}100 \qquad \text{Equation 3.15}$$

Where:

%AIR = acid insoluble residue

$W_{crucible+AIR}$ = weight of crucible with oven dried sample and filter

$W_{crucible}$ = weight of empty crucible

$W_{crucible+ash}$ = weight of crucible with furnaced sample and filter

$W_{protein}$ = weight of protein (obtained from protein analysis)

ODW_{sample} = weight of oven-dry sample

ASL was analysed by measuring the absorbance of the filtrate using a UV-Visible spectrophotometer (3000 series, Cecil Instruments Ltd., UK) at 325 nm wavelength a few hours after hydrolysis. Sample was diluted with 4% sulphuric acid for absorbance to fall between 0.7-1.0. The same solvent was used as blanks.

$$\%\mathrm{ASL} = \frac{\mathrm{UVabs\ x}\ V_{filtrate}\mathrm{xdilution}}{\varepsilon \mathrm{xODW_{sample}}xPathlenght}\mathrm{x100} \qquad \text{Equation 3.16}$$

Where:

UVabs = average UV absorbance for the sample

$V_{filtrate}$ = volume of filtrate

ε = absorptivity of substrate at specific wavelength (assumed to be 30 L g^{-1} cm^{-1} like corn stover).

Pathlength = pathlength of UV-Vis cell (1 cm)

Before sugar analysis samples were centrifuged at 13000 rpm for 7 minutes. The supernatant was diluted and placed in a 5 mL sample vial with a 0.45 µm nylon filter cap. Sugar analysis was carried out on a Dionex DX-500 HPLC system. In this glucose, xylose, galactose, arabinose, mannose and cellobiose were separated at 30^{o}C on a CarboPac PA1 column (250 x 4 mm) in combination with a CarboPac guard column (25 x 4 mm) (Dionex, Sunnyvale, CA, USA). The mobile phase components were 200 mM sodium hydroxide (A), distilled water (B) and 170 mM sodium acetate in 200 mM sodium hydroxide (C). The system set up used a 2.5 µL sample loop and 300 mM NaOH post column eluent at a pressure of 2.76 bar to aid sugar detection.

3.4.6 Lipids

Freeze-dried and milled ORS was used for this analysis. Lipid content was determined using Soxhlet extraction with hexane following method 9071B [273] with slight modifications. This procedure is used to quantify low concentrations of oil and grease in solid materials amenable to solvent extraction with hexane. The results of this method are appropriately termed ‘hexane extractable material’ (HEM). The Soxhlet apparatus containing the extraction thimble and sample was set up in a fume cupboard; and attached to a pre-weighed 250 mL round-bottom flask containing glass beads and 90 mL of hexane. The heating control on the heating mantle was adjusted to around 90°C so that a cycling rate of 20 cycles per hour was obtained for a period of 4 hours. After that, the flask was connected to the distilling head apparatus and the hexane was

distilled by immersing the lower half of the flask in a water bath. The hexane collected was disposed of and the remaining liquid was dried at 60°C for 45 min or until constant weight was achieved. The difference between the final weight and the weight of the flask with the beads is the mass of total dry lipids. This method is limited by what lipids can be extracted.

$$\%\text{lipids} = \left(\frac{F_0 - F_f}{ODW_{sample}}\right)\text{x}100 \qquad \text{Equation 3.17}$$

Where;

F_0 = mass of dry flask before extraction (g),

F_f = mass of dry flask after extraction (g),

ODW_{sample} = weight of oven-dry sample (g)

3.5 Characterisation of gas

3.5.2 Gas volume

Gas bag volumes were measured using a weight-type water displacement gasometer [274]. The initial height of solution in the gasometer (h_1) was recorded before the collected gas was introduced into the column through the top valve. After the bag was empty, the final height (h_2) and the weight of water (kg) were recorded, as well as the temperature (T) and pressure (P) in the room. All dry gas volumes reported are corrected to standard temperature and pressure of 273.15K and 101.325 kPa respectively according to the following equations [274]:

Height Gasometer Governing Equation Equation 3.18

$$\mathrm{V_{stp}} = \frac{\mathrm{T_{stp}A}}{\mathrm{T_{atm}p_{stp}}}\left[\left(\mathrm{p_{atm}} - \mathrm{p_{H_2O}(T_{atm})} - \rho_\mathrm{b}\mathrm{g}(\mathrm{h_{t2}} - \mathrm{h_{c2}})\right)\mathrm{h_{c2}} - \left(\mathrm{p_{atm}} - \mathrm{p_{H_2O}(T_{atm})} - \rho_\mathrm{b}\mathrm{g}(\mathrm{h_{t1}} - \mathrm{h_{c1}})\right)\mathrm{h_{c1}}\right]$$

Weight Gasometer Governing Equation Equation 3.19

$$\mathrm{V_{stp}} = \frac{\mathrm{T_{stp}A}}{\mathrm{T_{atm}p_{stp}}}\left[\left(\mathrm{p_{atm}} - \mathrm{p_{H_2O}(T_{atm})} + \rho_\mathrm{b}\mathrm{g}\left(\mathrm{H} - \mathrm{h_1} - \frac{\mathrm{m_b}}{\mathrm{A}\rho_\mathrm{b}}\right)\right)\left(\mathrm{h_1} + \frac{\mathrm{m_b}}{\mathrm{A}\rho_\mathrm{b}}\right) - \left(\mathrm{p_{atm}} - \mathrm{p_{H_2O}(T_{atm})} + \rho_\mathrm{b}\mathrm{g}(\mathrm{H} - \mathrm{h_1})\right)\mathrm{h_1}\right]$$

Where:

V = gas volume (m^3)

P = pressure (Pa)

T = temperature (K)

H = total height of column (m)

h = distance to liquid surface from a datum (m)

A = cross-sectional area of gasometer (m^2)

m_b = mass of barrier solution (kg)

ρ = density pf barrier solution (kg m^{-3})

g = gravitational acceleration (m s^{-2})

p_{H2O} = water vapour pressure (Pa)

1, 2, stp, atm, b, t, c subscripts refer to condition 1 (before addition of gas to column), condition 2 (after gas addition to column), standard temperature and pressure, atmospheric, barrier solution, collection trough and column respectively.

3.5.1 Gas composition

The biogas (CH_4, CO_2 and H_2) generated in the experiments was collected in gas-impermeable sampling bags. Biogas composition was quantified using a MG#5 Gas Chromatograph (SRI Instruments, USA) with a thermal conductivity detector. The GC instrument had two linked analytical lines with CH_4 and CO_2 separated by a Porapak Q column (80/100 mesh, 6ft). H_2 was separated using a molecular sieve 5A column (6ft). The GC was calibrated with standard gases: CH_4 (> 99.95%), 65% CH_4/35% CO_2, 50% CH_4/50% CO_2 and 60% CH_4/20% CO_2/20% H_2 (v/v). A sample of 10 mL was taken from bag used for sample collection and was injected into a gas sampling loop. If the sum of all CH_4, CO_2 and H_2 percentages was below 100%, the remaining volume was assumed to be air or N_2, depending on the experiment.

3.6 Characterisation of liquid fraction

3.6.1 pH and alkalinity

pH was measured using a Jenway 3010 meter (Bibby Scientific Ltd, UK) with a combination glass electrode, calibrated in buffers at pH 4, 7 and 9.2. The pH probe was rinsed with DI water in between measurements to avoid sample cross-contamination. Samples from fermentation experiments were measured immediately to prevent changes in pH due to the loss of dissolved CO_2.

Alkalinity was measured by titration based on Standard Method 2320B [268]. 2-5 g of sample was mixed with 40 mL of DI water using a magnetic stirrer. Titration was performed using a Schott Titroline Easy digital titration system (Xylem Inc, Germany), with sample being under constant stirring. A 0.25 N H_2SO_4 solution was used as titrant to determine endpoints of pH 5.7, 4.3 and 4.0, allowing calculation of total (TA), partial (PA) and intermediate alkalinity (IA) [275]. PA is a

measurement of bicarbonate buffering while IA is attributed to the buffering capacity of Volatile Fatty Acids (VFA).

The pH probe was calibrated before titration and washed with DI water between samples to avoid cross-contamination. Alkalinity was calculated according to the following equations:

$$\mathrm{TA} = \frac{(\mathrm{V}_{4.0} + \mathrm{V}_{4.3} + \mathrm{V}_{5.7})\ \mathrm{x\ N\ x\ 50000}}{\mathrm{V_s}} \qquad \text{Equation 3.20}$$

$$\mathrm{PA} = \frac{\mathrm{V}_{5.7}\ \mathrm{x\ N\ x\ 50000}}{\mathrm{V_s}} \qquad \text{Equation 3.21}$$

$$\mathrm{IA} = \frac{\mathrm{V}_{4.3}\ \mathrm{x\ N\ x\ 50000}}{\mathrm{V_s}} \qquad \text{Equation 3.22}$$

Where:

TA = total alkalinity (mg $CaCO_3$ L^{-1})

PA = partial or bicarbonate alkalinity (mg $CaCO_3$ L^{-1})

IA = intermediate or volatile fatty acid alkalinity (mg $CaCO_3$ L^{-1})

V_s = volume of sample (mL)

$V_{subscript}$ = volume of titrant required to reach the pH value indicated in the subscript (mL)

N = normality of the H_2SO_4 titrant, or the theoretical normality multiplied by a correction factor for the specific batch of titrant

3.6.2 Total ammonia nitrogen

Total ammonia nitrogen (TAN) analysis was based on Standard Method 4500-NH3 by APHA [276]. A sample aliquot of 5 mL was pipetted into a distillation/digestion tube and 50 mL of DI water was added. 50 mL of DI water were used as blanks Standards were prepared with 10 mL of 1000 mg N L^{-1} (3.82 g L^{-1} NH_4Cl) and 40 mL of DI water. Approximately 5 mL of ~50% NaOH was added to each digestion tube to guarantee a pH above 9.5 and the samples were distilled using a Büchi K-350 Distillation Unit (Büchi, UK). Erlenmeyer flasks previously filled with 25 mL of boric acid as an indicator were used to collect the distillate and progress of the distillation was indicated by a colour change from purple to green. Distillation took place for approximately 4 minutes and collected ~150mL of distillate. The distillate was titrated manually with 0.25 N H_2SO_4 using Schott Titroline Easy digital titration system (Xylem Inc, Germany) until an endpoint was reached as indicated by a colour change to purple at which point the volume of titrant added was recorded. Standards and blanks were distilled and titrated in the same way. The distillation system was cleaned in between runs by running the system with DI water in a clean digestion tube. The TAN concentration was calculated according to the following equation:

$$\mathrm{TAN} = \frac{(\mathrm{A} - \mathrm{B})\ \mathrm{x}\ \mathrm{MW}_N\ \mathrm{x}\ \mathrm{N}\ \mathrm{x}\ 1000}{\mathrm{V_s}} \qquad \text{Equation 3.23}$$

Where:

TAN = total ammonia nitrogen (mg L^{-1})

A = volume of titrant used to titrate the sample (mL)

B = volume of titrant used to titrate the blank (mL)

MW_N = molecular weight of nitrogen (14 g mol^{-1})

N = normality of the H_2SO_4 titrant, or the theoretical normality multiplied by a correction factor for the specific batch of titrant

V_s = volume of sample (mL)

3.6.3 Total organic carbon

Samples were centrifuged at 13000 rpm for 15 minutes to remove solids prior to analysis, using Thermo Sorvall Legend X1r refrigerated centrifuge at 4°C. Total organic carbon (TOC) was measured using a TOC-V_{CPH} (high-sensitivity model) system. In this system, a vertical quartz combustion tube packed with supported platinum catalyst receives a continuous flow of oxygen or air at about 150 mL min^{-1}. The furnace is maintained at 680°C. For total carbon (TC) measurement, samples are introduced into the combustion tube where they are combusted into CO_2 (and water) which is detected by a non-dispersive infrared detector (NDIR). For inorganic carbon (IC) analysis, the sample is automatically transferred to acidification chamber containing 20% (v/v) phosphoric acid, where carbon from carbonates, hydrogen carbonates and dissolved CO_2 is converted to CO_2 which is then purged by oxygen and transported to NDIR detector. The peak area is proportional to the TC/IC concentration of the sample. The TOC was quantified as the difference between TC and IC. Injection volume varied between 20-50 µL and a correction factor was applied accordingly. The linearity of the instrument was checked with 5 points (up to 50 times dilution) using standard solutions of 1000 mg TC L^{-1} of potassium hydrogen phthalate for TC and 1000 mg IC L^{-1} of NaHCO3/Na_2CO_3 for IC. Solutions of glucose and acetic acid of known concentrations were used as check standards.

3.6.4 Chemical oxygen demand

Chemical oxygen demand (COD) was measured by the closed tube reflux method with titrometric determination of the end point based on “The determination of chemical oxygen demand in waters and effluents (2007)” method by Environment Agency, UK [277]:

Sample dilution was carried out if the sample COD was expected to be higher than 400 mg L^{-1}. Either 2 mL of sample or 2 mL DI water for blanks was placed into round-bottom borosilicate reflux tubes followed by the addition of 3.8 mL of FICODOX-plus reagent (Fisher Scientific Ltd, UK), the composition of which is shown in Table 6.

Table 6. FICODOX-plus composition

Chemical	**Weight %**
$K_2Cr_2O_7$	0.1%
Ag_2SO_4	0.25-0.5%
H_2SO_4	85%
$KCr(SO_4)_2$	0.25-0.5%
Water	14%

Tubes were sealed with a PTFE screw cap and contents were mixed. Tubes containing samples and blanks were placed in heating block with holes of same diameter as tubes, cast aluminium 50 mm deep, at 150°C for 2 hours. After cooling, one or two drops of ferroin indicator (Table 7) were added to each tube (Fisher Scientific Ltd, UK).

Table 7. Ferroin indicator composition

Chemical	**Concentration**
1,10-phenantroline monohydrate	14.85 g L^{-1}
Iron (II) sulphate heptahydrate	6.95 g L^{-1}

The mixture was titrated with acidified (2% volume H_2SO_4) 0.025M ferrous ammonium sulphate (FAS) in solution ($(NH_4)_2Fe(SO_4)_2$). The end point was a colour change from blue-green to red-brown. FAS solution was regularly standardised using a mixture of 5 mL 0.021 M $K_2Cr_2O_7$, 55 mL DI water, 25 mL H_2SO_4 and two drops of ferroin indicator. COD values were calculated according to the following equations:

$$C = \frac{5}{8 \cdot V} \qquad \text{Equation 3.24}$$

$$F = \frac{C}{M} \qquad \text{Equation 3.25}$$

$$COD = (A - B) \cdot M \cdot F \cdot 4000 \cdot \text{dilution factor} \qquad \text{Equation 3.26}$$

Where:

C = actual molarity of FAS (M)

M = predicted molarity of FAS (M)

V = volume of FAS titrated in molarity measurement (mL)

F = correction factor of FAS molarity (no units)

COD = Chemical oxygen demand of sample (mg O_2 L^{-1})

A = average volume of FAS used for blank (mL)

B = volume of FAS used for sample (mL)

3.6.5 Volatile fatty acids

Volatile fatty acids (VFA) were analysed based on the method developed by the Standing Committee of Analysts [278]. Samples were centrifuged 13,000 rpm (micro-centrifuge, various manufacturers) for 15 minutes. 0.9 mL of the supernatant was transferred by pipette to 2 mL vials with 0.1 mL of pure (>98%) formic acid. Where dilution was necessary, DI water was used before adding to the vial with formic acid to give a final concentration of 10% formic acid.

Standard solutions containing acetic, propionic, iso-butyric, n-butyric, iso-valeric, valeric, hexanoic and heptanoic acids, at three dilutions to give individual acid concentrations of 50, 250 and 500 mg L^{-1} respectively in 10% formic acid, were used for calibration.

Quantification of the VFA was carried out by a Shimazdu GC-2010 gas chromatograph (Shimadzu, UK), using a flame ionisation detector and a capillary column type SGE BP-21. The carrier gas was helium with a linear velocity of 40 cm s^{-1}, at a split ratio of 100 giving a flow rate of 1.86 mL min^{-1} in the column and a 2 mL min^{-1} purge. The make-up gas was nitrogen at a flow rate of 30mL min^{-1}. The GC oven temperature was programmed to increase from 60 to 210°C in 15 minutes with a final hold time of 3 minutes. The temperatures of injector and detector were 235 and 250°C, respectively.

VFA mass values were converted to COD or TOC values using the appropriate conversion factors showed in Table 41, Appendix D. Degree of acidification (DoA) was defined as the TOC (or COD) corresponding to VFA over total TOC (or COD) of the solution:

$$\mathrm{DoA\%} = \frac{\mathrm{C}_{VFA}}{\mathrm{C}_{TOC}} \cdot 100 \qquad or \qquad \mathrm{DoA\%} = \frac{\mathrm{C}_{VFA}}{\mathrm{C}_{COD}} \cdot 100 \qquad \text{Equation 3.27}$$

3.6.6 Lactic acid and glucose

Glucose and lactic acid measurements were carried out using YSI 2700 Select biochemistry analyser (YSI Inc., US). Samples were centrifuged at 13000 rpm for 15 min and diluted before analysis.

Chapter 4 Batch experiments

This chapter focuses on the characterisation of the organic residual solids (ORS), the optimisation of the enzymatic pretreatment, and the batch acidogenic fermentation experiments.

4.1 Characterisation of organic residual solids

In this thesis, the ORS derived from the Fiberight municipal solid waste treatment process were used as substrate for acidogenic fermentation. Table 8 summarises the composition of the ORS.

Table 8. Characteristics of the ORS from Southampton pilot plant (A) and Maine (B)

Characteristic	Average value (A)	Standard deviation[1] (A)	Average value[2] (B)
Total solids (TS)	26.64%	±0.05%	-
Volatile solids on a dry basis (VS/TS)	75.94%	±0.96%	67.22%
$CaCO_3$ ash	5.00%	±0.41%	-
Glucan	33.33%	±2.94%	29.15%
Hemicellulose	5.84%	±0.25%	8.09%
Acid insoluble lignin (AIL)	11.10%	±0.07%	14.46%
Acid soluble lignin (ASL)	1.40%	±0.10%	0.66%
Proteins	8.11%	±0.29%	-
Lipids	4.71%	±0.22%	6.72%
Extractives	-	-	10.65%
Total food content[3]	64.49%	-	-

[1]Based on three sample replicates

[2]Data obtained from external laboratory Celignis Analytical [279]

[3]Total food content was calculated as the sum of glucan, hemicellulose, AIL, ASL, proteins and lipids. All values on a dry weight basis

The VS/TS of the ORS is about 10% lower than the average VS/TS of the organic fraction of municipal solid waste (OFMSW) around the globe (~85%) [2]. This difference can be explained by a washout of soluble volatiles and small suspended solids by the Fiberight washing process. High

VS/TS is desired for efficient AF as it can be correlated to organic-biodegradable material, i.e. volatile fatty acid (VFA) potential. VS/TS, however, is not a direct measure of biodegradability as VS analysis cannot differentiate between biodegradable volatiles (e.g. cellulose) and non-biodegradable volatiles (e.g. plastics). The carbohydrate analysis carried out in this work could not distinguish between the different glucose-containing carbohydrates (e.g. cellulose and starch). Therefore, all quantified glucose was presented as glucan, whose percentage varied between 29-33%, depending on the ORS source. Due to the fibrous nature of the ORS, and the fact that the washing process probably eliminated soluble starch, it is believed that most of the glucan is represented by cellulose. Hemicellulose (the sum of xylose, mannose, arabinose, galactose and cellobiose) content is 6-8%. Lignin content (AIL and ASL) represent 13-14%, which explains the recalcitrant nature (low solubility and digestibility) demonstrated in preliminary work (see Appendix B). In this preliminary work, methane yield in semi-continuous AD of ORS was only 0.15 L g^{-1} VS, which is considered low. Lignin degradation is unlikely to happen in AD/AF systems, unless the substrate is subjected to certain types of pretreatment [280]. Natural lignin degradation is generally carried out by fungal species [281] which are rarely present in AD.

Based on the results presented in Table 8, total 'food' (cellulose, hemicellulose, lipids and proteins) for AD microbes represents just over 50% of dry ORS, or 64% if lignin is included. Extractives are components that can be extracted by solvents (e.g. ethanol or acetone); for example fats, waxes, proteins, gums, resins, simple sugars, phenolics, pectins, and fatty acids. The main fatty acids detected in the ORS from Maine, USA were palmitic (42%), stearic (27%) and linoleic (18%) [279]. $CaCO_3$ provides buffering capacity in AD systems [209]. The measured $CaCO_3$ ash in the ORS from Southampton was 5%, which could have been derived from small pieces of eggshell (based on visual inspection). The $CaCO_3$ could also be derived from the paper fraction, since $CaCO_3$ is commonly used as paper filler [282].

Compositional differences are observed when comparing ORS from the commercial plant in Maine, USA, and ORS from the pilot plant in Southampton, UK. Replicability for this substrate, or any residue derived from MSW, cannot be guaranteed as MSW composition may vary depending on source location and season. In this case, replicability of composition is also difficult due to the difference in the scales of the two processes (i.e. pilot plant vs. large scale). The effect of different fermentation conditions can, however, be established by comparison to a control experiment or among replicates where the same source substrate has been used.

The ORS from Southampton was also subjected to elemental analysis. The results are shown in Table 9. As noted in section 3.4.3, the oxygen percentage was calculated as the difference between 100% and the sum of mass percentages of N, C, H and S, and should therefore be

considered as an estimated value. The results fall within the typical composition range of OFMSW: 20.3-45%C, 0.4-1.8% N, 5.9-7.8 %H, ~0% S, 31.0-47.3% O, on a dry weight basis [283, 284]. Some discrepancies between OFMSW and ORS are expected, as the ORS might contain a higher concentration of inorganic impurities (e.g. broken glass and grit).

Table 9. Elemental composition of ORS from Southampton on a dry basis

Element	**N**	**C**	**H**	**S**	**O**
Mass percentage (%)	1.0%	39.8%	5.4%	0.2%	53.7%
Molar percentage (%)	0.58%	27.49%	44.06%	0.04%	27.83%
Mol per mol of C	0.021	1	1.603	0.002	1.012

The elemental composition was used to calculate the theoretical bio-methane potential (BMP) using the modified Buswell equation [285]:

$$C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right)H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right)CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right)CO_4 + dNH_3 + eH_2O \quad \text{Equation 4.1}$$

The results for 1 kg of dry ORS are shown in Table 10. The sum of the products mass is slightly over 1 kg, as the mass balance includes water as reagent. The theoretical BMP is, based on the modified Buswell equation, 233 g of CH_4 per kg of dry ORS. Assuming ~70% VS/TS, the theoretical BMP is then 333 g CH_4 per g VS or 465 mL CH_4 per g VS. Assuming that one mol of acetic acid (AcH) is necessary to generate one mol of methane, the AcH potential has been estimated: 0.874 kg AcH kg^{-1} dry ORS or 1.249 g AcH g^{-1} VS. Based on first principles, a AcH potential higher than 1 g AcH g^{-1} VS is not possible, hence it is suspected that the theoretical AcH potential is biased by the presence of inorganic substances. One way to partially eliminate this bias would be to subject the ash resulting from VS analysis to elemental analysis and obtain the elemental composition of the VS fraction by deduction. For comparison purposes, the theoretical BMP of the OFMSW in one research study was 494 mL CH_4 g^{-1} VS, which resulted in an experimental BMP of 202 mL CH_4 g^{-1} VS [284] (0.543 g AcH g^{-1} VS). A higher experimental BMP of 344 mL CH_4 g^{-1} VS was achieved by Zhang et al., for a Buswell equation value of 557 mL CH_4 g^{-1} VS [286]. The values calculated by Zhang et al. were much lower using the biochemical composition of OFMSW, but still higher than experimental values.

Table 10. Gas products from the anaerobic digestion of ORS based on elemental composition, calculated using the modified Buswell equation

Element	**CO_2**	**CH_4**	**NH_3**	**H_2S**
Mol per kg of dry ORS	18.59	14.55	0.69	0.05
Kg per kg of dry ORS	0.818	0.233	0.012	0.002

4.2 TS and initial pH effect on AF

A batch experiment was carried out to study the effect of initial pH and TS content on the acidogenic fermentation (AF) of ORS at 35±2°C. Reactors with a 150 mL working volume were inoculated with digestate to reach a S/I ratio of 40 (g TS g^{-1} TS). Initial pH was modified by adding drops of 4M NaOH. Two replicates were used for daily solid-liquid sample collection and one replicate was connected to a gas bag for gas sampling at the end of the fermentation. Control reactors were run with inoculum alone. Initial pH values tested were 7 (approximately, i.e., no NaOH addition), 8.5 and 10. TS concentration were 2, 5 and 10%.

As can be seen from Figure 6, higher initial pH up to 10 resulted in higher VFA concentrations for all TS tested. Initial pH, however, did not have a significant effect on final VFA concentrations for 5% and 10% TS. For any initial pH, higher TS led to higher VFA concentrations. This is expected as high substrate concentration leads to high product concentration. Interestingly, significant VFA production was observed in the control reactor at initial pH 10. This could be attributed to the chemical effect of NaOH on the breakdown of proteinaceous substrates such as digestate, resulting in a release of soluble COD subjectable to AF. As chemical addition can add considerably to operational costs, optimum conditions were therefore 10% TS and unfixed initial pH. Although maximum VFA concentration was reached after 8 days, production rates slowed significantly after 3 days for 10% TS. Solid destruction was insignificant for all conditions after 14 days (<5% VSS reduction). This indicated that the hydrolysis may be the rate limiting step of the fermentation.

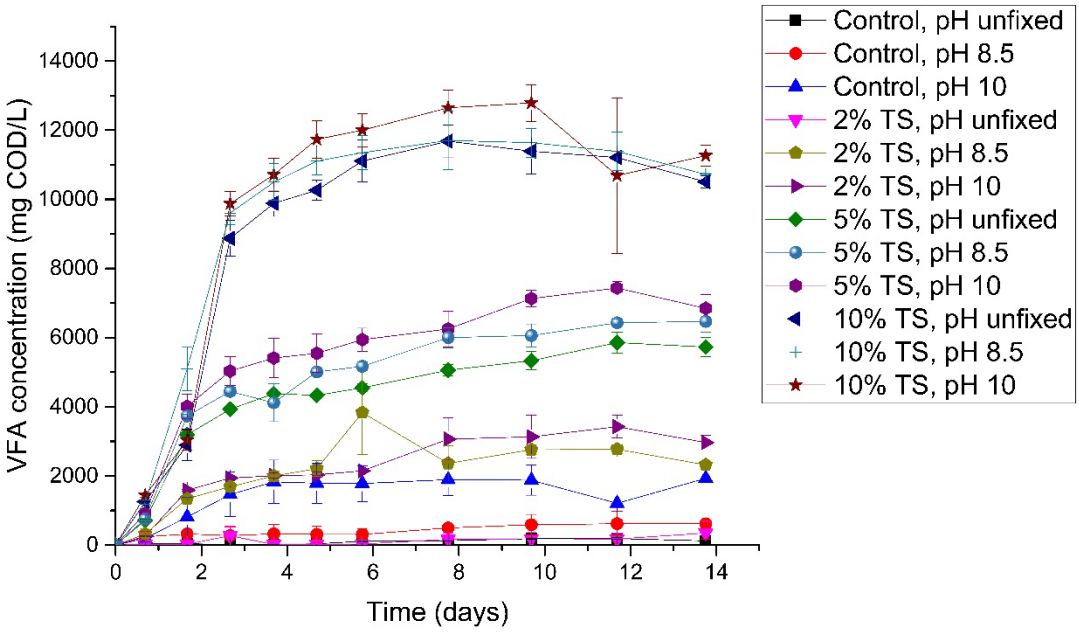

Figure 6. VFA concentration in mg COD L^{-1} through time at different TS concentrations and initial pH, at 35°C and S/I ratio of 40 g TS g^{-1} TS. Unfixed pH of ORS was roughly 6.6, 6.9 and 7.2 for 10%, 5% and 2% TS respectively. Error bars display standard deviation of two replicates

Figure 7 shows the VFA yields. Differences in yields were not significant during the first 6 days (maximum 130-160 mg COD g^{-1} VS), with the exception of 5% TS-pH unfixed which reached a maximum yield of 120 mg COD g^{-1} VS. Maximum VFA yield (~220 mg COD g^{-1} VS) was reached on day 12 with 2% TS and initial pH 10.

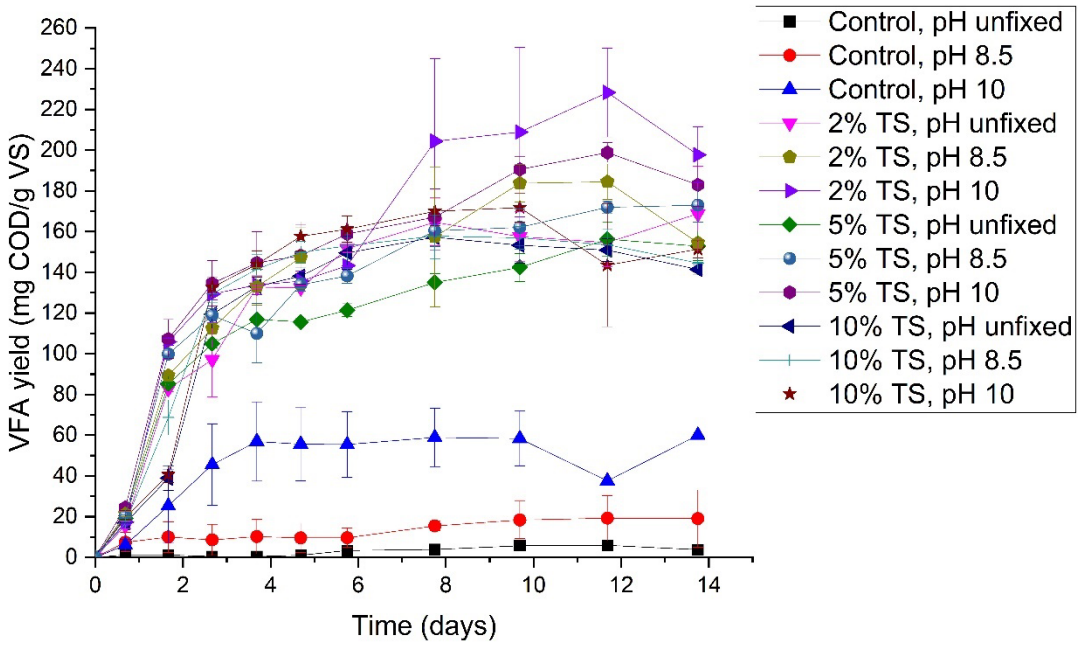

Figure 7. VFA yield in mg COD g^{-1} VS through time at different total solids concentrations and initial pH, at 35°C and S/I ratio of 40 g TS g^{-1} TS. Unfixed pH of ORS was roughly 6.6, 6.9 and 7.2 for 10%, 5% and 2% TS respectively. Error bars display standard deviation of two replicates

As observed in Figure 8, accumulated CH_4 in all cases for the ORS was either negligible or at undetectable levels. The primary gas accumulated was CO_2 as this is a by-product of the acidogenic fermentation. H_2 and CH_4 yields after 14 days were insignificant (<1 L kg^{-1} VS) regardless of pH for all ORS samples. The highest H_2 yield was observed for 10% TS and initial pH 10. Therefore, controlling pH in alkaline levels may have a more important role in dark fermentation where hydrogen production is desired. In the case of the control reactors, adjusting the initial pH to 10 completely inhibited the methanogenic activity and no biogas was detected. A significant CH_4 production was observed for initial pH 8.5 in comparison to unfixed pH. This, again, is probably due to the chemical effect of NaOH addition breaking down insoluble materials. At pH 8.5, however, methanogenic activity was not inhibited, and released VFA are degraded to biogas.

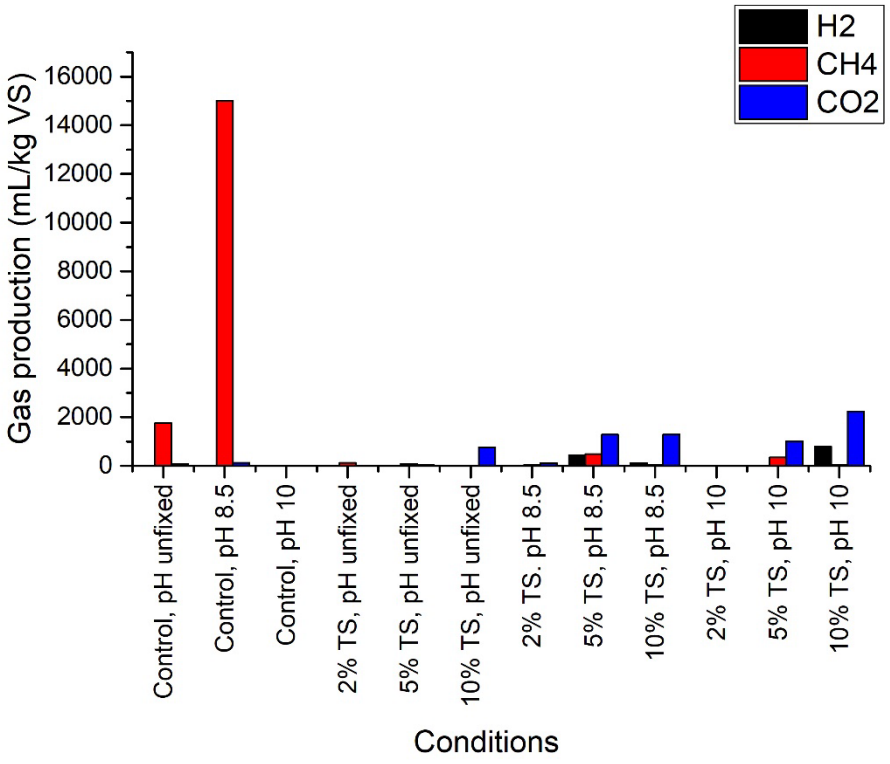

Figure 8. Accumulated volume of H_2, CH_4 and CO_2 after 14 days of batch fermentation at different TS and initial pH, at 35°C and S/I ratio of 40 g TS g^{-1} TS. Unfixed pH of ORS was roughly 6.6, 6.9 and 7.2 for 10%, 5% and 2% TS respectively.

Figure 9 shows the pH measurement through time. For all reactors containing ORS, pH declined significantly within 24 hours. Despite the differences in VFA concentrations, there were no significant differences in final pH (5.5-6.0) for different TS content and initial pH. For the control reactors, the pH did not change much with respect to initial values. The control with initial pH 10 has the same concentration of VFA as the replicates with 2% TS-initial pH 10 on day 4, however the pH of the control remained practically constant. Higher pH stability indicates that the digestate (the inoculum) has better buffering properties than the ORS. The high substrate to inoculum ratio (S/I ratio), however, did not appear to affect the VFA production negatively, and could explain methanogenic activity inhibition.

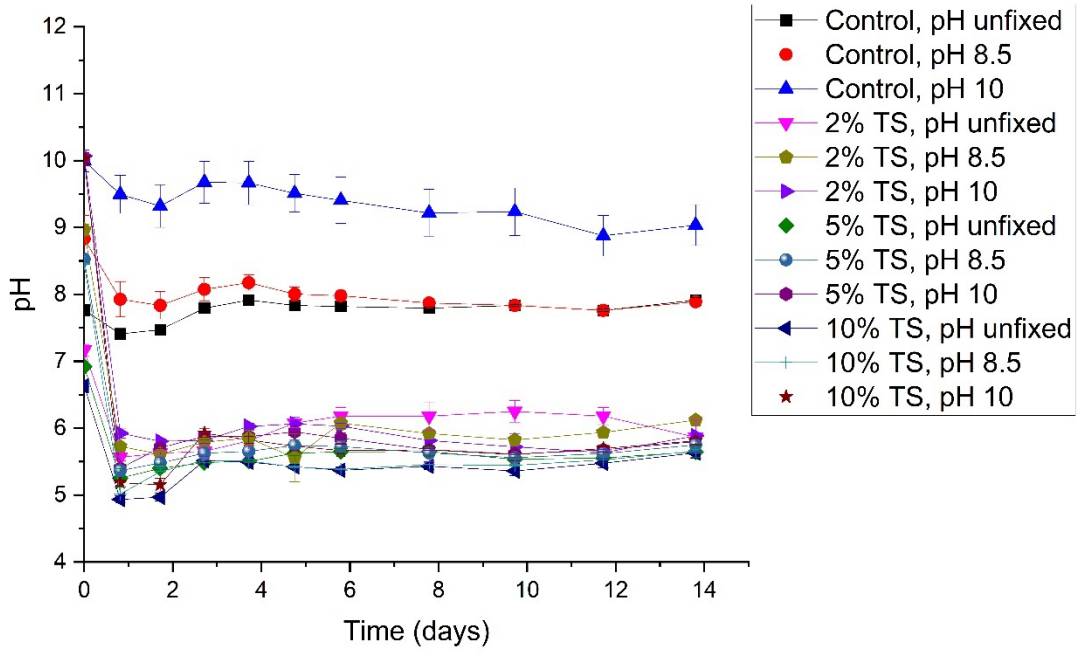

Figure 9. Measurements of pH vs. fermentation time for a batch experiment at different TS concentration and initial pH, at 35°C and S/I ratio of 40 g TS g^{-1} TS. Error bars display standard deviation of two replicates

Table 11 shows the VFA profile generated in this experiment. As observed, the TS and initial pH had a significant effect on the type of VFA produced. The main acid in all conditions was acetic (>32%), followed by butyric (11-34%) and propionic (13-20%). Valeric acid was more abundant in 10% TS conditions for all initial pH values. In contrast, iso-valeric acid was more abundant in 2% TS conditions. Overall, initial TS had a more significant effect on VFA profile than initial pH. This is mostly due to the fact that pH rapidly changed and settled at similar levels for all replicates. This is an indication that organic concentration might have a strong influence on AF.

Table 11. VFA profile on a COD basis on day 8 of fermentation at different initial pH and TS, at 35°C and S/I ratio of 40 g TS g^{-1} TS. Percentages are the average of two replicates

	Unfixed pH			**pH 8.5**			**pH 10**		
Acid/conditions	**2% TS**	**5% TS**	**10% TS**	**2% TS**	**5% TS**	**10% TS**	**2% TS**	**5% TS**	**10% TS**
Acetic	35.7%	37.4%	33.0%	37.5%	32.8%	38.1%	40.8%	34.4%	35.3%
Propionic	17.1%	13.0%	16.5%	13.7%	13.9%	20.1%	14.6%	13.1%	17.9%
Iso-Butyric	5.4%	2.9%	1.1%	4.4%	1.9%	1.0%	2.8%	1.7%	1.2%
n-Butyric	22.5%	33.7%	15.1%	26.0%	33.0%	11.4%	26.8%	31.9%	12.5%

Iso-Valeric	13.9%	5.2%	3.2%	10.6%	4.6%	2.8%	6.2%	4.1%	3.2%
n-Valeric	4.1%	2.9%	11.9%	4.4%	3.9%	12.1%	3.6%	2.9%	12.7%
Hexanoic	1.2%	4.6%	12.3%	2.1%	9.1%	8.5%	3.9%	11.2%	10.3%
Heptanoic	0.0%	0.2%	6.8%	1.3%	0.8%	6.0%	1.1%	0.8%	6.9%

To conclude, optimal conditions to achieve a VFA concentration of 10 g COD L^{-1} in batch tests of ORS are pH unfixed, 10% TS and 3 days HRT. Although maximum concentration of over 12 g COD L^{-1} is achieved for pH 10, 10% TS and 8 days HRT. Solid destruction achieved in this experiment was not significant due to the recalcitrant nature of the ORS. Therefore, it was concluded that a pretreatment could be beneficial to maximise conversion and reduce the final solid amount sent to landfill.

4.3 Liquid fraction removal effect on AF

It has been established that product inhibition and degradation are disadvantages of AF processes. Removing part of the liquid fraction containing the VFA and replacing it with VFA-free medium (e.g. water) could help avoid inhibition and degradation. This is similar to industrial reactors which have a solids recirculation system, or to a leachate reactor. In the laboratory, liquid removal can be achieved via centrifugation. In systems of this kind, hydraulic retention time (HRT) and solids retention time (SRT) are uncoupled and will depend on the operational mode.

An experiment was carried out to study the effect of liquid removal on the acidogenic fermentation of the ORS at 35±2°C and 10% TS (pH unfixed). Reactors with a 200 mL working volume were inoculated with digestate to reach a substrate to inoculum ratio of 40 g TS g^{-1} TS. Reactor contents were centrifuged at 4350 rpm for 10 min and a proportion of supernatant was removed as shown in Table 12. Conditions were tested in duplicate for solid-liquid samples. Two control replicates were run without liquid removal. HRT was defined as the average time the liquid fraction remains inside the reactor. Limited loss of solid was observed when removing supernatant, therefore, SRT could be considered equal to the fermentation time for this experiment. The experiment was carried out over 12 days.

Table 12. Conditions tested for the effect liquid fraction removal on the AF of ORS

Operational mode	**Discontinuous**	**Semi-continuous**	**Continuous**	**Discontinuous**	**Discontinuous**
Volume of supernatant removed	100% every 3 days	66% every 2 days	33% every day	100% every 2 days	100% every 4 days
Approximate HRT (days)	5.5	5.5	5.5	3.6	7.3

Figure 10 shows the VFA concentration versus time for different HRT and operational modes. These results reflect the VFA concentration inside the reactors. Two concentration values are observed at the same times for all test, with the exception of the control test: one before removing part of the liquid fraction and one after adding fresh water.

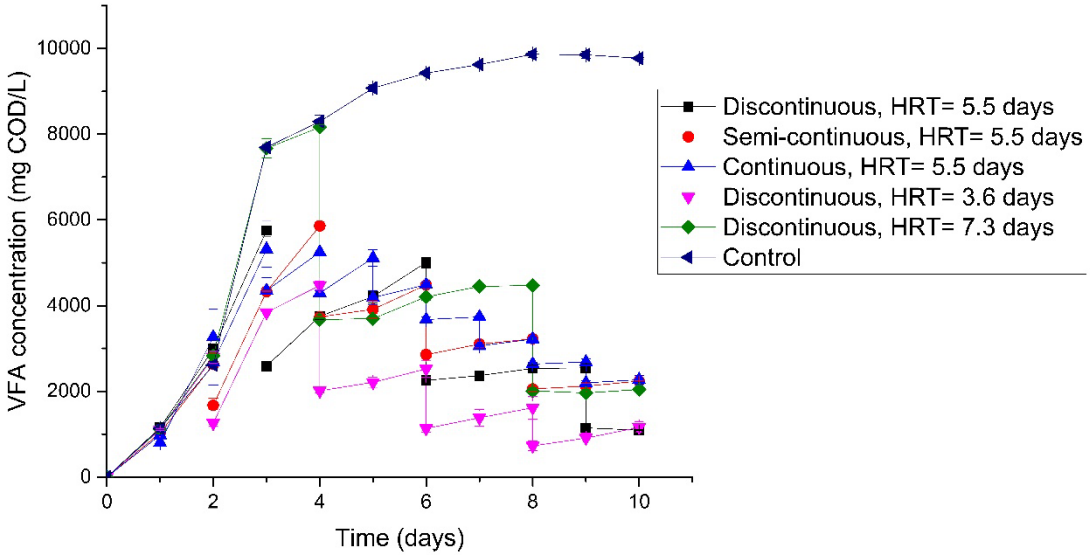

Figure 10. VFA concentration vs. fermentation time for different HRT and operational modes of liquid removal, at 10% TS, unfixed pH, 35°C, and S/I ratio of 40 g TS g^{-1} TS. Error bars display standard deviation of two replicates

Figure 11 shows VFA yield through time. It should be noted that the yield is not a representation of the VFA accumulated in the reactor, but rather the cumulative amount of VFA produced, which was calculated as the VFA remaining in the reactor plus the VFA that have been removed. In principle, VFA yields should have been the same in all cases, however, all case studies have a lower VFA yield than the control test, probably due to microbial wash out. Shorter HRTs led to

lower VFA yields, drifting away from control values. Operational mode (i.e. continuity of liquid fraction removal) did not have significant effect on the VFA yields for 5.5 day HRT.

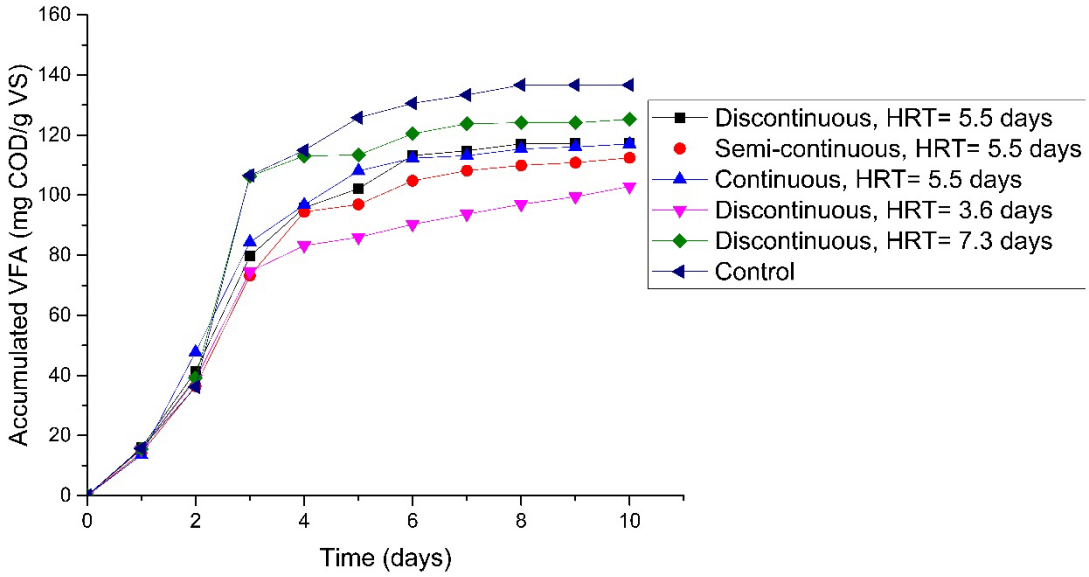

Figure 11. Cumulative VFA yield versus time for different HRT and operational modes of liquid removal, at 35°C and S/I ratio of 40 g TS g^{-1} TS

Figure 12 shows the VFA speciation through time for each operational mode. On day 1 and 2, the predominant VFA was acetic acid for all conditions tested. On day 3, butyric acid prevails but from thereon the butyric acid percentage start to decline. In the last stages of the fermentation on day 8-10 clear distinctions in VFA profile can be observed. Continuity of liquid removal did not seem to have a significant effect on VFA profile: For HRT= 5.5, continuous, semi-continuous and discontinuous modes all gave similar VFA profiles, with propionic and acetic being the predominant acids. Propionic acid becomes even more predominant at lower HRT of 3.6 days at the expense of valeric, hexanoic and heptanoic which were found in larger proportions in the control. These results may indicate that liquid removal could be used as strategy to bio-engineer a system to produce a targeted VFA. Based on the control experiment, length of fermentation has a significant effect on VFA profile.

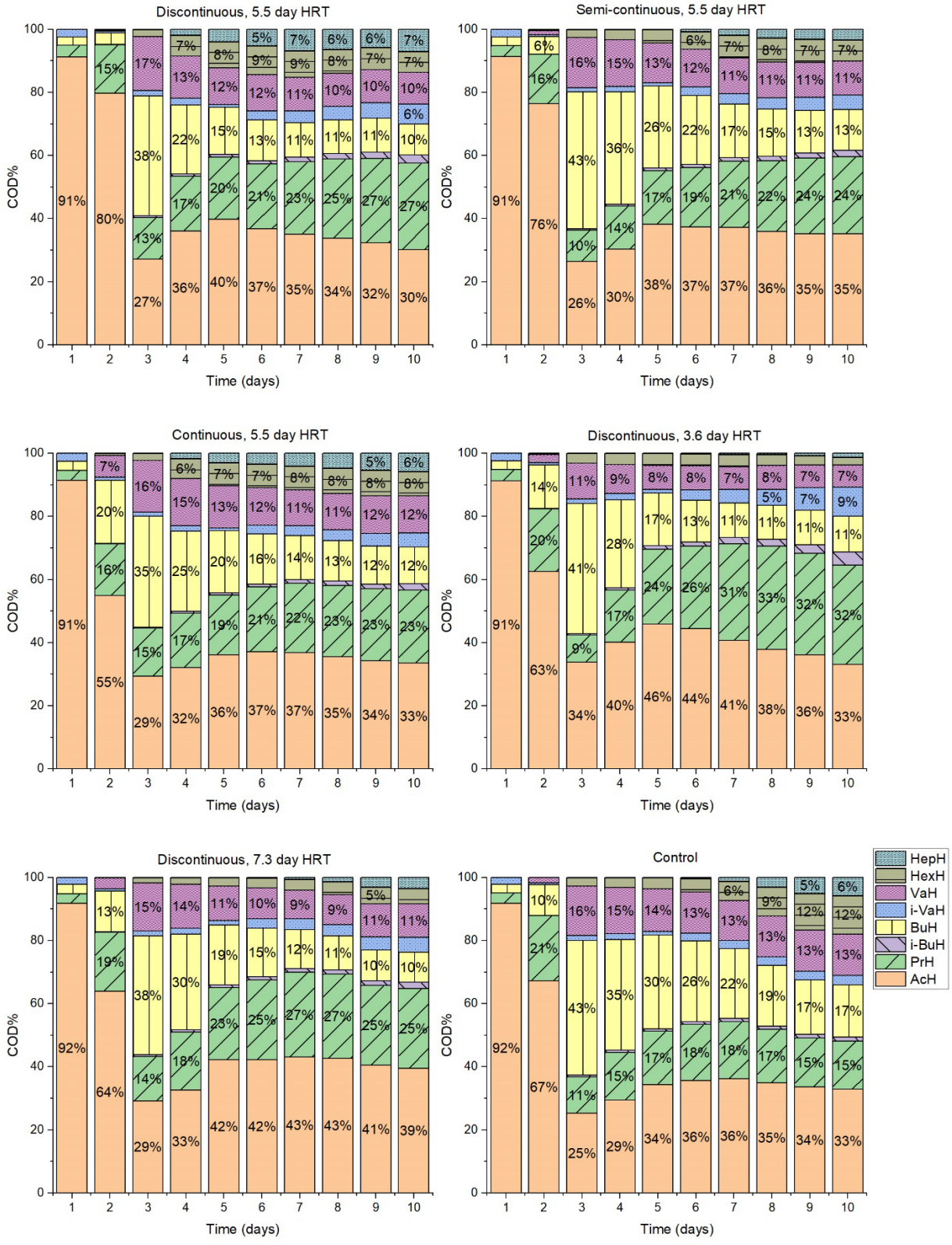

Figure 12. VFA profile on a COD basis for different HRT and operational modes of liquid removal, at 35°C and S/I ratio of 40 g TS g^{-1} TS. Percentages are the average of two replicates

Figure 13 displays the degree of acidification (DoA) of the reactors on day 8 of the fermentation. The DoA values are very similar to the control for all cases except for HRT of 3.6 days. It is then

recommended to operate at HRT higher than 3.6 days to reach maximum DoA possible. The DoA is lower than 70% in all cases, probably due to the presence of other fermentation products like alcohols and other carboxylic acids.

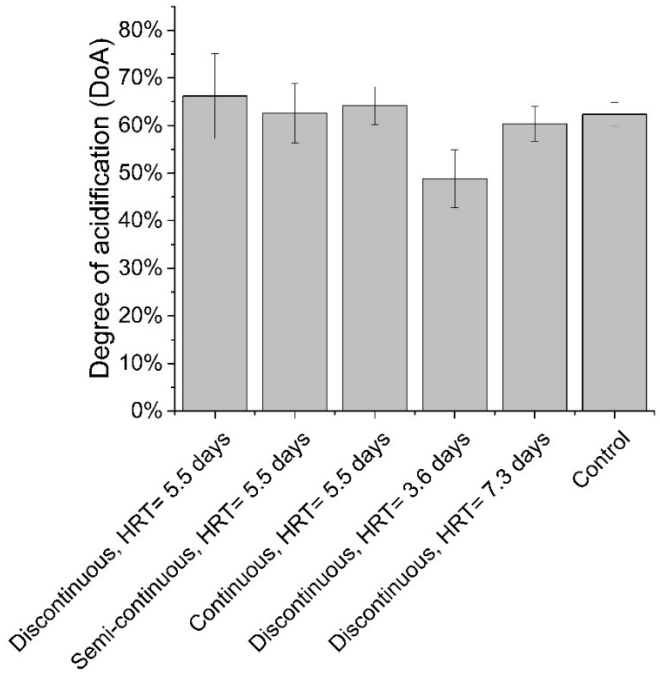

Figure 13. Degree of acidification (DoA) after 8 days of fermentation based on sCOD, for different HRT and operational modes of liquid removal, at 35°C and S/I ratio of 40 g TS g^{-1} TS. Error bars display standard deviation of two replicates

These results suggest that VFA concentration and yield limits are reached due to the recalcitrant nature of the waste, as opposed to product inhibition or degradation. This means pretreatment is required to improve the rate-limiting hydrolysis step.

Table 13 shows the alkalinity values for this experiment. It can be seen that total alkalinity (TA) is significantly lower in all tests compared to the control values, due to a reduced intermediate alkalinity (IA). The highest pH of 6 was reached by 3.6 day HRT after 11 days, compared to pH 5.4 in the control reactors. It was therefore concluded that, although liquid removal did not improve VFA yield, it did restore pH values thanks to a reduction in intermediate/partial alkalinity.

Table 13. Total (TA), partial (PA) and intermediate (IA) alkalinity values on day 12, for different HRT and operational modes of liquid removal, at 35°C and S/I ratio of 40 g TS g^{-1} TS. Values are the average of two replicates

Operational mode	**TA (mg/L)**	**PA (mg/L)**	**IA (mg/L)**	**IA/PA**
Discontinuous, HRT= 5.5 days	3600	550	2333	4.58
Semi-continuous, HRT= 5.5 days	4616	500	3466	6.94
Continuous, HRT= 5.5 days	2933	317	1967	6.18
Discontinuous, HRT= 3.6 days	2766	567	1700	3.00
Discontinuous, HRT= 7.3 days	3100	283	1933	6.82
Control	6800	100	4783	47.83

4.4 Enzyme pretreatment optimisation

As previously discussed, the ORS is recalcitrant and may benefit from pretreatment for efficient VFA production. Among the variety of different feedstock pretreatments, enzymatic pretreatment was chosen in this work. Despite the high cost of enzymes, they are compatible with biological processes, i.e. they are not inhibitory nor do they produce inhibitory compounds. Compared to physical and chemical pretreatments, enzyme pretreatment could therefore be consider 'sustainable' due to the biodegradable composition of enzymes and the low energy required.

In previous work, a TS content higher than 10% led to poor mixing and therefore, experiments were carried out at 10% TS or lower. As the enzymes break down the substrate, the solid-liquid mixture becomes less viscous and the mixing improves.

The aim of this experiment was to optimise enzymatic pretreatment of ORS for VFA production. To simplify the experimental work, the experiment was divided in two parts: one part aimed to optimise the composition of the enzyme mixture and the other part to optimise the enzyme loading, substrate concentration (TS) and the hydrolysis time.

PPCO centrifuge containers were used as hydrolysis reactors, with a 100 mL working volume. The shaker was set at 200 rpm. The enzymes used in this experiment were complex A (cellulase/beta-glucosidase/hemicellulase), complex B (arabinase/cellulase/beta-glucanase/hemicellulase/xylanase), amylase, lignase and lipase. In accordance with the enzyme

manufacturer's recommendations, pH was maintained at 5.5 using a buffer solution of 96% (v/v) of 0.1M KH_2PO_4 and 4% (v/v) 0.1M Na_2HPO_4, and the reactors were incubated at 50°C.

The first part of the experiment was carried out at 7.5% TS and 24 hours of incubation time. The enzyme loading was 2% (defined as g of wet enzyme per g of TS). The proportion of each enzyme in the mixture was defined on a wet weight basis. For the second part of the experiment, the optimum enzyme mixture was used to test different TS (5-10%), enzyme loading (1-5%) and incubation times (16-48h).

The design of experiments (DoE) was developed using the JMP software from SAS [287]. The DoE was carried out to minimise experimental bias, develop a model and study interactions between parameters. The model was set to maximise soluble COD (sCOD), which is a direct and measurable result of enzymatic pretreatment performance.

4.4.1 Optimisation of enzyme mixture

In this part of the experiment, the variables (enzyme proportions) were defined as "mixture" in the JMP software. In preliminary work, it was found that the most suitable enzyme to treat the ORS is complex A and, therefore, complex A values were set to fall between 0.5 and 1 in the mixture. The other enzymes were therefore set between 0 and 0.5. The DoE was set to test the individual effect of each enzyme and the interactions between them up to 5 levels, with up to two replicates. For this input, JMP suggested 33 runs in total with the random combinations summarised in Table 14. In addition to the suggested runs, a further run (run 34) with no enzyme addition was used as a control.

Table 14 also presents the experimental results from each run. The highest sCOD yield (368 mg g^{-1} VS) was achieved for 0.75 complex A and 0.25 amylase mixture. This yield is more than double the value of the control (155 mg g^{-1} VS), which demonstrates the efficacy of the enzymatic pretreatment.

Table 14. Design of experiment suggested by JMP for different enzyme mixtures and experimental sCOD concentration and yields

	Proportion in mixture					**Experimental results**		
Run	Complex A	Amylase	Complex B	Lignase	Lipase	sCOD (mg L^{-1})	sCOD (mg g^{-1} VS)	STD
1	0.50	0.00	0.25	0.25	0.00	17,064	303.37	0.00%
2	0.50	0.00	0.00	0.50	0.00	13,338	237.12 (lowest)	#N/A
3	0.50	0.25	0.25	0.00	0.00	16,100	286.22	0.27%

4	0.67	0.00	0.17	0.00	0.17	16,195	287.91	5.12%
5	0.63	0.13	0.13	0.12	0.00	16,656	296.11	1.57%
6	0.67	0.17	0.00	0.00	0.17	18,250	324.46	4.08%
7	0.50	0.00	0.00	0.50	0.00	13,612	242.00	#N/A
8	0.50	0.00	0.00	0.25	0.25	16,482	293.01	5.82%
9	1.00	0.00	0.00	0.00	0.00	18,628	331.16	1.41%
10	0.75	0.00	0.00	0.25	0.00	17,258	306.82	1.52%
11	0.50	0.50	0.00	0.00	0.00	17,495	311.02	0.75%
12	0.63	0.00	0.13	0.12	0.12	18,462	328.21	3.56%
13	0.63	0.12	0.00	0.13	0.13	18,104	321.85	13.53%
14	0.50	0.00	0.17	0.17	0.17	18,718	332.76	1.88%
15	0.50	0.00	0.25	0.00	0.25	19,390	344.72	0.00%
16	0.50	0.17	0.17	0.00	0.17	19,082	339.23	3.23%
17	0.67	0.17	0.00	0.17	0.00	17,585	312.62	1.74%
18	0.75	0.00	0.00	0.00	0.25	16,702	296.92	3.92%
19	0.50	0.25	0.00	0.00	0.25	18,858	335.25	1.86%
20	0.50	0.00	0.00	0.00	0.50	15,975	284.01	1.64%
21	0.67	0.00	0.17	0.17	0.00	19,007	337.90	1.62%
22	0.75	0.25	0.00	0.00	0.00	20,733	368.58 (highest)	2.12%
23	0.50	0.00	0.50	0.00	0.00	19,519	347.00	1.36%
24	0.75	0.00	0.00	0.25	0.00	19,546	347.49	7.62%
25	0.62	0.12	0.12	0.00	0.13	19,741	350.95	3.79%
26	0.50	0.17	0.00	0.17	0.17	18,486	328.63	0.95%
27	0.60	0.10	0.10	0.10	0.10	17,540	311.82	6.23%
28	0.50	0.25	0.00	0.25	0.00	15,617	277.63	2.78%
29	0.67	0.17	0.17	0.00	0.00	19,005	337.86	5.77%
30	0.50	0.17	0.17	0.17	0.00	17,592	312.75	3.48%
31	0.50	0.13	0.13	0.13	0.13	16,954	301.41	1.03%
32	0.67	0.00	0.00	0.17	0.17	17,384	309.04	0.50%
33	0.75	0.00	0.25	0.00	0.00	19,297	343.06	0.23%

34	0.00	0.00	0.00	0.00	0.00	8,740	155.38 (control)	8.90%

Table 15 shows the statistical parameters for models with interactions levels 1-5. The original model was designed to provide information on up to 5 levels of interactions, however, only 2-level interactions showed significance (P<0.05). The Akaike information criterion (AIC) considers the number of parameters (complexity) and the maximum of likelihood. The model with the lowest AIC value is typically considered the best option, as it would be a compromise between fitness and simplicity. The 2-level model was therefore adopted, as the R^2 value is significantly higher compared to the 1-level model.

Table 15. Statistical parameters (AIC, R^2 and P value) of models with different levels of interactions for optimum enzyme mixture

Interactions levels	AIC	R^2	P Value
1	307	0.47	<0.05
2	318	0.81	<0.05
3	483	0.87	0.11
4	1190	0.97	0.18
5	2246	0.97	0.37

The 2-level model equation given by the software was as follows:

$$\begin{aligned}\text{sCOD}\left(\frac{\text{mg}}{\text{gVS}}\right) = {} & 336.27\left(\frac{complex\ A-0.5}{0.5}\right)+311.67\left(\frac{amylase}{0.5}\right)+347.22\left(\frac{complex\ B}{0.5}\right)\\ & +240.23\left(\frac{lignase}{0.5}\right)+284.87\left(\frac{lipase}{0.5}\right)+\left(\left(\frac{complex\ A-0.5}{0.5}\right)\left(\frac{amylase}{0.5}139.01\right)\right)\\ & +\left(\left(\frac{complex\ A-0.5}{0.5}\right)\left(\frac{complex\ B}{0.5}(-22.95)\right)\right)\\ & +\left(\left(\frac{complex\ A-0.5}{0.5}\right)\left(\frac{lignase}{0.5}139.70\right)\right)\\ & +\left(\left(\frac{complex\ A-0.5}{0.5}\right)\left(\frac{lipase}{0.5}(-104.93)\right)\right)\\ & +\left(\left(\frac{amylase}{0.5}\right)\left(\frac{complex\ B}{0.5}(-131.05)\right)\right)+\left(\left(\frac{amylase}{0.5}\right)\left(\frac{lignase}{0.5}(-4.34)\right)\right)\\ & +\left(\left(\frac{amylase}{0.5}\right)\left(\frac{lipase}{0.5}(171.32)\right)\right)+\left(\frac{complex\ B}{0.5}\right)\left(\left(\frac{lignase}{0.5}\right)65.28\right)\\ & +\left(\frac{complex\ B}{0.5}\right)\left(\left(\frac{lipase}{0.5}\right)94.81\right)+\left(\frac{lignase}{0.5}\right)\left(\left(\frac{lipase}{0.5}\right)146.34\right)\end{aligned}$$

Equation 4.1

Equation 4.1 was used with the software to predict values of sCOD and these were plotted against actual experimental values in Figure 14. The model fit is adequate (R^2>0.8) and the results are statistically significant (P <0.05). The variance observed could be explained by substrate heterogeneity in composition and/or COD analysis variability.

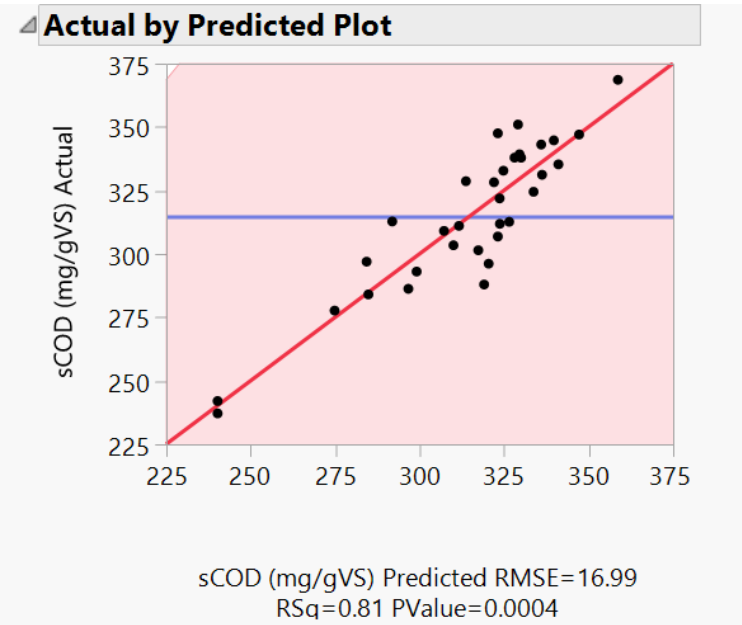

Figure 14. Fitness of predicted model (2 levels) by JMP for enzyme mixture optimisation. RMSE refers to root mean square error

Figure 15 shows the predicted sCOD by each individual enzyme proportion in the mixture, based on the 2-level model. According to the model, the maximum sCOD that can be achieved is 360 mg g^{-1} VS for a 0.81 complex A and 0.19 amylase combination. This is slightly lower than the highest sCOD achieved experimentally (369 mg g^{-1} VS) with 0.75 complex A and 0.25 amylase. Despite the experimental values being higher, the optimum values predicted by the model were adopted in this work, as there is a higher for replicability, eliminating any experimental bias.

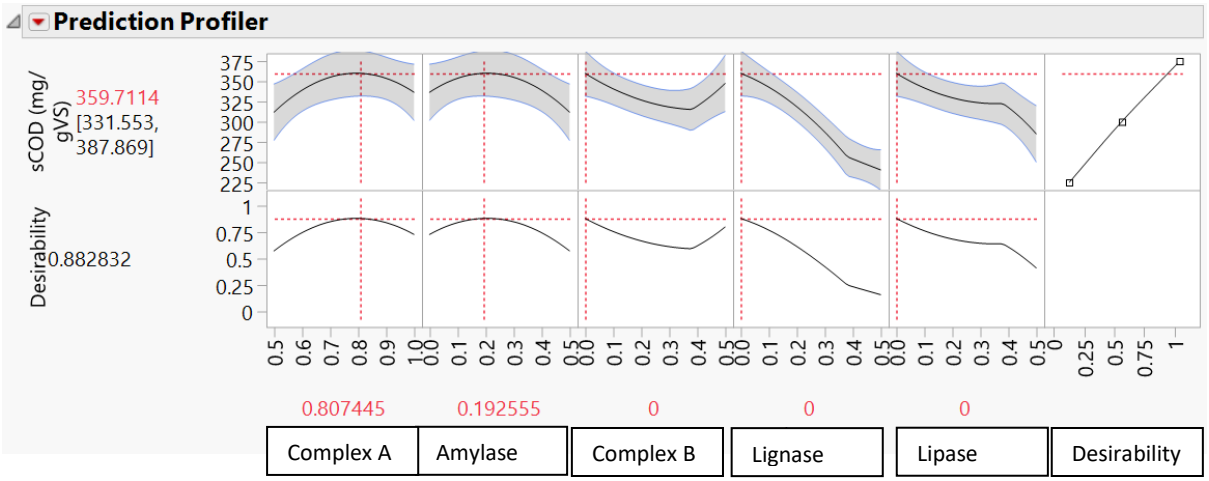

Figure 15. Prediction profiler of sCOD using the 2-level model by JMP software for enzyme mixture optimisation

4.4.2 Optimisation of enzyme loading, TS and hydrolysis time

This part of the experiment was carried out using the optimum mixture from the previous experiment: 0.81 complex A and 0.19 amylase. Different enzyme loadings (1%-5%), TS (5%-10%) and hydrolysis time (16h-48h) were tested. In the DoE, the variables were set as continuous, and the aim was to test up to three levels of interaction. For this setup, the software suggested a total of at least 13 combinations. An extra combination was added as control with the same condition as the first part of the experiment (7.5% TS, 24h of incubation, 2% enzyme loading).

Table 16. Design of experiment suggested by JMP for different TS, enzyme loading and hydrolysis; experimental sCOD yields

Run	TS	Enzyme loading	Time (h)	sCOD (mg g^{-1} VS)
1	0.1	0.05	48	383.5256
2	0.075	0.01	24	261.7643
3	0.1	0.01	16	269.2483
4	0.1	0.05	16	334.5394
5	0.05	0.01	16	254.9607
6	0.1	0.01	48	344.0884
7	0.05	0.01	48	298.504
8	0.1	0.03	24	280.4624
9	0.05	0.05	16	354.9503

10	0.1	0.03	24	345.097
11	0.075	0.03	16	348.0453
12	0.1	0.01	48	337.2848
13	0.05	0.05	48	360.3932
14	0.075	0.02	24	223.261

The results for models which included interactions showed that these were not statistically significant (P>0.05). Therefore, a model without parameters interaction was adopted in the end, see Equation 4.2. The AIC of this model is 151.42.

$$\text{sCOD}\left(\frac{\text{mg}}{\text{gVS}}\right) = 322.06 + 8.72\left(\frac{\text{TS} - 0.075}{0.025}\right) + 36.66\left(\frac{Loading - 0.03}{0.02}\right) + 23.64\left(\frac{Time(h) - 32}{16}\right)$$

Equation 4.2

Figure 16 shows the experimental sCOD values obtained against the sCOD values predicted using this model. The fit of this model was moderate (R^2=0.62), but it is statistically significant (P=0.019). The high variability, i.e. the distances between the points and the red line, might be explained by analytical errors and the heterogeneity of the substrate. The extra variability, compared to the previous optimisation test, could be linked to substrate concentration (TS%). Changes in TS% might lead to changes in other operational variables which have not been measured in this experiment. For example, different TS% can lead to differences in viscosity, creating mass transfer limitations, as well as different final acid concentrations (if natural fermentation occurs during hydrolysis), causing changes in pH. Changes in these variables can lead to different enzymatic performances.

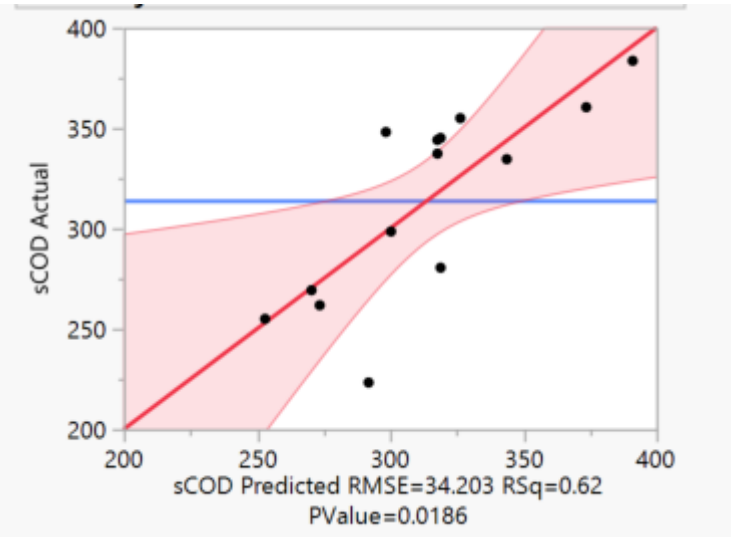

Figure 16. Fitness of predicted 1-level model by JMP for hydrolysis conditions optimisation

The effect of individual variables on the model, in terms of log-worth, are summarised in Figure 17. As expected, the variable of larger effect (largest log-worth) is the enzyme loading. Time also appears to have a great effect, but it is not considered significant (log-worth<2). TS shows insignificant effect in the range tested compared to time and enzyme loading.

Figure 17. Effect of individual variables on the predicted model for hydrolysis conditions optimisation

Figure 18 shows the prediction profiler for sCOD values predicted by the model based on each individual variable; TS (%), enzyme loading (%) and time (h). The image was obtained from the JMP software. As expected, the maximum sCOD that can be achieved, according to the model, is 391 mg g^{-1} VS for the largest TS, enzyme loading and time tested (10% TS, 5% enzyme loading and 48h hydrolysis). However, the predicted sCOD is not much greater (<10%) than the predicted sCOD obtained (360 mg g^{-1} VS) by the model from the previous part for the experiment with 7.5% TS, 2% enzyme loading and 24h hydrolysis time. To get an increase of around 8.6% in sCOD would require 2.5 times as much enzyme per unit of TS plus twice the volume of treatment vessel, so this would be economically disadvantageous by a large margin. Therefore, it was concluded that adopting the conditions predicted by the previous model would be more economic as a result of using lower quantity of enzymes and lower heating requirements (shorter incubation time). TS, however, should be kept at the maximum value, since this results in more concentrated VFA solutions after fermentation.

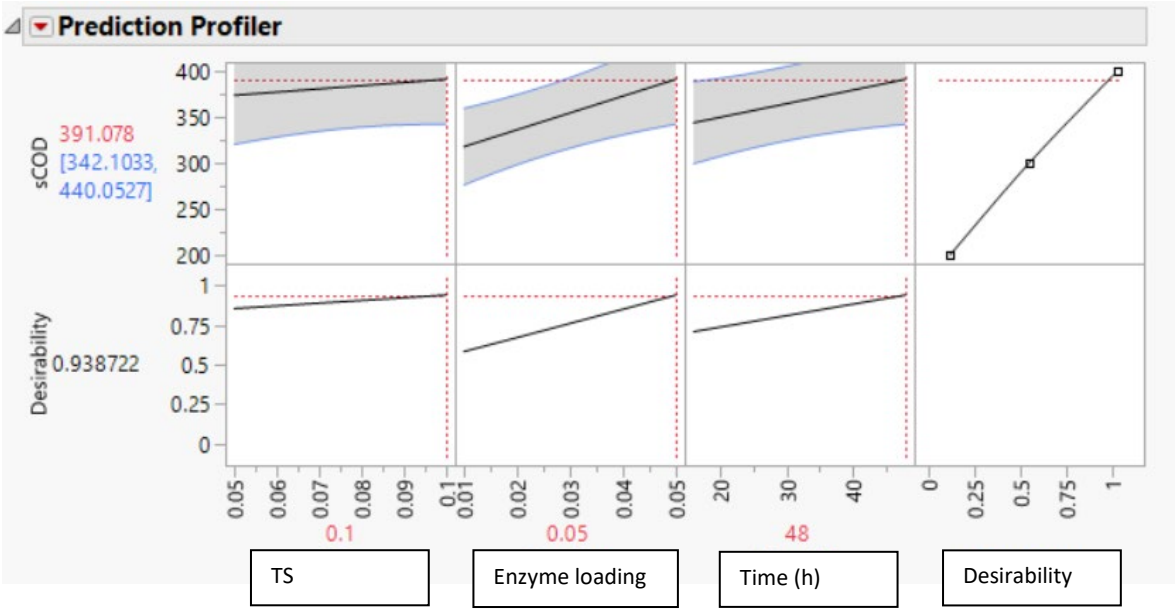

Figure 18. Prediction profiler of sCOD 1-level model for hydrolysis conditions optimisation

The solubility improvement achieved by enzymatic pretreatment can be quantified by sCOD increase, but it can also be qualitatively observed by the rheological properties of the substrate, which appeared less viscous after pretreatment. Image 4 shows the differences between pretreated and untreated ORS after centrifuging at 13000 rpm for 15 min. As seen, the pretreated ORS occupy less volume as a fraction of them have been solubilised. In addition, a small fraction of low-density particulates can be observed suspended in the liquid surface in the pretreated reactors. This would mean that, in a large scale process, the solid-liquid separation system would require an additional step to remove suspended solids which do not settle in gravity separation systems (e.g. clarifiers and centrifuges).

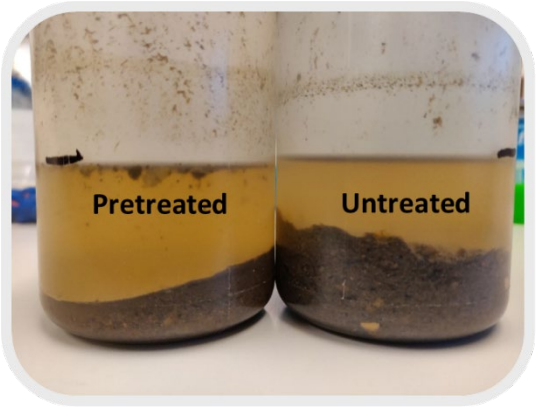

Image 4. Enzyme pretreated (7.5% TS, 2% enzyme loading and 24h hydrolysis time) and untreated diluted ORS after centrifugation

4.5 Enzyme pretreatment effect on AF

In the previous experiments, it was demonstrated that enzymatic pretreatment significantly increases soluble COD of the ORS. It was also suggested in the literature (see section 2.5) that higher solubilisation does not always result in improved yields for AF/AD systems. This section presents the results from an experiment designed to test the effect of the enzymatic pretreatment on the acidogenic fermentation of ORS.

In this experimental setup, tap water was used instead of buffer solution to avoid any potential negative side effects on the fermentation and to simulate what would be closer to the conditions of an industrial process. The ORS were pretreated at 50°C for 24 hours, 10% TS, and 2% enzyme loading (0.81 complex A and 0.19 amylase). Control replicas were incubated at 50°C for 24 hours, (10% TS) without enzyme addition. Afterwards, fermentation took place at 35°C and 200 rpm for 8 days. Substrate to inoculum ratio was 40 g TS g^{-1} TS. This experiment was carried out two times: in the first part fermentation was tested without any pH adjustment, and in the second with pH

adjustment by 4 M NaOH addition. Control replicas were also added NaOH to maintain pH in the second part of the experiment.

4.5.1 Without pH adjustment (pH<5.5)

As can be seen in Figure 19, VFA concentrations were always lower in the pretreated replicates than in the untreated controls , reaching a maximum on day 5. Lactic acid was observed immediately after pretreatment at concentrations between 5-7.5 g/L. This is an indication that the ORS contain lactic acid producing bacteria which thrive under hydrolysis conditions as sugars are released. Lactic acid is a stronger acid (lower pKa) than the other VFA (acetic, propionic, butyric, etc.). The pH in each reactor remained approximately constant during the run, at around 4.5-5 and 5-5.5 for pretreated and control reactors respectively. It is therefore likely that a lower pH and relatively high concentrations of lactic acid in pretreated reactors inhibited the acidogenesis process, despite its improved solubility (i.e. higher sCOD) compared to untreated ORS.

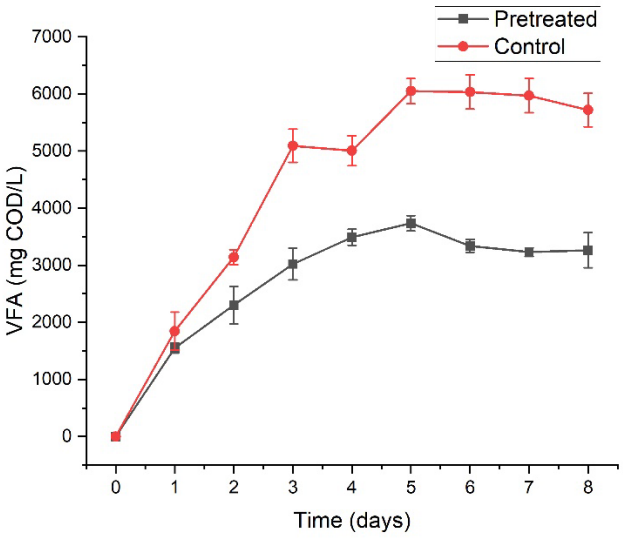

Figure 19. Total VFA concentration of untreated ORS (Control - 50°C for 24 hours) and enzyme pretreated ORS (50°C for 24 hours, 2% enzyme loading) without pH adjustment, at 35°C and S/I ratio of 40 g TS g^{-1} TS. Error bars display standard deviation of three replicates for pretreated conditions and two replicates for control conditions

Figure 20 shows the VFA profile of this experiment for both treated and untreated ORS. The main acids in the control reactor are acetic and n-butyric, with traces of propionic, iso-butyric, iso-valeric, and hexanoic. In contrast, the main VFA in the pretreated reactor is acetic with some traces of propionic.

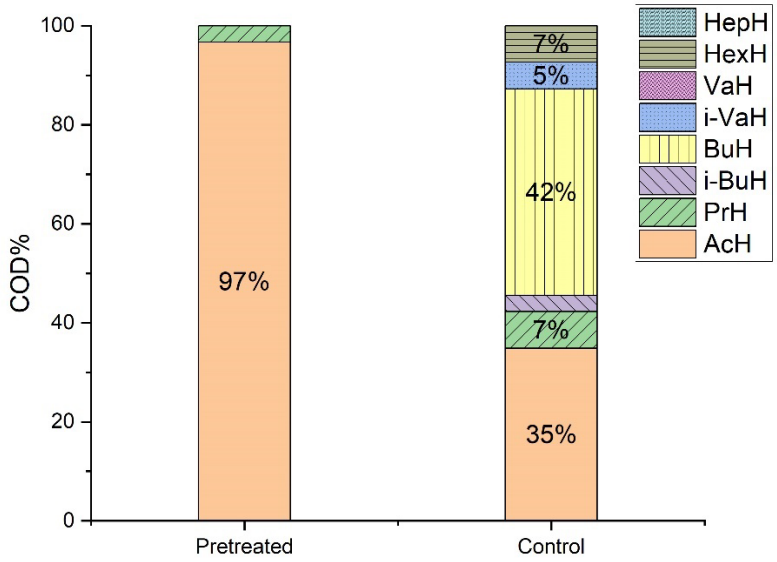

Figure 20. VFA profile of untreated ORS (Control - 50°C for 24 hours) and enzyme pretreated ORS (50°C for 24 hours, 2% enzyme loading) without pH adjustment on day 7 of the fermentation, at 35°C and S/I ratio of 40 g TS g^{-1} TS. Percentages are the average of three replicates for pretreated conditions and two replicates for control conditions

The acidification degree (DoA) was significantly lower in the pretreated reactors (13%) compared to the control (50%). This indicates that, under the pretreatment conditions, the concentration of by-products, proven to be mostly lactic acid, is higher than that of the targeted VFA.

Figure 21 shows the soluble TOC values after pretreatment and fermentation. All reactors show a TOC loss through fermentation. This is expected as some degree of conversion of organic carbon into CO_2 is unavoidable as part of the acidogenesis fermentation. In addition, there could have been some methanogenic activity. At the anticipated pH values, however, methane production was expected to be minimal and was not measured. The pretreated reactors TOC loss was higher than the control reactors.

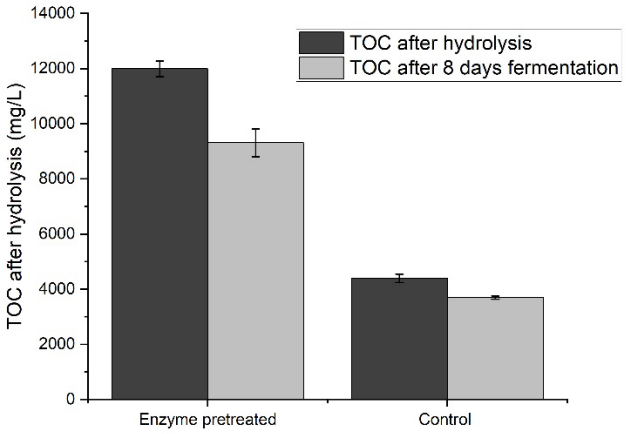

Figure 21. Soluble TOC concentration after pretreatment and fermentation of untreated ORS (Control - 50°C for 24 hours) and enzyme pretreated ORS (50°C for 24 hours, 2% enzyme loading) without pH adjustment, at 35°C and S/I ratio of 40 g TS g^{-1} TS. Error bars display standard deviation of three replicates for pretreated conditions and two replicates for control conditions

Figure 22 shows the ammonia concentration resulting from this experiment. The ammonia release in the enzyme pretreated replicates was negligible, probably due to inhibition of protein-hydrolysis enzymes as a result of low pH. In contrast, the control reactors showed a considerable increase in ammonia concentration from 0.08 to 0.28 g NH_4 L^{-1}.

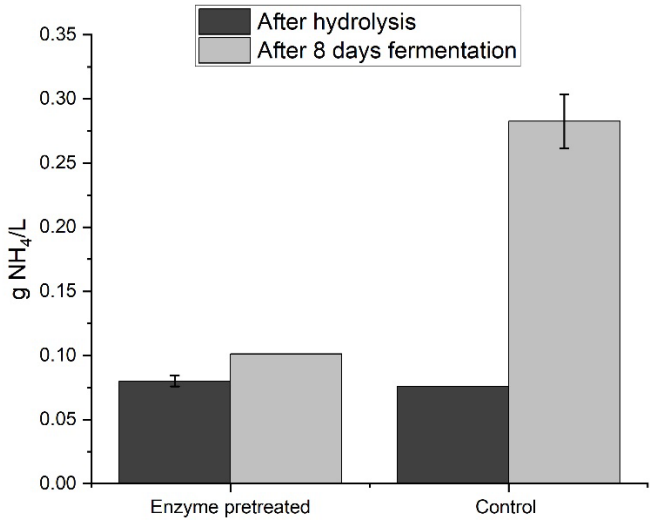

Figure 22. Ammonia concentration after pretreatment and fermentation of untreated ORS (Control - 50°C for 24 hours) and enzyme pretreated ORS (50°C for 24 hours, 2% enzyme loading) without pH adjustment, at 35°C and S/I ratio of 40 g TS g^{-1} TS. Error bars display standard deviation of two replicates. Results with no error bars are based on one replica

4.5.2 With pH adjustment (pH>5.5)

In this part of the experiment, the reactors were inoculated after hydrolysis and pH was maintained above 5.5 by daily 4M NaOH addition. As can be seen in Figure 23, the pretreated reactors reached higher VFA concentrations than the control reactors. This is explained by the increased sCOD of the substrate after pretreatment and improved acidogenic fermentation performance when pH is maintained above 5.5. A lag phase of approximately 3 days was observed in the pretreated reactors. This could have been due to an inoculum adaptation to the measured high lactic acid concentrations.

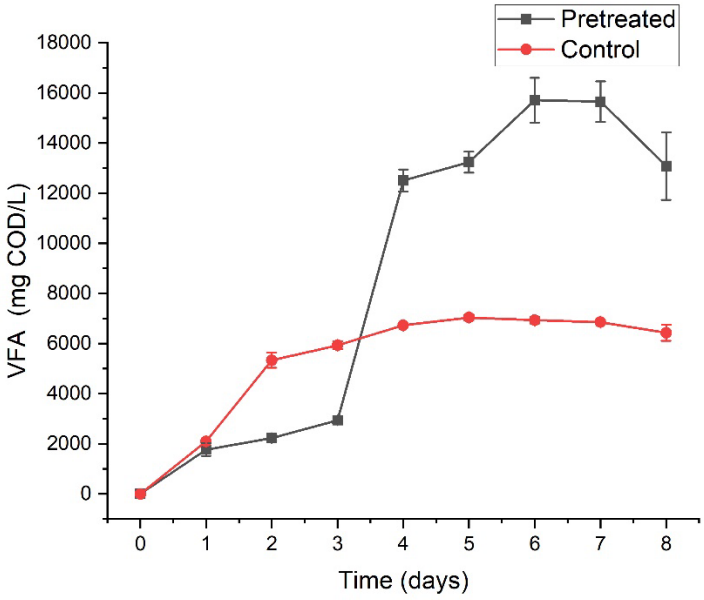

Figure 23. Total VFA concentration of untreated ORS (Control - 50°C for 24 hours) and enzyme pretreated ORS (50°C for 24 hours, 2% enzyme loading) with pH adjustment to keep above 5.5, at 35°C and S/I ratio of 40 g TS g^{-1} TS. Error bars display standard deviation of three replicates for pretreated conditions and two replicates for control conditions

Figure 24 shows the VFA profile for this part of the experiment. The pretreated reactors mainly produced butyric acid, with acetic acid in the next highest concentration. The control reactors produced similar quantities of butyric and acetic, and traces of propionic, iso-butyric, iso-valeric and hexanoic. Despite the addition of NaOH, the control reactors behaved very similar to the control reactors in the previous experiment with regards to VFA concentration and profile.

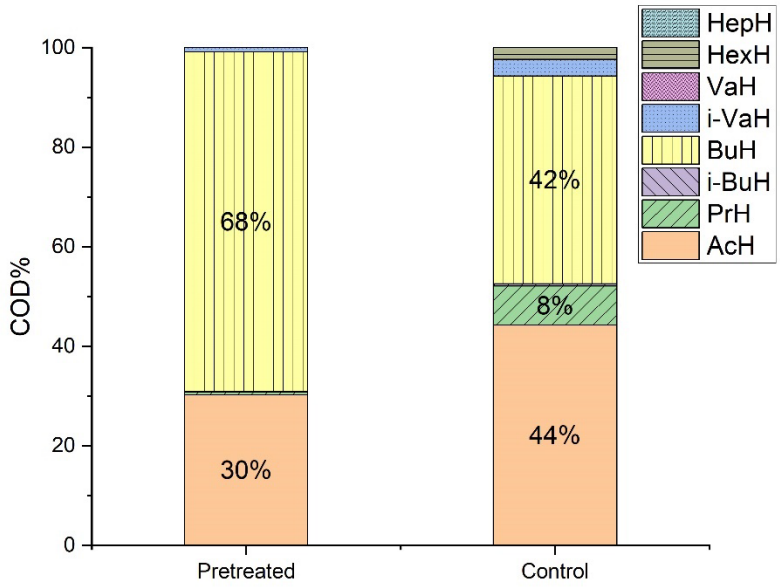

Figure 24. VFA profile of untreated ORS (Control - 50°C for 24 hours) and enzyme pretreated ORS (50°C for 24 hours, 2% enzyme loading) with pH adjustment to keep above 5.5, at 35°C and S/I ratio of 40 g TS g^{-1} TS, after 7 days of batch fermentation. Percentages are the average of three replicates for pretreated conditions and two replicates for control conditions

Figure 25 shows soluble organics concentrations (VFA, glucose and lactic acid) in terms of COD for the three enzyme pretreated replicates. As can be seen, all the glucose is consumed within 24 hours of fermentation. This sudden glucose concentration drop does not coincide with an increase in VFA concentration in any of the replicates. This probably indicates that glucose is following a different pathway to any of the acidogenesis pathways, e.g. ethanol fermentation, or it is being utilised for cell growth. Lactate concentrations remained relatively constant until day 3-4 when it started to be consumed, reaching negligible concentrations within 24 hours. This lag phase could be explained by an adaptation of the inoculum, where a lactate oxidation bacterial population outgrew that of the glucose oxidation bacteria. The rapid increase in butyric acid strongly indicates lactic acid metabolisation towards butyric. One specie that can metabolise lactate to butyrate is *Clostridium acetobutylicum* [16]. With the information available, it is unclear which was the main pathway for acetic acid production. The VFA profile also shows that, after reaching a maximum concentration, butyric acid starts to be metabolised, coinciding with an acetic acid increase, which happens at neutral pH (see Figure 26). It can therefore be concluded that neutral pH is detrimental for butyric acid production.

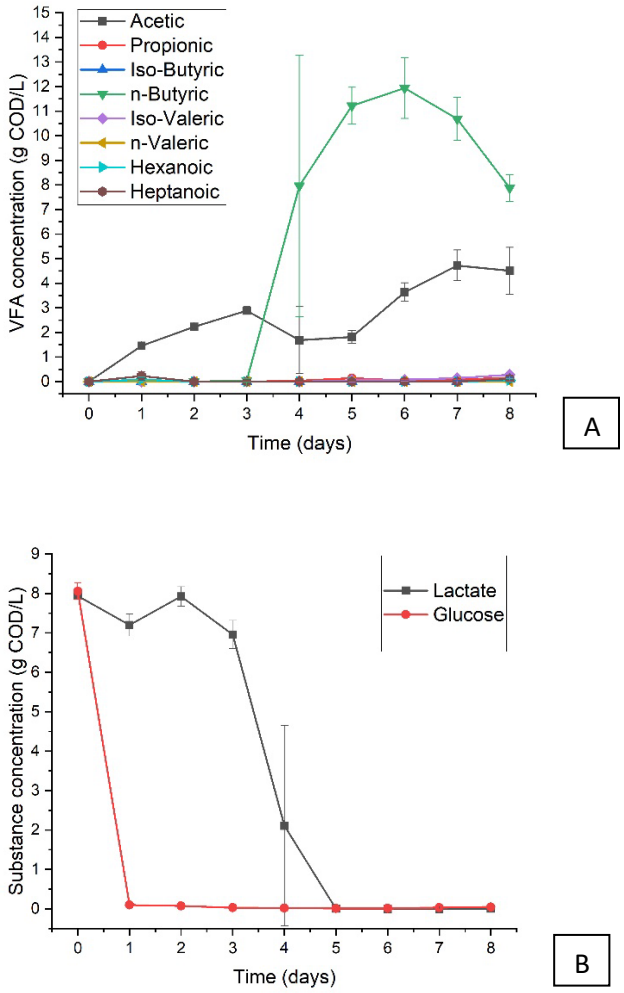

Figure 25. VFA (A) and soluble organics (B) of enzyme pretreated ORS (50°C for 24 hours, 2% enzyme loading) with pH adjustment to keep above 5.5, at 35°C and S/I ratio of 40 g TS g^{-1} TS. Error bars display standard deviation of three replicates

Figure 26 shows the pH profiles for this part of the experiment. Between day 3 and 5, the pH of the pretreated reactors naturally increased to approximately 7. These pH increases coincide with an increase in VFA concentration as well as in the pressure in the reactors' headspace. Pressure in the headspace was not quantified but was evident from the noise resulting from pressure release as reactors are opened. The increase in VFA concentrations, especially acetic acid, may indicate the slight pressurisation is due to H_2 and/or CO_2 (by-products of acidogenic fermentation) rather than CH_4. The pH increase can be explained by the pKa of the acids. Lactic acid is a stronger acid with a pKa of 3.86 compared to 4.82 and 4.76 for butyric and acetic respectively (see Table 3 and Table 4). As lactic acid is metabolised, the concentration of H^+ decreases, causing a pH increase.

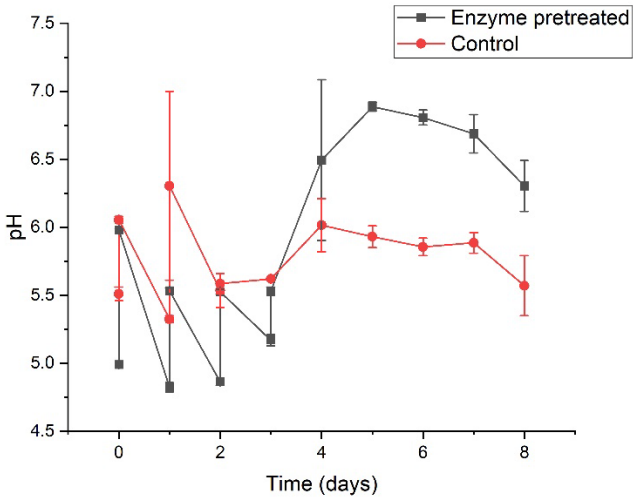

Figure 26. pH profile of untreated ORS (Control - 50°C for 24 hours) and enzyme pretreated ORS (50°C for 24 hours, 2% enzyme loading) with pH adjustment to keep above 5.5, at 35°C and S/I ratio of 40 g TS g^{-1} TS. Error bars display standard deviation of three replicates for pretreated conditions and two replicates for control conditions

In contrast to the first part of the experiment where pH was not adjusted, the DoA of pretreated reactors was similar to that of the control reactors. Around 40-45% of soluble organic carbon is represented by VFA.

As can be seen in Figure 27, TOC loss is similar to previous experiment for pretreated reactors. However, it is significantly lower for the control reactors. This could be explained by the NaOH addition. As NaOH is a lignin removing agent and breaks down proteins, it could have acted as pretreatment, helping with the hydrolysis and dissolution of recalcitrant fractions. This extra release of TOC might have balanced any TOC loss due to CO_2 production.

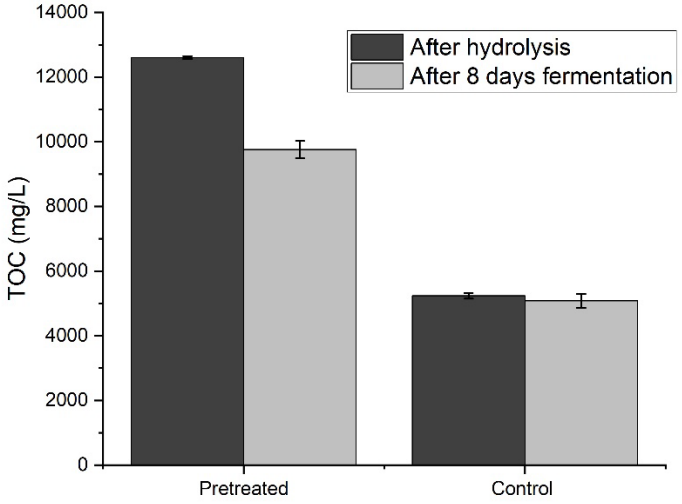

Figure 27. TOC concentration in supernatant after pretreatment and fermentation of untreated ORS (Control - 50°C for 24 hours) and enzyme pretreated ORS (50°C for 24 hours, 2% enzyme loading) with pH adjustment to keep above 5.5, at 35°C and S/I ratio of 40 g TS g^{-1} TS. Error bars display standard deviation of three replicates for pretreated conditions and two replicates for control conditions

Figure 28 shows the ammonia concentrations in this experiment before and after the fermentation step. A similar ammonia concentration increase to about 0.31-0.33 g NH_4 L^{-1} is observed in both pretreated and control reactors, suggesting there is enough nitrogen for biomass growth.

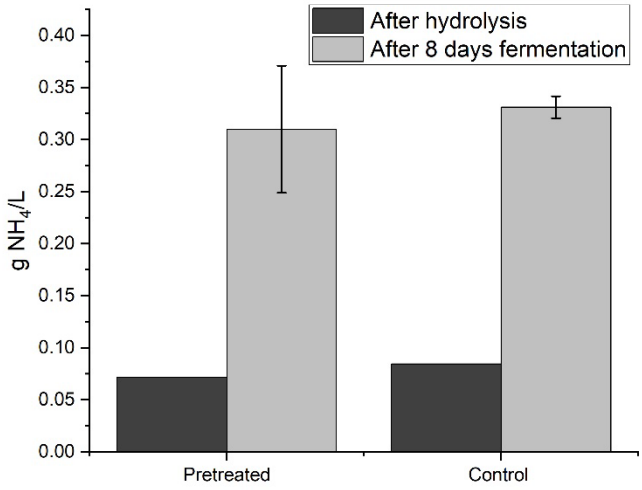

Figure 28. Ammonia concentration before and after 8 days of fermentation of untreated ORS (Control - 50°C for 24 hours) and enzyme pretreated ORS (50°C for 24 hours, 2% enzyme loading) with pH adjustment to keep above 5.5, at 35°C and S/I ratio of 40 g TS g^{-1} TS. Error bars display standard deviation of two replicates

In conclusion, the pretreated reactors with pH adjustment produced 128% and 385% more VFA than the control reactors and the pretreated without pH adjustment, respectively. Enzymatic pretreatment released sugars that were quickly metabolised to lactic acid by the microbes present in the substrate during the incubation (50°C). This resulted in low pH (below 5) which, in combination with the presence of lactic acid, inhibited acidogenic activity. Based on these results, it can therefore be concluded that, in order to carry out an efficient consecutive hydrolysis and fermentation (CHF), pH values must be maintain higher than 5.5, which can be achieved by alkaline substance addition.

4.6 Nitrogen addition effect

In previous experiments, it was established that acidogenic fermentation of pretreated ORS was inhibited due to undesired lactic acid production during hydrolysis/pretreatment and a consequent acidic pH (<5.5). The addition of NaOH helped increase the pH and promoted lactic acid conversion to butyric acid.

Some literature suggests that sodium cations (Na^+) can be inhibitory at 0.4 M to most bacteria [288] and at 0.27 M for AD species in particular [289]. The estimated NaOH (or Na^+) concentration in previous experiments was 0.23 M, which is below inhibitory levels. Further NaOH addition would, however, be necessary to achieve the desired higher VFA concentrations. Therefore, it is important to find alternative alkaline substances to control acidity/alkalinity in AF processes.

This experiment was designed to provide further insight into the ammonia release mechanisms from urea and the effect of different C/N ratios achieved by urea addition on the AF of ORS. Some literature discussion on this topic can be found in Section 2.6.6. The experiment was divided in four parts: the first was designed to check the effect of different pH on urea hydrolysis using AD inoculum. The second, third and forth parts were designed to test different C/N ratios on AF of untreated ORS, AF of pretreated ORS and simultaneous hydrolysis and fermentation (SHF) of ORS, respectively.

4.6.1 pH effect on urea hydrolysis

This part of the experiment was carried out to study the effect of pH on the bio-hydrolysis of urea into ammonia. Tested pH values were 4.5, 6 and 8, which were adjusted daily using 4 M HCl or 4 M NaOH. Each pH condition was tested in duplicate, each containing 1 g of urea, 1.5 g of glucose and 150 mL of inoculum. Control replicates contained inoculum alone and the pH was not adjusted.

Figure 29 shows the pH values for each condition tested. On day 0, pH adjustment by acid addition to pH 4.5 and 6 replicates was followed by foaming. This foaming could have been caused by the release of dissolved CH_4 and CO_2 as a result of acidification. From thereon, only one major adjustment was needed on day 2 for the pH 6 replicates.

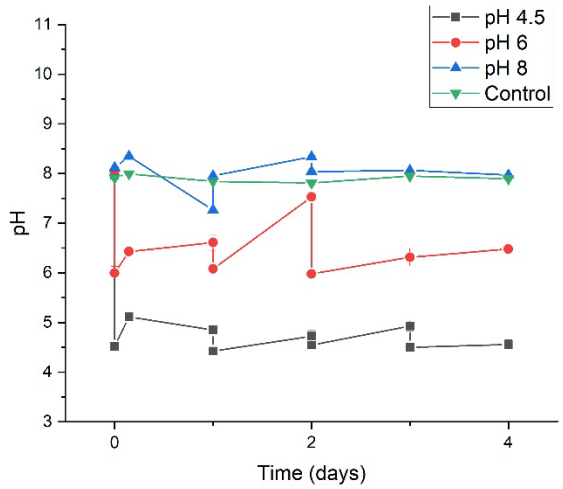

Figure 29. pH readings from urea hydrolysis experiment, adjusted by HCl or NaOH addition (1 g of urea and 1.5 g of glucose in 150 mL of AD digestate). pH values are the average of two replicates

Figure 30 shows the ammonia/ammonium concentration for each pH condition tested. Ammonia release plateaued within 2 days in all cases, with a slight increase on day 4 for pH 8 and pH 6. The highly acidic pH (pH 4.5) reached a maximum of 4 g NH_4^+ L^{-1}, whereas pH 6 and 8 resulted in 6 g NH_4^+ L^{-1}. Approximately 97% of urea was hydrolysed at pH 8 and pH 6, whereas only 51% was hydrolysed at pH 4.5 after 3 days. These results confirm that highly acidic pH inhibits biological ammonia release by AD species from nitrogen sources and urea. This inhibition is caused by a protonation of both ionizable groups in the urease enzyme at pH<5.3 [290]. Therefore, careful consideration must be taken when choosing the fermentation conditions if using urea and other natural nitrogen sources as a methanogenic inhibitor or buffering agent.

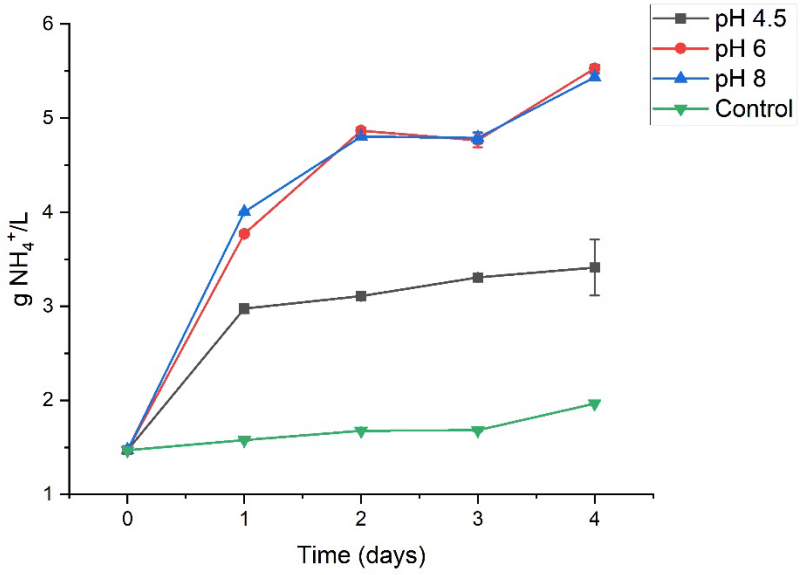

Figure 30. Ammonia/ammonium concentration resulting from urea hydrolysis at different pH values (1 g of urea and 1.5 g of glucose in 150 mL of AD digestate). Error bars display the standard deviation of two replicates

4.6.2 C/N ratio effect on AF of untreated ORS

It was previously suggested that urea addition can help inhibit methanogenic activity and promote acidogenesis, partly due to increases in pH to alkaline levels and mostly to the presence of ammonia to which methanogens are especially sensitive. The C/N ratio can be fixed using different quantities of urea at the beginning of the experiment. It should be noted that available/soluble carbon in the ORS is dependent on the performance of the enzyme pretreatment. In this work, C/N ratio has been defined based on total carbon.

This part of the experiment looked at the effect C/N ratios adjusted by urea addition on the AF of untreated ORS. An ORS concentration of 7.5% TS and a S/I of 40 g^{-1} TS g^{-1} TS was used in a working volume of 150 mL. The fermentation took place for 7 days. Two replicates were used to test each C/N ratio by urea addition: 25, 10 and 5 (g C g^{-1} N). The pH of these replicates was measured, and the same number of replicates were run without urea addition but adjusting the pH (using 4 M NaOH or 4 M HCl) to match the pH values of the urea-containing replicates. Two control replicates were used without any urea addition or pH adjustment (equivalent to a C/N ratio of 40).

The measured ammonia concentrations after 7 days of fermentation were 0.68, 2.99, 7.49 and 0.16 g NH_4^+ L^{-1} for C/N of 25, 10, 5 and control (40), respectively. In all cases, the ammonia release represented ≥90% of theoretical values based on the mass of urea added.

Figure 31 shows the pH values for each condition tested. C/N 10 and 5 replicates had a pH drop in the first 24 hours and a sudden rise in the next 24 hours. This is explained by a quick acidification (which was usually observed in all batch experiments) followed by a neutralisation as ammonia is being released at a slower rate. In the case of C/N 5, the pH increased to 8.4 after 4 days. The pH equivalent replicates for C/N 10 and 5 had very unstable pH after 2 days, with pH dropping by 0.5-1.5 units within 24 hours. This could simply be explained by the fact that NaOH is added on a daily basis, as opposed to a slow release of buffering agent in urea replicates. However, this instability is also observed in the late stages of the fermentation (day 5 to 7), when VFA yields/concentrations have reached a maximum. This may mean that there are other interactions with NaOH that are not happening with ammonia (e.g. CO_2 absorption). It can be concluded that ammonia (or urea) offers better buffering capacity than NaOH in the AF of ORS.

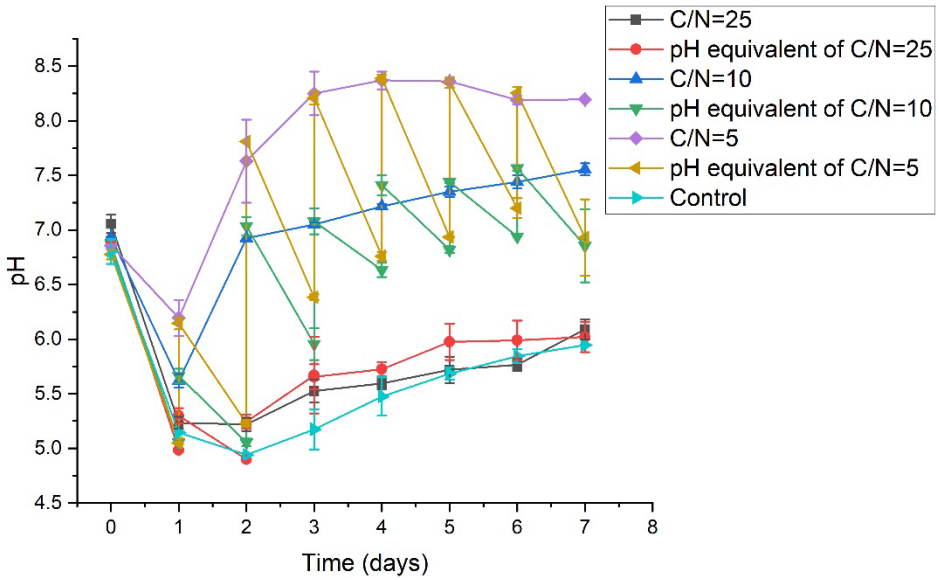

Figure 31. pH readings of different C/N ratios modified by urea addition, using untreated ORS at 7.5% TS, S/I of 40 g^{-1} TS g^{-1} and 35°C. The pH of 'pH equivalent' replicas was modified using NaOH or HCl. Error bars display the standard deviation of two replicates

Figure 32 shows the VFA yields for each condition tested. Based on these results, nitrogen addition seemed to have a positive impact on VFA yields from untreated ORS. Maximum yield was achieved at C/N 10 on day 5 (0.19 g COD g^{-1} VS), closely followed by C/N 5 on day 6 (0.18 g COD g^{-1} VS). When studying the pH equivalent replicates, a similar pattern is observed, with increased alkalinity/pH leading to higher yields in the last days of the fermentation and reaching similar levels to the corresponding C/N replicates. However, the replicates adjusted with NaOH took longer to reach maximum yields, indicating that daily NaOH addition as buffering strategy can lag

VFA production when compared to ammonia release from urea. Continuous automated NaOH dosing might eliminate this lag, however, this was not investigated.

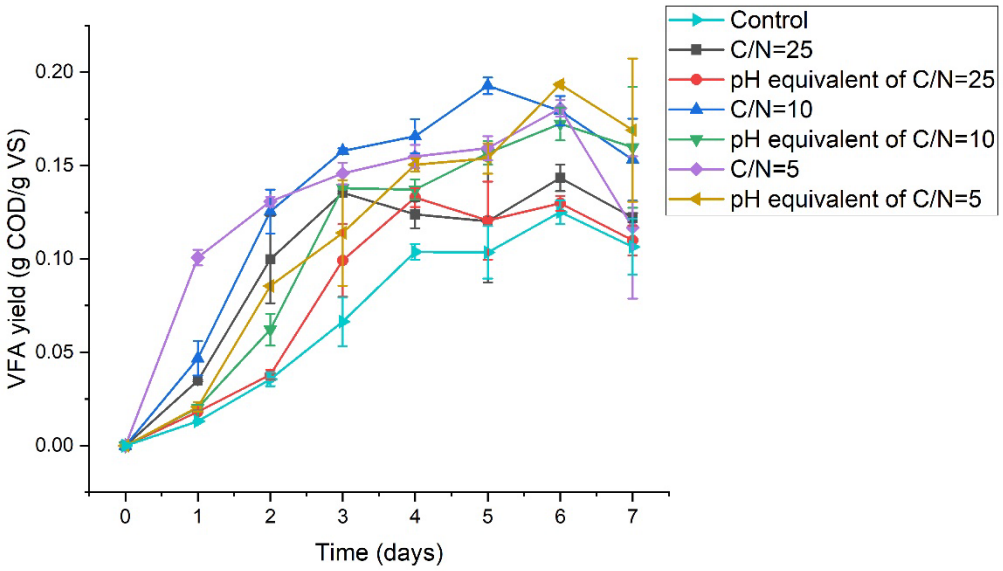

Figure 32. VFA yield of different C/N ratios modified by urea addition, using untreated ORS at 7.5% TS, S/I of 40 g^{-1} TS g^{-1} and 35°C. The pH of 'pH equivalent' replicas was modified using NaOH or HCl. Error bars display the standard deviation of two replicates

Nitrogen/urea addition had a significant effect on the VFA profile (Figure 33). Decreasing C/N ratio, i.e. increasing ammonia concentration, resulted in a switch from mostly butyric acid production towards a balanced production of acetic and propionic acid. This is explained by the changes in pH, as butyric acid production is predominant in slightly acidic pH. However, some differences are observed comparing to the pH equivalent replicates. The C/N 25 replicates had a balanced composition of acetic, propionic and butyric, whereas the C/N 25 pH equivalent replicates were predominantly comprised of acetic and butyric with small proportions of propionic and valeric acids. This difference is observed despite following the same pH profile as C/N 25 replicates throughout the experiment. This proved that VFA profile is also affected by ammonia concentration, independently of pH values.

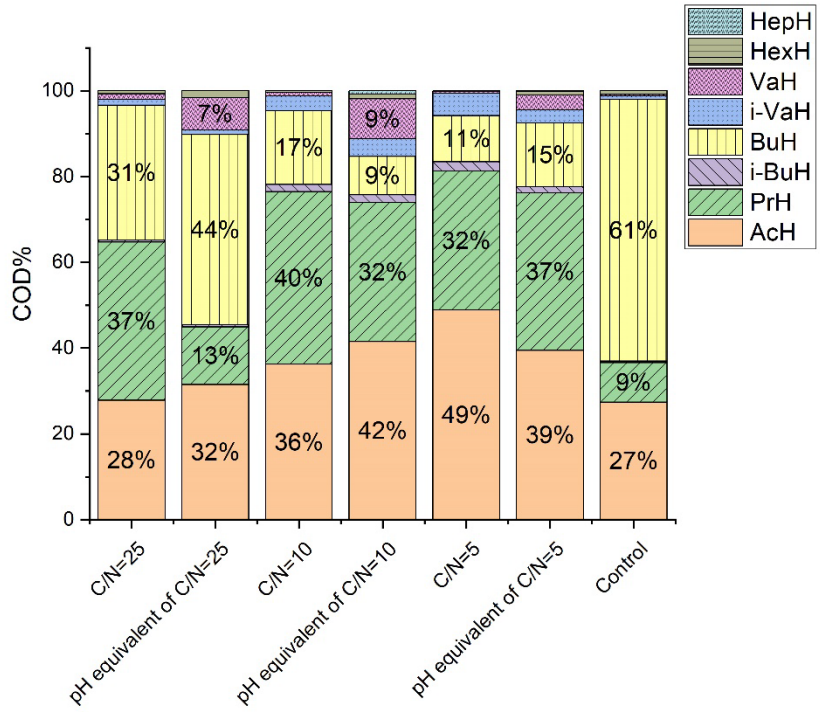

Figure 33. VFA profile on day 6 of the fermentation of different C/N ratios modified by urea addition, using untreated ORS at 7.5% TS, S/I of 40 g^{-1} TS g^{-1} and 35°C. The pH of 'pH equivalent' replicas was modified using NaOH or HCl. Percentages are the average of two replicates

4.6.3 C/N ratio effect on AF of pretreated ORS

For this part of the experiment, the ORS was first pretreated with 2% enzyme loading during 24 hours at 50°C prior to the fermentation. An ORS concentration of 7.5% TS and a S/I of 40 g^{-1} TS g^{-1} TS was used in a working volume of 150 mL. Inoculum and urea were added after pretreatment. Fermentation took place for 7 days. Following the same pattern as in the previous section, two replicates were used to test each C/N ratio by urea addition: 25, 10 and 5 (g C g^{-1} N). The pH of these replicates was measured, and the same number of replicates were run adjusting the pH (using 4 M NaOH or 4 M HCl) to match pH values of the urea-containing replicates. Two control replicates were used without any urea addition or pH adjustment (equivalent to a C/N ratio of 40).

The pH of all replicates fell to 3.8-4.3 after enzymatic pretreatment. It is believed, as demonstrated in previous experiments, that this low pH after pretreatment results from lactic acid production, which is a stronger acid than the targeted VFA. This low pH was maintained throughout the fermentation despite the urea addition. After 7 days of fermentation the ammonia concentrations were 0.23, 0.57, 1.35 and 0.13 g NH_4^+ L^{-1} for C/N of 25, 10, 5 and control

(40), respectively, meaning urea hydrolysis only yielded 20-40% of the maximum theoretical value. These low hydrolysis yields are likely to be a result of the low pH since, as previously demonstrated, acidic pH inhibits the action of urease enzymes. As a result, acidogenesis was inhibited resulting in undesirably low VFA concentrations the end of the fermentation (<1 g COD L^{-1}). The yields are plotted in Figure 34, and were one order of magnitude lower than in the previous experiment.

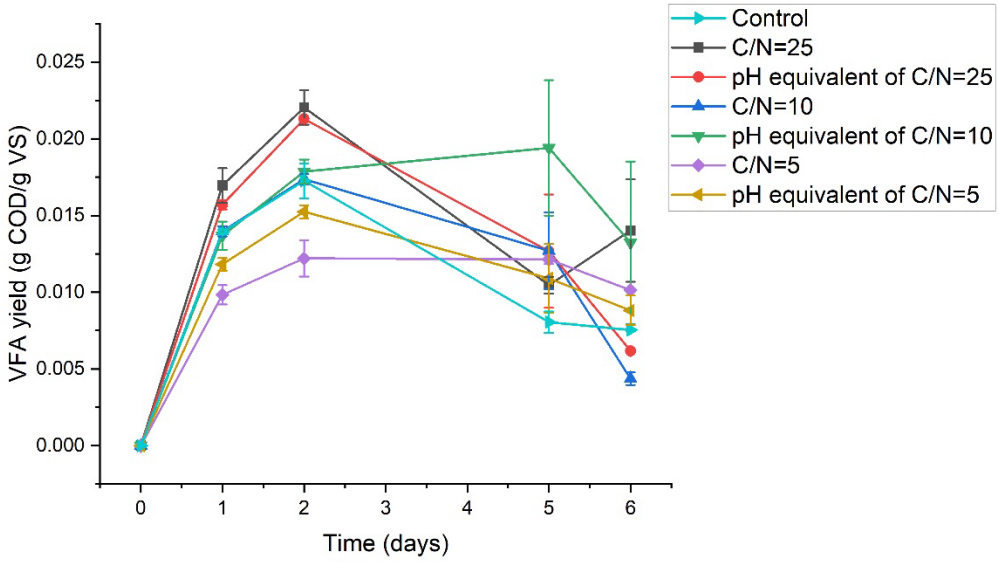

Figure 34. VFA yield of different C/N ratios modified by urea addition, using pretreated ORS (Pretreatment: 2% enzyme load, 24 hours at 50°C) at 7.5% TS, S/I of 40 g^{-1} TS g^{-1} and 35°C. The pH of 'pH equivalent' replicas was modified using NaOH or HCl. Error bars display the standard deviation of two replicates

4.6.4 C/N ratio effect on SHF of ORS

In the previous section, the aim was to increase the alkalinity/pH of pretreated ORS with the use of urea and to test the effect of different C/N ratios by urea additions. Unexpectedly, urea hydrolysis was inhibited due to low pH, and thus pH change after pretreatment was negligible as a result of low ammonia release, causing the acidogenic fermentation to fail. Simultaneous hydrolysis and fermentation (SHF) could be an alternative strategy to avoid conversion of sugars to lactic acid and the subsequent sudden pH drop.

It is important to highlight that with SHF there is a higher risk of enzymes being degraded by AD microbes, and temperature conditions are not optimum for hydrolysis (50°C). In this experiment, the same conditions were tested as in the previous one, except that enzymes were added at the

same time (or as close as practically possible) as the inoculum. The fermentation took place for 7 days.

Figure 35 shows the pH values from this experiment. All reactors experienced a rapid pH drop within a day of fermentation as a result of VFA release. After that, C/N 25 replicates did not show a pH rise with pH remaining below 5 throughout the fermentation. C/N 10 replicates reached a neutral pH (6.5-7) by day 5. One C/N 5 replicate reached pH 8 by day 5 of the fermentation, whereas the identical replicate at stayed at slightly acidic pH (pH<6 throughout the fermentation), leading to large error bars for C/N 5 conditions. After 7 days of fermentation the ammonia concentrations were 0.63, 2.82, 5.18 (average of 7.24 and 3.11) and 0.10 g NH_4^+ L^{-1} for C/N of 25, 10, 5 and control (40), respectively. Urea hydrolysis yield was approximately 100% in all cases except for one of the C/N 5 replicates (only 46% of urea hydrolysed), which explains the differences in pH. It is possible that an experimental error occurred when weighing the amount of urea that was meant to be added to the replica.

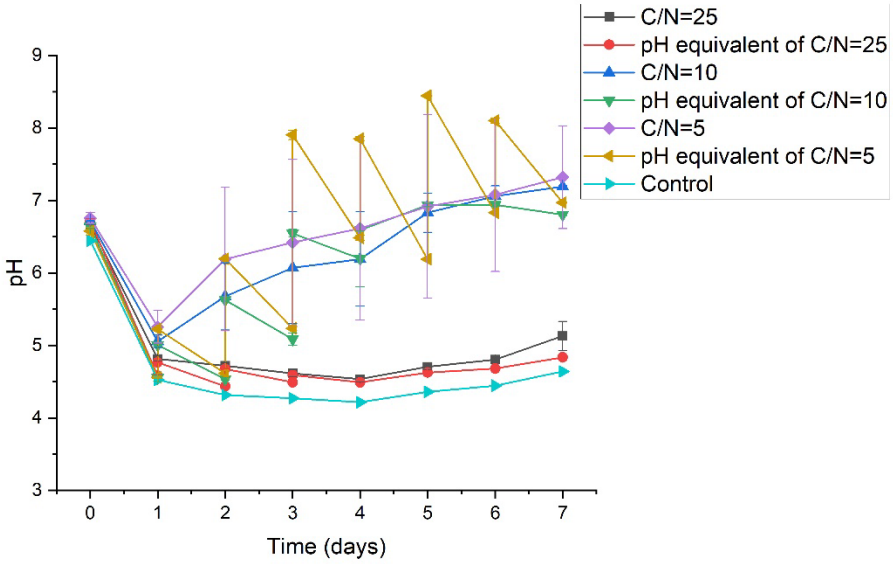

Figure 35. pH readings of different C/N ratio for SHF of ORS at 7.5% TS, S/I of 40 g^{-1} TS g^{-1}, 35°C and 2% enzyme load. The pH of 'pH equivalent' replicas was modified using NaOH or HCl. Error bars display the standard deviation of two replicates

Figure 36 shows the VFA yields for this experiment. A lag of at least 2 days in VFA production was observed for all replicates, probably as a result of pH instability in the first 2 days. Towards the end of the fermentation, C/N 10 and 5 had higher VFA yields than C/N 25 and the control. In this case, pH equivalent replicates behaved much more like their corresponding C/N replicates, with the exception of C/N 5, whose pH equivalent showed higher yields. This is explained by the high

pH variability in C/N 5 pH equivalent replicates, as seen in Figure 35, which resulted in higher average pH.

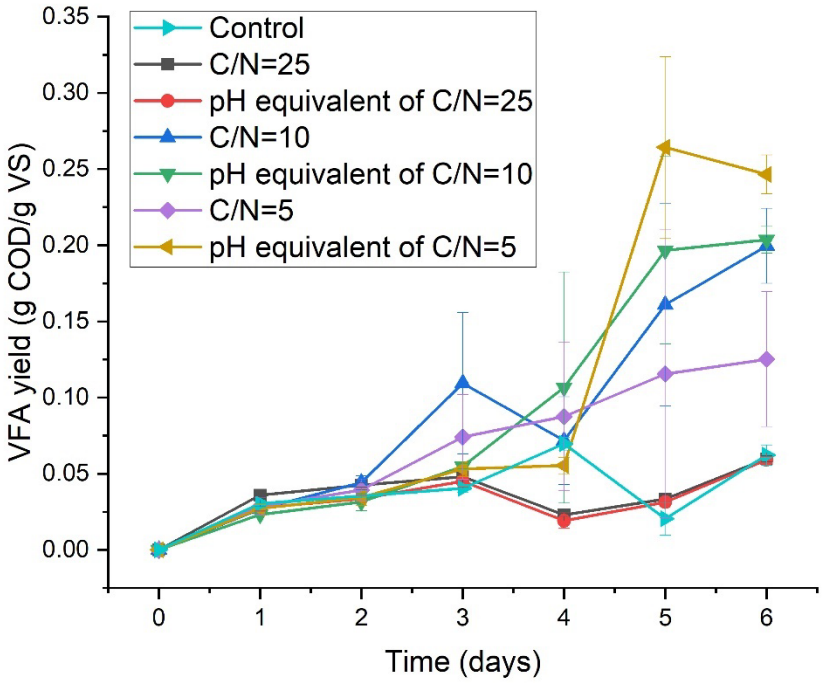

Figure 36. VFA yield of different C/N ratio for SHF of ORS at 7.5% TS, S/I of 40 g^{-1} TS g^{-1}, 35°C and 2% enzyme load. The pH of 'pH equivalent' replicas was modified using NaOH or HCl. Error bars display the standard deviation of two replicates. C/N 5 results are based on one replicate only (replicate with 100% urea hydrolysis)

Figure 37 shows the VFA profiles for each C/N condition tested. Compared to the VFA profile of untreated ORS (Figure 33), there are significant differences for all conditions. This is due to the extra available carbon that result from the action of enzymes. In the case of C/N 25 its pH equivalent and control, VFA concentrations were low (<4 g COD L^{-1}), potentially due to inhibition. Under these conditions, acetic acid was the predominant acids (>70%). Butyric acid and acetic acid were the predominant acids for C/N 10 and 5. Significant differences were observed with the pH equivalent replicates, particularly for C/N 5. Butyric acid percentage was 40% for the C/N 5 replicates, whereas it was only 11% for the C/N 5 pH equivalent replicates. This is likely explained by the higher average pH in the pH equivalent replicates, as butyric acid production is promoted under acidic pH.

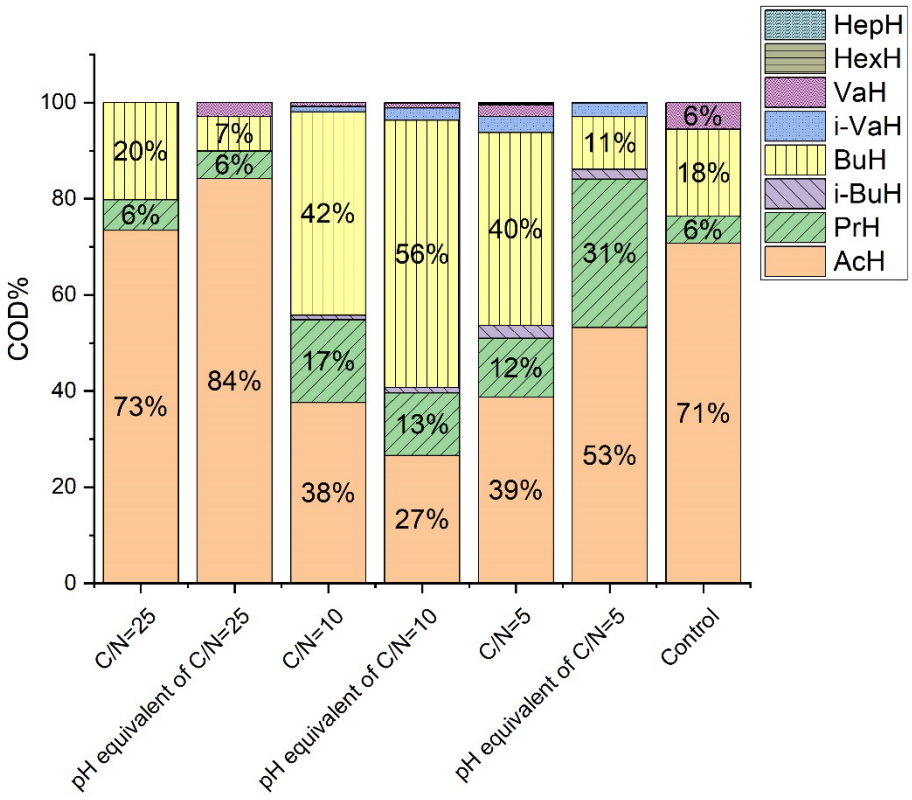

Figure 37. VFA profile on day 6 of different C/N ratio for SHF of ORS at 7.5% TS, S/I of 40 g^{-1} TS g^{-1}, 35°C and 2% enzyme load. The pH of 'pH equivalent' replicas was modified using NaOH or HCl. Percentages are the average of two replicates

It can be concluded that, while urea is an excellent buffering agent and C/N modifier, the hydrolysis process is highly dependent on pH conditions which can affect the release of ammonia, and, consequently, the behaviour of the fermentation. Therefore, urea should only be used as buffering agent when fermentation conditions have been optimised around neutral pH (6-8). In addition, pH, C/N ratios and operational mode (simultaneous vs. consecutive hydrolysis and fermentation) play a key role in type of VFA produced and by-product formation.

4.7 Inoculum thermal pretreatment

It is common practice to subject AD inoculum to heat shock or thermal treatment prior to AF. The theory is that high temperatures (above 80°C) kill most methanogens whereas acidogens have the ability to form spores. Therefore, heat shock is used as a strategy to inhibit methanogenic activity and promote VFA accumulation. However, there is limited information on optimal conditions to carry out this heat shock [186] and what effect it has on VFA profile. Often, the conditions used seem arbitrary or based on those used to promote H_2 production [187, 188].

The aim of this experiment was to test the effectiveness of thermal treatment for methanogenic activity inhibition and to study its effect on VFA profile and H_2 production. The inoculum was boiled using a hot plate, then transferred to bottles and incubated at 37°C for 24h to re-activate the anaerobic microbial community. Reactors of 150 mL working volume were used with an ORS concentration of 10% TS and 2% enzyme loading. On this occasion, a S/I of 5 g TS g^{-1} TS was used to ensure methanogenic activity is not completely inhibited by excessively high S/I. Initial pH was adjusted to 7 using 4 M NaOH. Reactors were flushed with N_2 and connected to gas bags. Incubation took place for 6 days. Two replicates were used to test each of three conditions A, B and C with inoculum boiled for 15, 45 and 90 min, respectively. The control reactors contained untreated fresh inoculum.

After 6 days, the pH of the control reactors was 6.8. The pH of replicates A, B and C had fallen to below 4.5, which has already been proven detrimental for VFA production. This was probably due to, as noted earlier, the production of lactic acid, which resulted from the growth of the microbe population present in the ORS. This pH is also disadvantageous for hydrogen production [291].

The gas yield for each inoculum pretreatment is presented in Figure 38. Methane yield was negligible for all conditions tested, including the control (untreated inoculum). Interestingly, a significant hydrogen volume was observed in the control samples. This indicates that, under these conditions, methanogens were inhibited, and hydrogen producers were favoured. Carbon dioxide production was proportional to VFA and hydrogen yields.

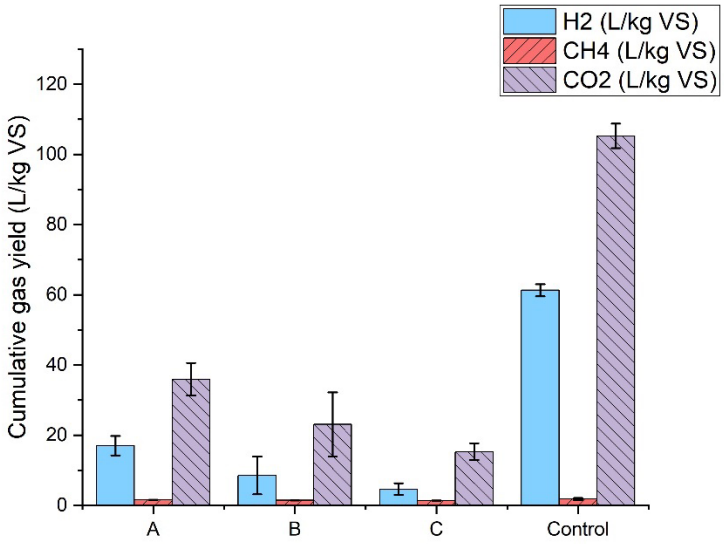

Figure 38. Cumulative gas yield of SHF after 6 days of incubation for different inoculum thermal treatment time at boiling point: A) 15 min B) 45 min C) 90 min, at 10% TS, 2% enzyme loading, 37°C and S/I of 5 g TS g^{-1}. Error bars display the standard deviation of two replicates

Figure 39 shows TS and VS values after 6 days of fermentation. Initial VS percentage was estimated to be 6.3% for all replicates. VS and TS values after 6 days increased and reached the same values for all conditions, except the control replicates in which values were lower. An increase in TS% (or VS%) can only be a result of water evaporation. A decrease is a result of production of methane, hydrogen or carbon dioxide.

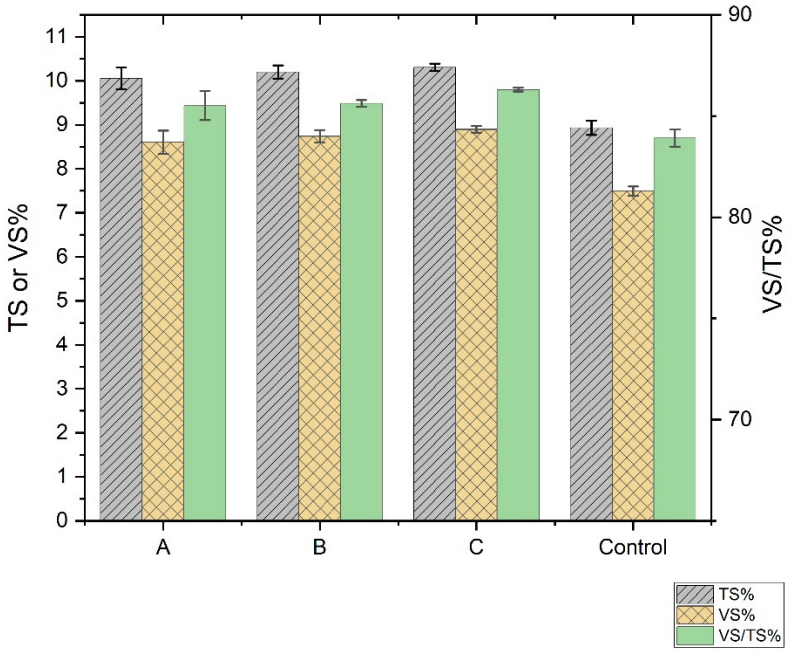

Figure 39. TS and VS of SHF after 6 days of incubation for different inoculum thermal treatment time at boiling point: A) 15 min B) 45 min C) 90 min, at 10% TS, 2% enzyme loading, 37°C and S/I of 5 g TS g^{-1}. Error bars display the standard deviation of two replicates

Figure 40 shows the DoA and the VFA yields. A strong correlation between DoA and VFA yield is observed, indicating that low VFA yields are a result of by-product production. DoA and VFA yield are inversely proportional to the heat shock time. This indicates that heat shock can negatively affect acidogens present in the inoculum, not just methanogens. Inoculum pretreatment might be beneficial for relatively clean substrates. In the case of the ORS, substrate populations (e.g. lactic acid producers) take over, resulting in by-product production. Under these conditions, the control replicates did not produce as much VFA as expected, which is explained by the additional H_2 produced, although DoA were not maximised.

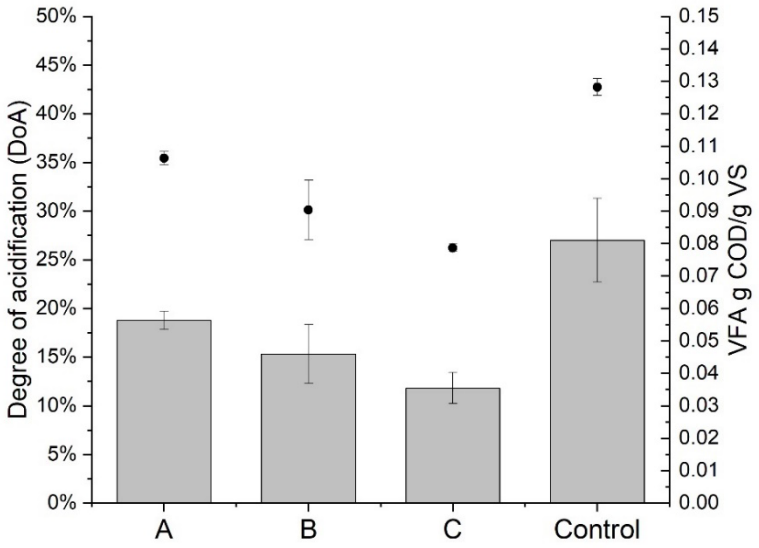

Figure 40. DoA (bar plot) and VFA yields (dot plot) of SHF after 6 days of incubation for different inoculum thermal treatment time at boiling point: A) 15 min B) 45 min C) 90 min, at 10% TS, 2% enzyme loading, 37°C and S/I of 5 g TS g^{-1}. Error bars display the standard deviation of two replicates

Figure 41 shows the VFA profile after 6 days of incubation. As can be seen, there are no significant differences in the profile for different inoculum pretreatment times. The main VFA produced is acetic acid, representing more than 80% of total VFA. The profiles in the control samples were similar to those in other experiments, with butyric acid as the predominant VFA followed by acetic acid.

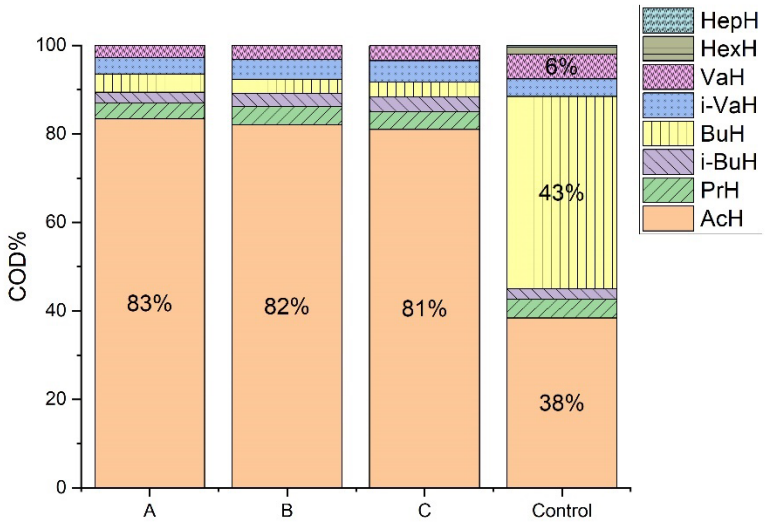

Figure 41. VFA profile in terms of COD percentage of SHF after 6 days of incubation for different inoculum thermal treatment time at boiling point: A) 15 min B) 45 min C) 90 min, at 10% TS, 2% enzyme loading, 37°C and S/I of 5 g TS g^{-1}. Percentages are the average of two replicates

4.8 Inoculum preadaptation

Typically, in batch AF studies, the inoculum used is a digestate from an anaerobic digestor (AD) designed/adapted to treat waste activated sludge (WAS) derived from wastewater. The AD systems have been optimised for biogas production and, therefore, contain a population of methanogens among other microbes. The compositions of WAS and ORS are significantly different. The literature suggests that adaptation of the inoculum to the substrate can be done by running continuous (or semi-continuous) reactors, i.e., by feeding fresh substrate and withdrawing reactor contents. Ideally, the new/adapted microbial consortia should have: hydrolytic bacteria that can produce enzymes to break down the structure of the lignocellulose material within the ORS, a higher proportion of acidogens compared to typical AD inoculum, and a negligible proportion of methanogens.

For this experiment, inoculum derived from 0.5L working volume CSTR reactors which were run for 4 weeks under mesophilic temperatures at increasingly OLR up to 8 g VS L^{-1} day^{-1} (HRT = 19-7 days), was compared to the original AD inoculum. The CSTR reactors had negligible methane production at the time of inoculum collection, therefore, methanogenesis was inhibited. In this experiment, an ORS concentration of 7.5% TS, S/I 40 g TS g^{-1} TS and 2% enzyme loading were used in a working volume of 150 mL. Initial pH was adjusted to 8.5 using NaOH to provide extra alkalinity. Each inoculum was tested in duplicate. Control reactors contained inoculum alone. Reactors were connected to gas bas and fermentation took place for 7 days.

Figure 42 shows the cumulative gas yields for this experiment. There were no significant differences between the adapted inoculum and the AD inoculum in terms of gas production. These results indicate that adaptation of the inoculum does not present an advantage in this situation, when methanogens are inhibited by other variables such as high S/I ratio, acidic pH, short retention times or a combination of these. Interestingly, the control replicates for adapted inoculum produced significant quantities of biogas. This means that, although methanogens are inhibited in the CSTR system, they could re-activate once transferred to a batch system with decreased VFA concentrations, or if retention times are increased. Reactors were run for 16 days in total. No gas samples were taken on day 16, however, VS/TS% reduction can give an indication of the conversion of VS into biogas. VS/TS% on day 16 did not differ much from day 7 except for “control – AD inoculum” which saw a reduction from 68% to 63%.

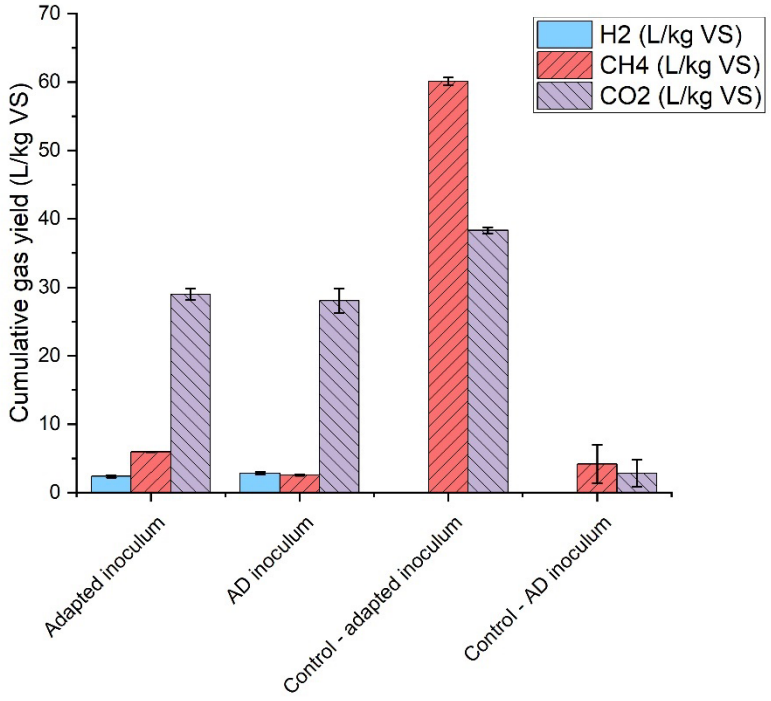

Figure 42. Cumulative gas yield for different inoculum types after 7 days of SHF at ORS concentration of 7.5% TS, S/I 40 g TS g^{-1} TS, 35°C and 2% enzyme load. Control replicas contain inoculum alone. Error bars display the standard deviation of two replicates

Despite the additional alkalinity provided by NaOH addition, the pH of the main replicates dropped from 8.3-8.7 to 5.0-5.1 in 7 days of fermentation, indicating acid production. In contrast, the control replicates maintained a stable pH throughout (6.1-7.5). The pH of the control for adapted inoculum increased from 6.1 to 7. This is in accordance with biogas production, as VFA are being consumed. The pH of the control reactors for AD inoculum remained stable at approximately 7.5.

Figure 43 shows the VFA yields and DoA results. A strong relationship is observed between VFA yields and DoA percentages. No significant differences were observed between adapted inoculum and AD inoculum after 7 days of fermentation. This is another indication that adapting the inoculum does not signify an advantage in the batch AF of ORS.

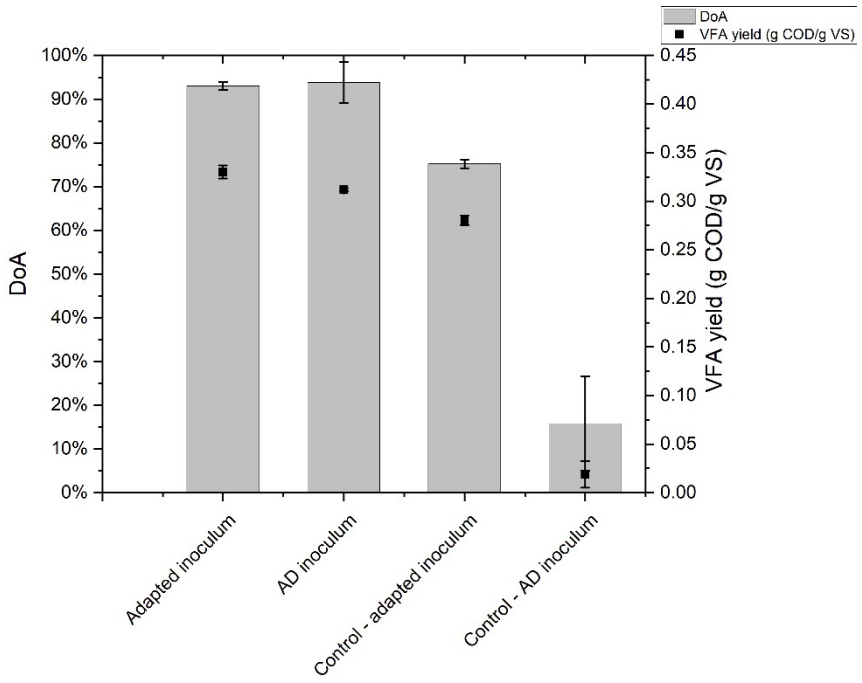

Figure 43. DoA and VFA yield for each inoculum type after 7 days of SHF at ORS concentration of 7.5% TS, S/I 40 g TS g^{-1} TS, 35°C and 2% enzyme load. Control replicas contain inoculum alone. Error bars display the standard deviation of two replicates

Figure 44 shows the VFA distribution for each inoculum type on day 0 and day 7 of Figure 44 shows the VFA profile for each inoculum type on day 0 and day 7 of the fermentation. There are no significant differences in VFA profile after 7 days of fermentation of each type of inoculum. The main VFA were acetic and butyric, followed by propionic, valeric and hexanoic. These profiles differ greatly from the control replicates on day 0. These results indicate that VFA profile is highly dependent on fermentation conditions and substrate composition, regardless of what type of inoculum is used.

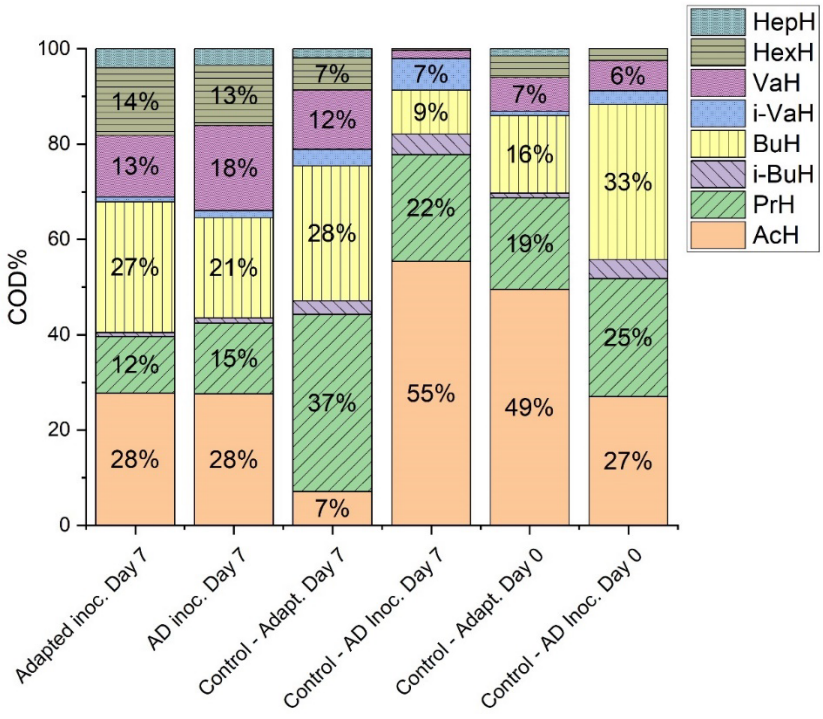

Figure 44. VFA profile for different inoculum types of SHF at ORS concentration of 7.5% TS, S/I 40 g TS g^{-1} TS, 35°C and 2% enzyme load. Control replicas contain inoculum alone. Percentages are the average of two replicates

The use of adapted inoculum did not seem to affect hydrolysis performance. The VS% of the liquid fraction over the total VS% after 7 days of fermentation was the same for the adapted inoculum and the AD inoculum (~16%).

4.9 Substrate to inoculum ratio

For this experiment, an ORS concentration of 7.5% TS was used in a working volume of 150 mL. Two replicates were used to test S/I of 5, 22 and 40 g TS g^{-1} TS (equivalent to 5.3, 23.3 and 42.4 g VS g^{-1} VS, respectively). The same number of replicates were used with N_2 flushing at the beginning of the experiment and every time the reactor contents were exposed to air. Control replicates only contained inoculum and enzyme. In this experiment, pH was adjusted daily to above 6 using 4 M NaOH during the first 6 days, to ensure inhibition of methanogenesis is not due to pH. Reactors were connected to gas bags and incubated for 12 days. Samples were taken only on day 3, 6 and 12.

Figure 45 shows the VFA yields from this experiment. For all conditions tested, VFA yield increased until day 6. On day 6, S/I 5 replicates without N2 flushing had the highest yield, reaching 0.3 g COD g^{-1} VS. This is in agreement with previous research, where S/I values of 4-6 g VS g^{-1} VS

resulted in higher VFA yields from food waste [220]. A lower S/I of 1.5 g VS g^{-1} VS, however, was reported as optimum for the AF of agroindustrial wastes [219]. On day 12, VFA yields of S/I 5 and S/I 5 with N2 flushing decreased significantly, whereas VFA yields of other replicates plateaued or increased slightly. This indicates that S/I 5 can only successfully inhibit methanogenic activity if retention time is kept under 6-8 days. The effect of N2 flushing was only significant for S/I 5 replicates on day 6. As acidogens are facultative anaerobes, it can be concluded that N_2 flushing is not necessary for VFA production.

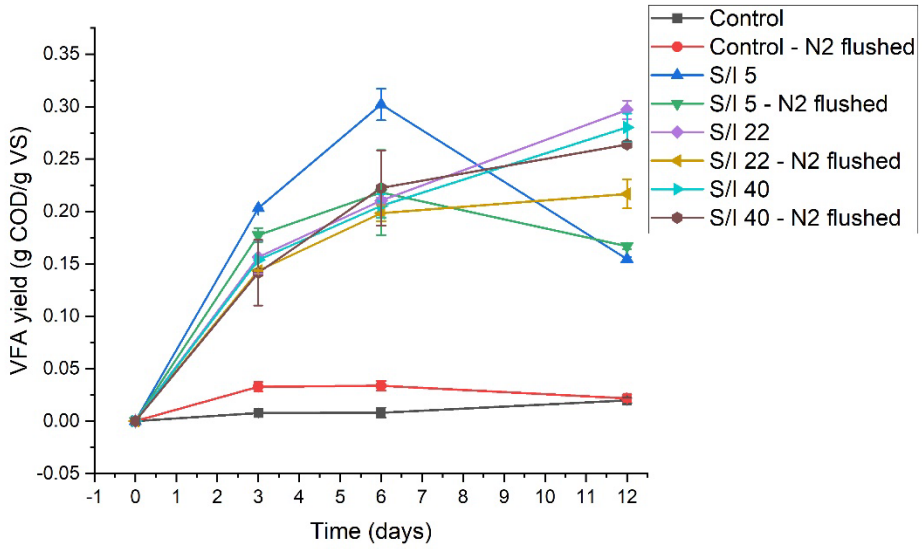

Figure 45. VFA yields for different S/I ratios with or without N_2 flushing of SHF at ORS concentration of 7.5% TS, 35°C and 2% enzyme load. Error bars display the standard deviation of two replicates

Figure 46 shows the cumulative methane and carbon dioxide volume after 12 days of fermentation. In accordance with VFA yield decrease after day 6, S/I 5 resulted in highest methane production. S/I 22 and S/I 40 appeared to inhibit methanogenic activity to some extent, but significant methane production still occurred in 12 days. There were no major differences between the replicates with and without N_2 flushing. Therefore, it can be concluded that methanogenic activity was not inhibited under non-strict anaerobic conditions. It is likely that the presence of small quantities of air in the headspace was insufficient to cause a change in the redox potential to which methanogens can be sensitive. Production of CO_2 might also have contributed to maintaining the redox potential within the comfortable range for methanogenic activity.

Hydrogen production was not detected in this experiment. In section 4.7, it was observed that a S/I ratio of 5 g TS g^{-1} TS with N_2 flushing leads to H_2 production at initial pH 7. This shows that

minor variations at start conditions may lead to slightly different behaviours in term of biogas production.

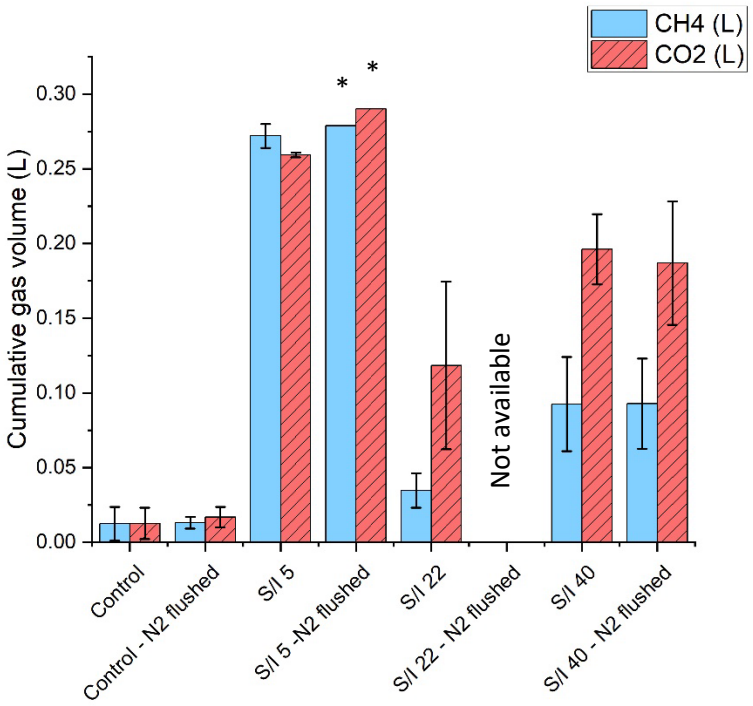

Figure 46. Cumulative gas volume after 12 days fermentation for different S/I ratios with or without N_2 flushing of SHF at ORS concentration of 7.5% TS, 35°C and 2% enzyme load. Error bars display the standard deviation of two replicates. *S/I – N2 flushing results are based on one replica only

Figure 47 shows the VFA profiles on day 6 and 12. The proportion of acetic acid decreased considerably from day 6 to 12 for S/I 5 replicates, coinciding with the methanogenic activity as previously noted. Acetic acid also decreased to some extent for the other conditions. No major differences were observed between VFA profiles on day 6 (before methanogenic activity) among all the conditions tested. The small variations might be due to differences in pH and alkalinity. Even smaller differences are observed when comparing replicates of same S/I with or without N2 flushing. It is very likely that, N2 flushing did not make a difference in redox potential compared to reactors exposed to air as previously discussed.

The VFA profile in control – N_2 flushed replicates was very different to the replicates containing ORS. Although it is not clear to what extent this is due to 'food' availability (i.e. enzymes and soluble COD derived from digestion of inoculum material) and pH (see Figure 48), it can be said that substrate has a significant impact on VFA profile. Profile results from the control (without N2 flushing) replicates were not considered meaningful and were not discussed in detail due to the

high variability in VFA profile between the two replicates; for example, the acetic acid proportion varied between 2% and 70% on day 12. On day 6, valeric acid represented 23% of the VFA in one replicate and was not detected in the other. Average values have been included in the graph for comparison purposes only. It is unclear what caused these differences in the control replicates, but the main conclusions drawn from this experiment are not affected.

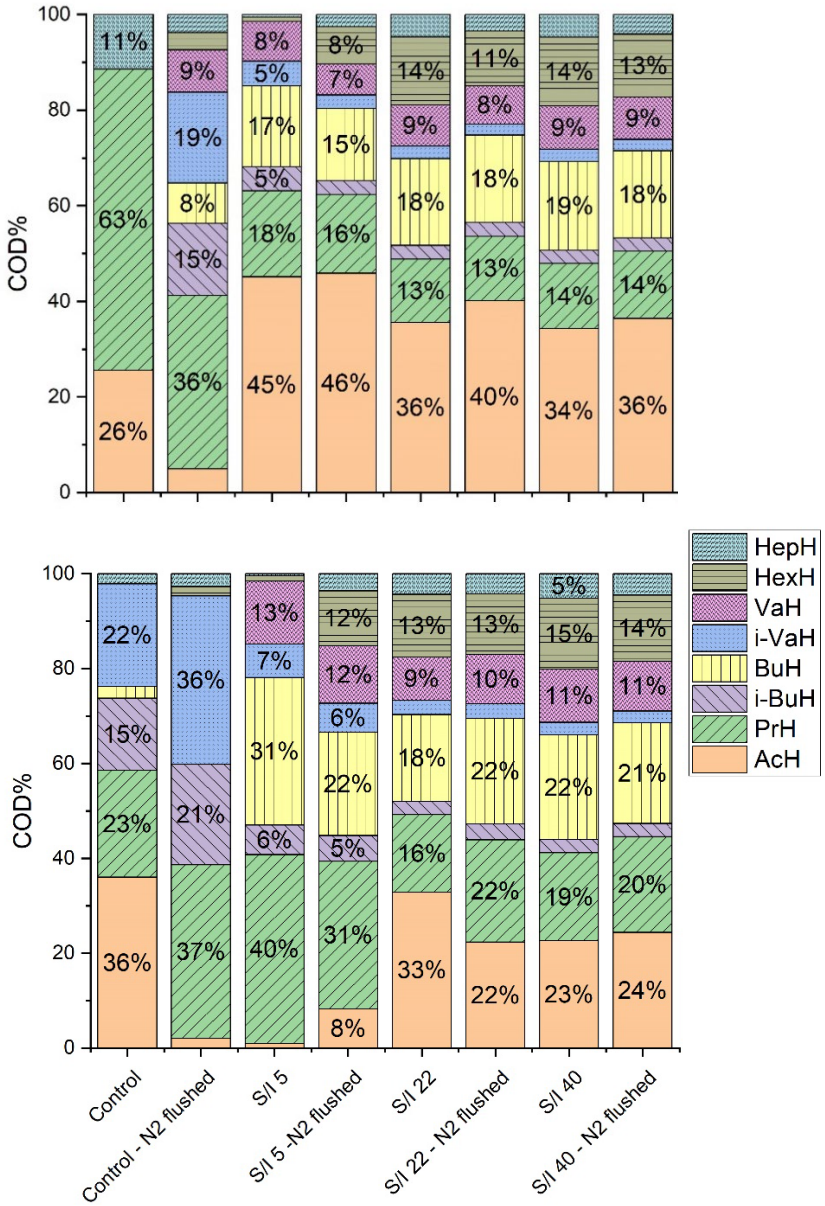

Figure 47. VFA profile in terms of COD% for different S/I ratios with or without N_2 flushing on day 6 (top) and day 12 (bottom), of SHF at ORS concentration of 7.5% TS, 35°C and 2% enzyme load. Percentages are the average of two replicates. Note: Profile results from control (without N2 flushing) replicates were significantly different, but the average values are display for comparison purposes

Figure 48 shows the pH values during this experiment. pH was maintained above 6 using NaOH to ensure that methanogenesis was not inhibited by acidic pH. Control replicates had a relatively stable pH explained by low VFA production. The S/I replicates had variable pH during the first 6 days when VFA production was occurring. Once VFA production plateaued, pH measurements were stopped until day 12 to avoid further exposure to air. pH values on day 6 and 12 were generally similar, with the exception of S/I 5 (with and without N2 flushing) replicates, which increased up to 7.5. This can be explained by the degradation of VFA into biogas.

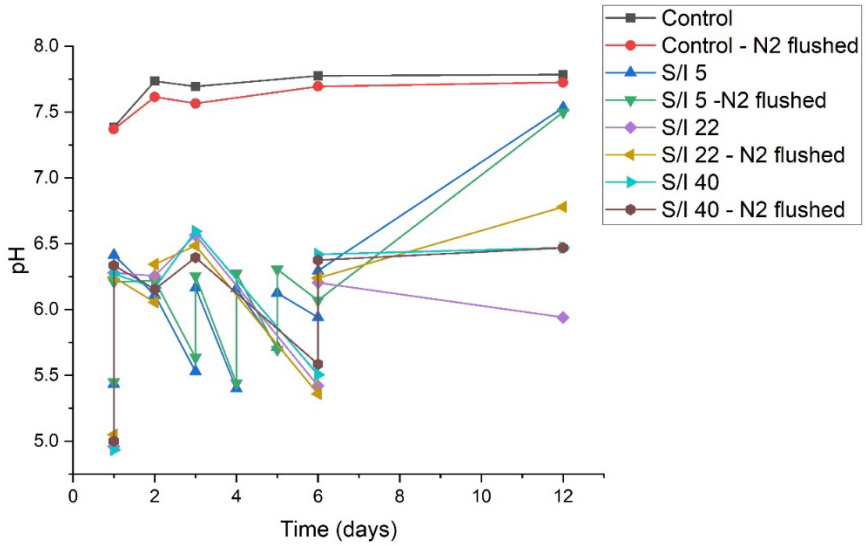

Figure 48. pH readings for different S/I ratios with or without N_2 flushing FA yields for different S/I ratios with or without N_2 flushing of SHF at ORS concentration of 7.5% TS, 35°C and 2% enzyme load.

Figure 49 shows the DoA on day 6 for this experiment. The highest DoA was achieved at a S/I of 5 g TS g^{-1} TS (without N2 flushing), coinciding with the highest VFA yield. Therefore, it was concluded that higher S/I can lead to more generation of by-products.

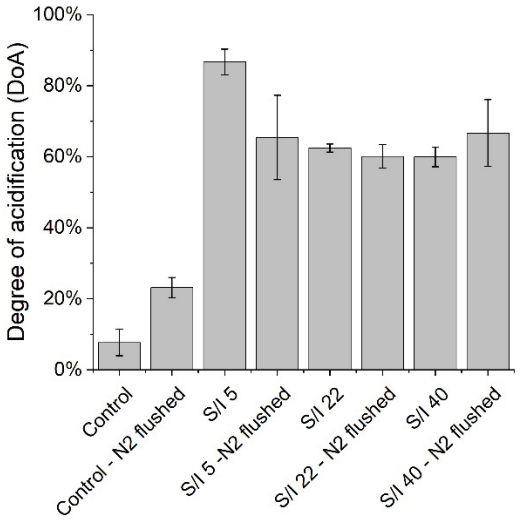

Figure 49. DoA on day 6 for different S/I ratios with or without N_2 flushing of SHF at ORS concentration of 7.5% TS, 35°C and 2% enzyme load. Error bars display the standard deviation of two replicates

4.10 pH and alkalinity effect on AF

In a previous experiment (Section 4.2) the initial pH was adjusted immediately before incubation using NaOH. The pH dropped sharply within 24 hours as VFA were produced. The results from that experiment indicate that initial pH had some effect on VFA yields and profile. Manually controlling the pH throughout the duration of an experiment proved difficult in this work, due to the rapid falls in pH as VFA are generated. Lab scale pH control systems are limited and, therefore, only one pH at a time can be tested using these systems, making optimisation tests tedious and impractical.

It should be noted that there is a difference between alkaline pH and alkalinity. Alkaline pH is any pH value between 8-14. Alkalinity is the resistance of a solution to pH changes as strong acid is added. It is common in AF studies to mix these two concepts. The addition of base (e.g. NaOH) in order to increase pH results in increased alkalinity. However, the addition of salts can also influence alkalinity without a significant effect on pH. For example, $CaCO_3$ only increases pH to maximum value of 6.4 [25]. This experiment was designed to test the effect pH with daily NaOH addition and the presence of $CaCO_3$ (i.e. added alkalinity) on simultaneous hydrolysis and fermentation (SHF) of ORS.

An ORS concentration of 7.5% TS and a S/I of 40 g^{-1} TS g^{-1} TS was used in a working volume of 200 mL. Fermentation took place for 8 days. Two replicates were used to test each pH: 10, 8.5 and

7 by NaOH addition. The same number of additional replicates was used with the addition of 2.2 g of $CaCO_3$. No control replicates were used in this experiment.

After 24 hours of hydrolysis/fermentation, it was observed that the pH 10 and pH 10 + $CaCO_3$ replicates did not have a significant reduction in viscosity (poor mixing when shaken), unlike the other replicates at lower pH which showed signs of turbulence when shaken. This indicates that enzymes activity was negatively affected by pH 10.

Figure 50 shows the alkalinity results from this experiment. As expected, the replicates with higher (total) alkalinity levels are pH 10 + $CaCO_3$, followed by the other replicates with $CacO_3$. Higher NaOH addition, resulted in higher partial alkalinity and higher pH. The presence of $CaCO_3$ resulted in higher intermediate alkalinity for all pH tested. This is an indication that $CaCO_3$ might be useful as buffering agent for slightly acidic pH (~5.7). Error bars are large for replicates pH 10 and pH 10 + $CaCO_3$. This might be attributed to poorer sample homogeneity due to the reduced degree of hydrolysis. However, a clear correlation between pH and alkalinity can be seen.

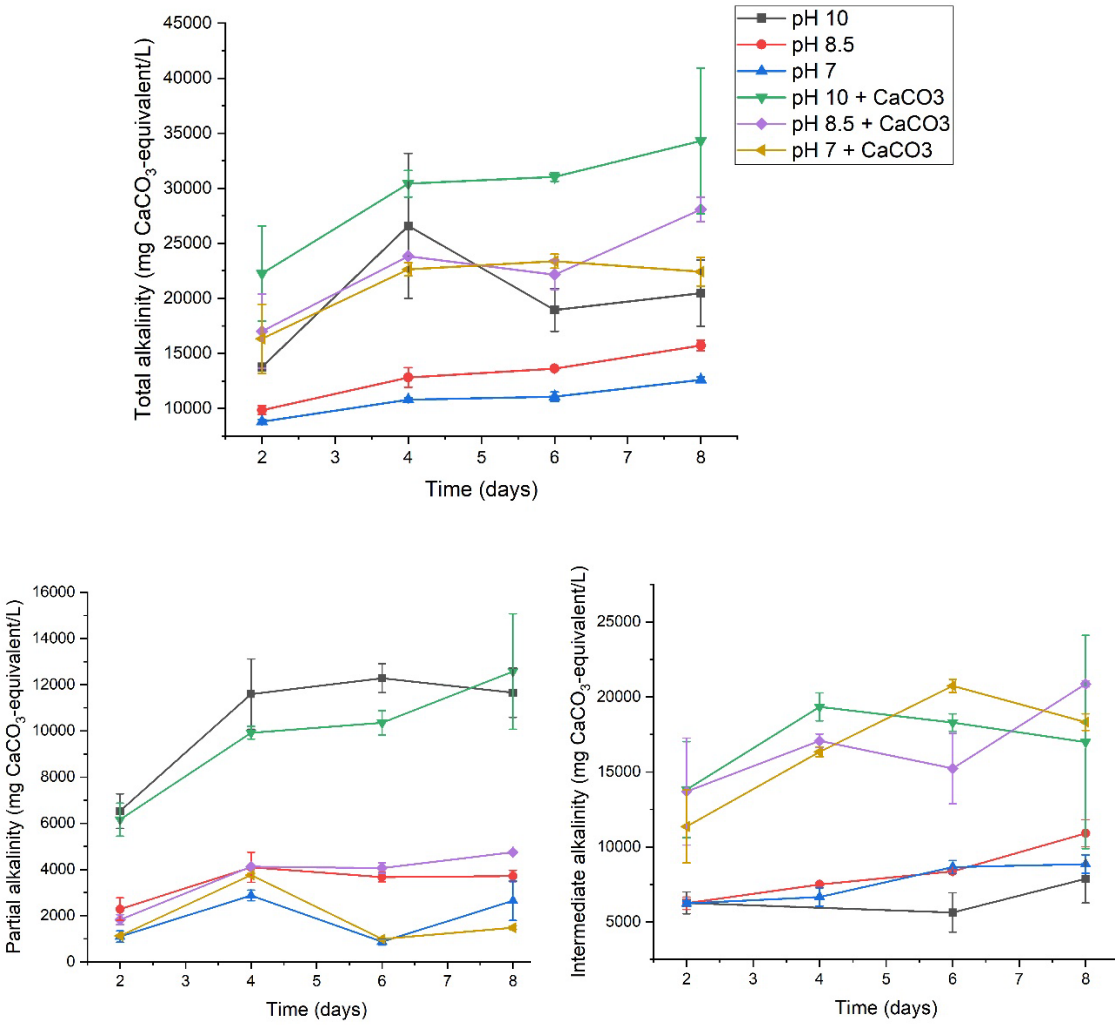

Figure 50. Total, partial and intermediate alkalinity for different controlled pH with and without $CaCO_3$ addition of SHF at 7.5% TS, S/I of 40 g^{-1} TS g^{-1} TS, 35°C and 2% enzyme loading. Error bars display the standard deviation of two replicates

Figure 51 shows the VFA yields resulting from this experiment. The negative effect of pH 10 on hydrolysis is reflected in the low yields achieved (~0.1 g COD g^{-1} VS after 8 days). There are no significant differences in yields for pH 7 and 8.5, whether $CaCO_3$ is present or not, with the exception of pH 7 + $CaCO_3$ on day 6, whose yield is higher than the rest. Unlike previous experiments, yields did not plateau after 4-5 days. This could be due to additional COD release by the action of semi-continuous NaOH addition.

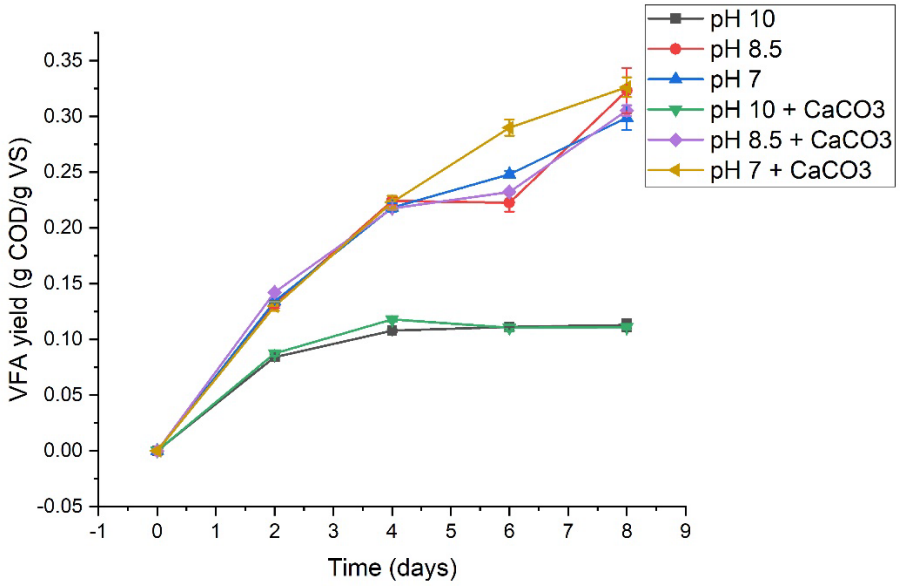

Figure 51. VFA yields for different controlled pH with and without $CaCO_3$ addition of SHF at 7.5% TS, S/I of 40 g^{-1} TS g^{-1} TS, 35°C and 2% enzyme loading. Error bars display the standard deviation of two replicates

Figure 52 shows the VFA profile on day 8 of the SHF. As can be seen, the conditions tested had a significant effect on VFA profile, with acetic acid being the predominant VFA in all cases (≥44%). Compared to previous experiments, these conditions proved unbeneficial for butyric acid production. Clearly, pH had the strongest influence on VFA profile; however, $CaCO_3$ presence had a significant effect on pH 7 replicates. While the main acids were AcH, PrH and BuH for pH 7 with or without $CaCO_3$, the percentage of HexH was significantly higher (by more than 10%) for the replicates with $CaCO_3$. PrH production mostly benefited at pH 8.5 (with or without $CaCO_3$).

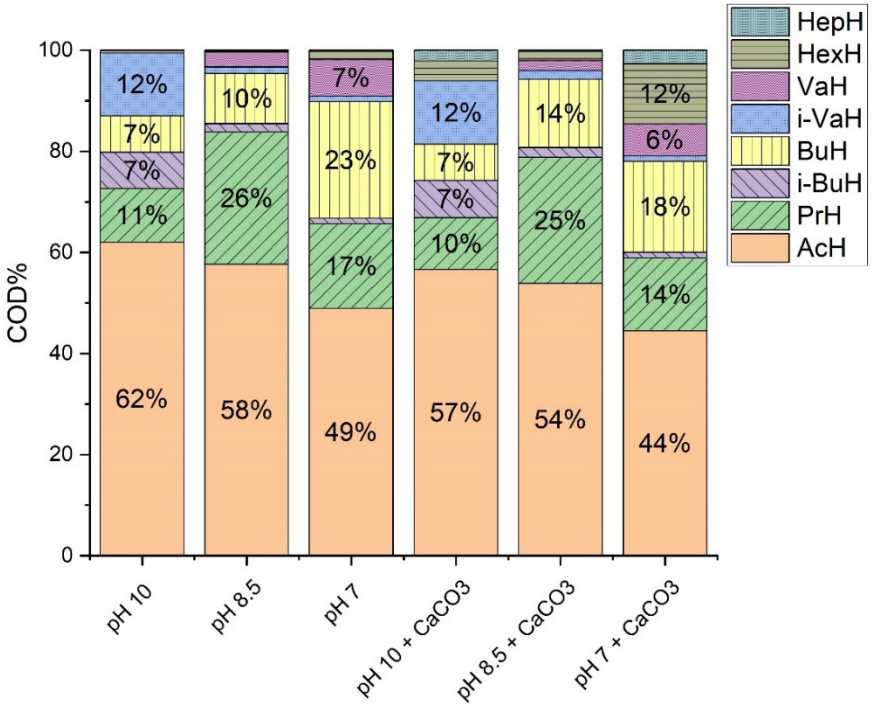

Figure 52. VFA profile on day 8 for different controlled pH with and without $CaCO_3$ addition of SHF at 7.5% TS, S/I of 40 g^{-1} TS g^{-1} TS, 35°C and 2% enzyme loading. Percentages are the average of two replicates

Controlling pH was challenging, particularly during the first 2 days (see Figure 53). pH 10 replicates had a more stable pH after day 5, probably due to the fall in VFA production. As can be seen the presence of $CaCO_3$ did not have a significant influence on pH.

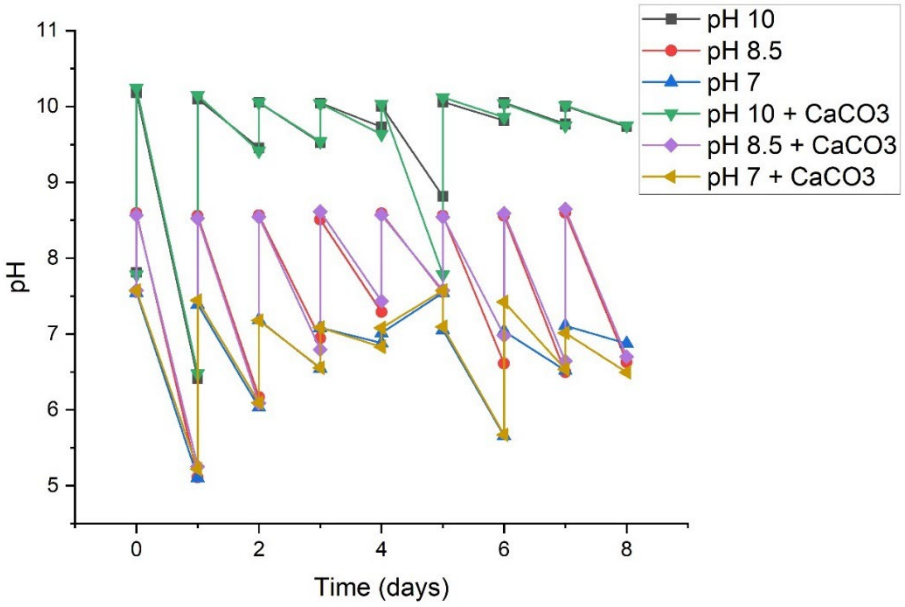

Figure 53. pH reading for different controlled pH with and without $CaCO_3$ addition of SHF at 7.5% TS, S/I of 40 g^{-1} TS g^{-1} TS, 35°C and 2% enzyme loading

The average of all pH readings taken for each replicate was estimated, and the results are shown in Table 17. The average pH of pH 8.5 replicates was far from the design value with an average of 7.7. This could explain the similarities in VFA yield for pH 7 and pH 8.5 replicates. However, the 0.8 difference in average pH was still sufficient to affect the VFA profile. It is likely that more significant differences would have been observed if pH was continuously monitored and controlled, but it was not possible in this work.

Table 17. Average pH for different controlled pH replicates with and without $CaCO_3$ addition

Condition	**Average pH**
pH 10	9.7
pH 8.5	7.7
pH 7	6.9
pH 10 + CaCO3	9.6
pH 8.5 + CaCO3	7.7
pH 7 + CaCO3	6.9

Figure 54 shows the DoA for each condition tested in this experiment. As can be seen, the DoA achieved by pH 10 replicates is very low, indicating that pH 10 not only affected hydrolysis, but

also fermentation. This could be a side effect of high viscosity as opposed to a result from pH, as it is known from the literature that acidogens are not inhibited at pH 10.

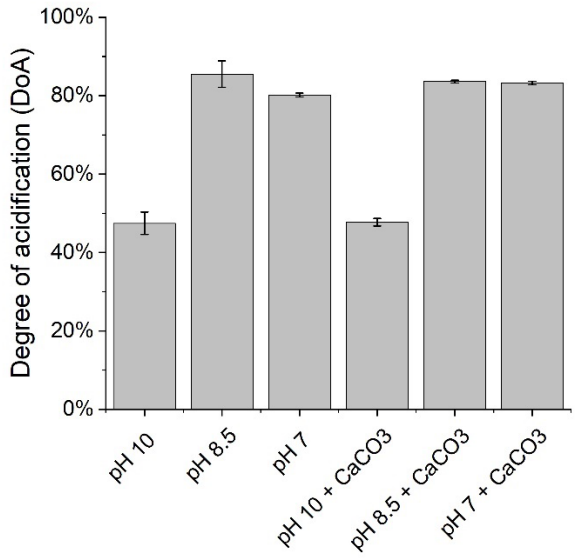

Figure 54. DoA for different controlled pH with and without $CaCO_3$ addition of SHF at 7.5% TS, S/I of 40 g^{-1} TS g^{-1} TS, 35°C and 2% enzyme loading. Error bars display the standard deviation of two replicates

Figure 55 shows the TS and VS results from day 8. Higher pH resulted in higher TS due to NaOH addition. Similarly, replicates with $CaCO_3$ had higher TS% for all pH values. VS% were the same for replicates with or without CaCO3. pH 8.5 and 7 replicates had lower VS% than pH 10 replicates, probably as a result of CO_2 release from acidogenic fermentation.

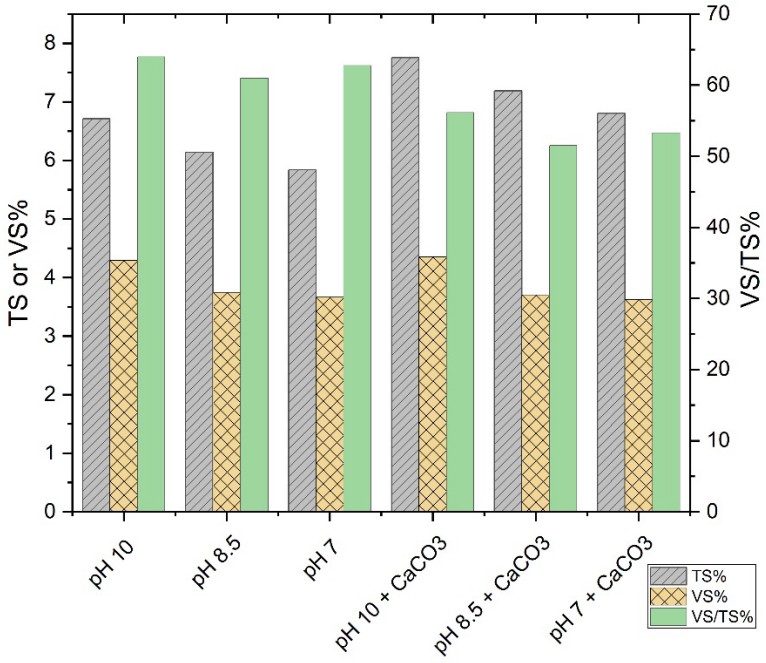

Figure 55. TS and VS values on day 8 for different controlled pH with and without $CaCO_3$ addition of SHF at 7.5% TS, S/I of 40 g^{-1} TS g^{-1} TS, 35°C and 2% enzyme loading

From these results, it can be concluded that pH 10 is highly unfavourable for SHF aiming at VFA production. Since there were no significant differences in VFA yields for pH 8.5 and 7, pH 7 is recommended to reduce NaOH usage. However, pH 7, as opposed to slightly acidic pH, will benefit acetic acid production over butyric acid.

4.11 Conclusions

Results from batch mode experiments show that ORS is a promising substrate for biological VFA production. The findings from this chapter gave further insight into fermentation conditions and how they affect VFA production, by-product generation and methanogenic inhibition. These results were used in the design of the following semi-continuous and fed-batch experiments.

Chapter 5 Semi-continuous and fed batch experiments

This chapter discusses the results from semi-continuous and fed-batch experiments. Due to the relevance of the results discussed in this chapter, a brief introduction has been included to provide a better understanding of the decision making in the experimental design. Relevant discussion on the literature focusing on continuous (or semi-continuous) mode and fed batch mode can be found in Section 2.6.4.

5.1 Introduction

In the preceding chapter, experiments were carried out in batch mode. Batch mode has many advantages, and it is ideal to study the effect of different fermentation variables for optimisation purposes. At large scale, batch mode offers a certain level of operational flexibility, although it normally results in higher operating costs. Generally, continuous operation is desired for higher productivities. In continuous systems, two extra variables are introduced: organic loading rate (ORL) and hydraulic retention time (HRT). As discussed in Chapter 2, OLR and HRT can significantly impact VFA production and methanogenic activity.

In previous batch experiments, it was found that the pretreatment of ORS with enzymes can lead to the production of lactic acid, which causes acidogenic fermentation to fail unless pH is controlled above 5.5. Simultaneous enzyme hydrolysis and fermentation (SHF) helps to overcome this problem by 'forcing' the conversion of organic soluble material into VFA by the inoculum population. In continuous (or semi-continuous) mode, the enzyme can be fed along with the substrate. In this way, the batch pretreatment step is also avoided, giving a saving in operating costs.

Fed-batch mode is typically applied in fermentations when the feed is toxic to the bacteria at high concentrations. This is rarely the case in acidogenic fermentation studies and, therefore, this strategy is hardly used in this field. In this work, the product concentration in batch experiments was limited by the substrate concentration (maximum 10% TS for appropriate mixing) and its biodegradability. After enzyme pretreatment, the solid-liquid mixture is less viscous, and more ORS and enzymes could be added to increase substrate concentration, without removing the broth, essentially becoming a fed-batch system.

5.2 Semi-continuous experiment

For the semi-continuous experiment, six stirred tank reactors (A to F) were used, each with a 0.5 L working volume. The reactors were connected to 5 L gas bags and were fed every 48 hours. Gas measurements (volume and composition) took place every 2-6 days, depending on the saturation of the bas bags. The daily gas yield was calculated by dividing the methane production by the time between sampling. The pH was adjusted using 4 M NaOH after every feed. The ORS used was packed in 500 g bags and frozen: bags were defrosted as necessary and kept refrigerated for less than four days. Different HRT and OLR were tested in duplicate as described in Table 18. The feed was prepared with an approximate ORS concentration of 75% TS and the total volume (i.e. flowrate Q) was varied in order to obtain different HRT and OLR. OLR of all reactors were increased stepwise until day 22 and kept constant from day 22 to day 36 at 8 g VS L^{-1} day^{-1}. Different OLR values were introduced from day 38 and kept constant until day 70.

Table 18. Target conditions of the semi-continuous experiment

Stage	**Conditions/reactors**	**A & B**	**C & D**	**E & F**
Inoculum adaptation – 38 days	**Q (L day^{-1})**	0-0.071	0-0.071	0-0.071
	HRT (days)	19-7	19-7	19-7
	OLR (g VS L^{-1} day^{-1})	0-8	0-8	0-8
OLR and HRT at constant TS concentration – 32 days	**Q (L day^{-1})**	0.107	0.071	0.044
	HRT (days)	4.7	7	11.3
	OLR (g VS L^{-1} day^{-1})	12	8	5

The volume of all reactors was kept constant between 500 and 550 mL. On day 6, due to a pressurisation resulting from unexpected excess biogas production, which saturated gas bags, some content was lost. The volume was then adjusted back to normal after 3-4 feedings by maintaining approximate OLR.

The TS/VS of the ORS was slightly variable, depending on the bag/batch used, due e.g. to condensation during defrosting. As TS analysis takes a few hours, the TS% of the feed was calculated retrospectively as 7.5%, 6.4% and 5.8% for days 0-26, 28-62 and 64-68, respectively. Exact quantities of ORS, enzyme solution and water fed to reactors are summarised in Table 36 (Appendix C). The VS/TS% of the ORS (0.7) was used to calculate actual OLR values based on measured TS%. The actual HRT and OLR values are plotted in Figure 56. On day 70, the

experiment was terminated due to the Covid-19 lockdown imposed by the UK government and no further feeding took place, hence HRT and OLR values on day 70 are not displayed on the graph.

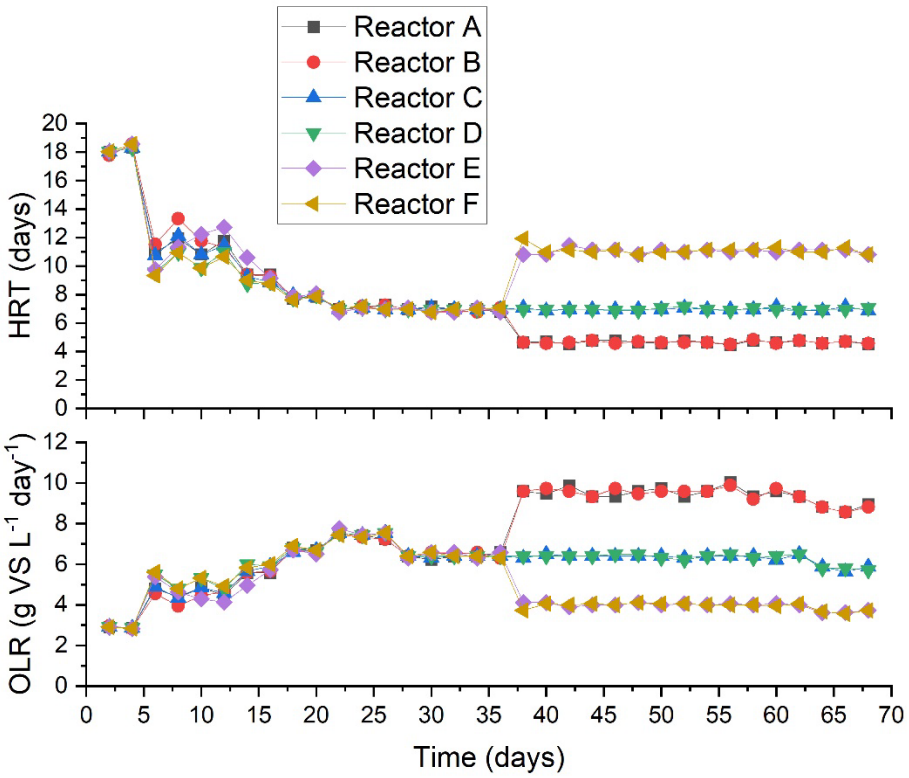

Figure 56. HRT (top) and OLR (bottom) for each semi-continuous reactor

Figure 57 shows the pH of the effluent of each reactor. During the first 12 days, the pH fell slowly for all reactors. This was expected since the pH of the substrate was slightly acidic, there was an anticipated increase in VFA concentration, and the alkalinity of the inoculum was being washed out. From thereon, pH rapidly dropped below 5.5, so NaOH addition was introduced to control pH values in the reactors to reach 5.9-6.0 immediately after feeding. From day 26 to day 36, pH was maintained relatively stable by the addition of 30-50 drops of 4 M NaOH every time after feeding. After day 40, reactors A-B needed 20-30 drops every time after feeding. Reactors C-D and E-F did not need adjustment. Based on pH, three different phases can be clearly distinguished: pH decrease (phase I - day 0 to 20), constant pH (phase II – day 21 to 40) and change in pH based on operational variables (phase III – day 41 to 70).

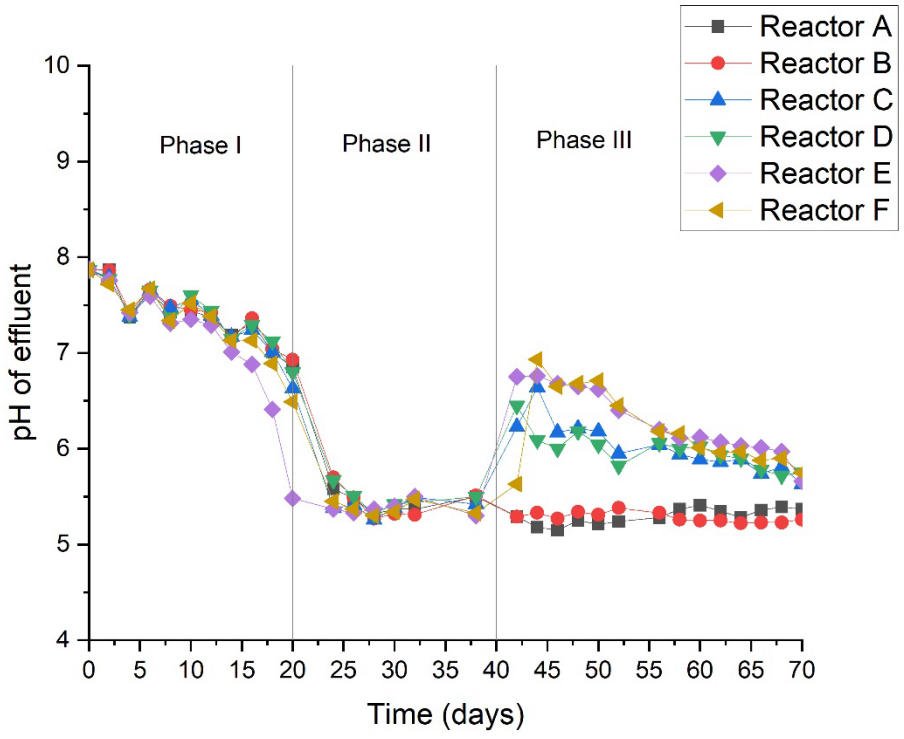

Figure 57. pH readings of effluent for each semi-continuous reactor

Effluent TS and VS are shown in Figure 58. On day 10, TS concentration of reactors was higher than on day 0 except for reactor E. A small crack in the reactor vessel was detected, where water from the water bath was infiltrating, resulting in a reduction in TS%. The reactor vessel was then replaced. On day 30, all reactors had a similar TS content and from thereon TS% showed small variations for all reactors. These small oscillations are probably due to heterogeneity in the solid distribution of the sample/effluent. On phase III of the experiment, slight differences in terms of VS (dry basis) are observed. The pattern clearly indicates the VS is slightly lower for lower OLR (or higher HRT). This is expected since lower OLR is preferred by methanogens to convert VFA into biogas.

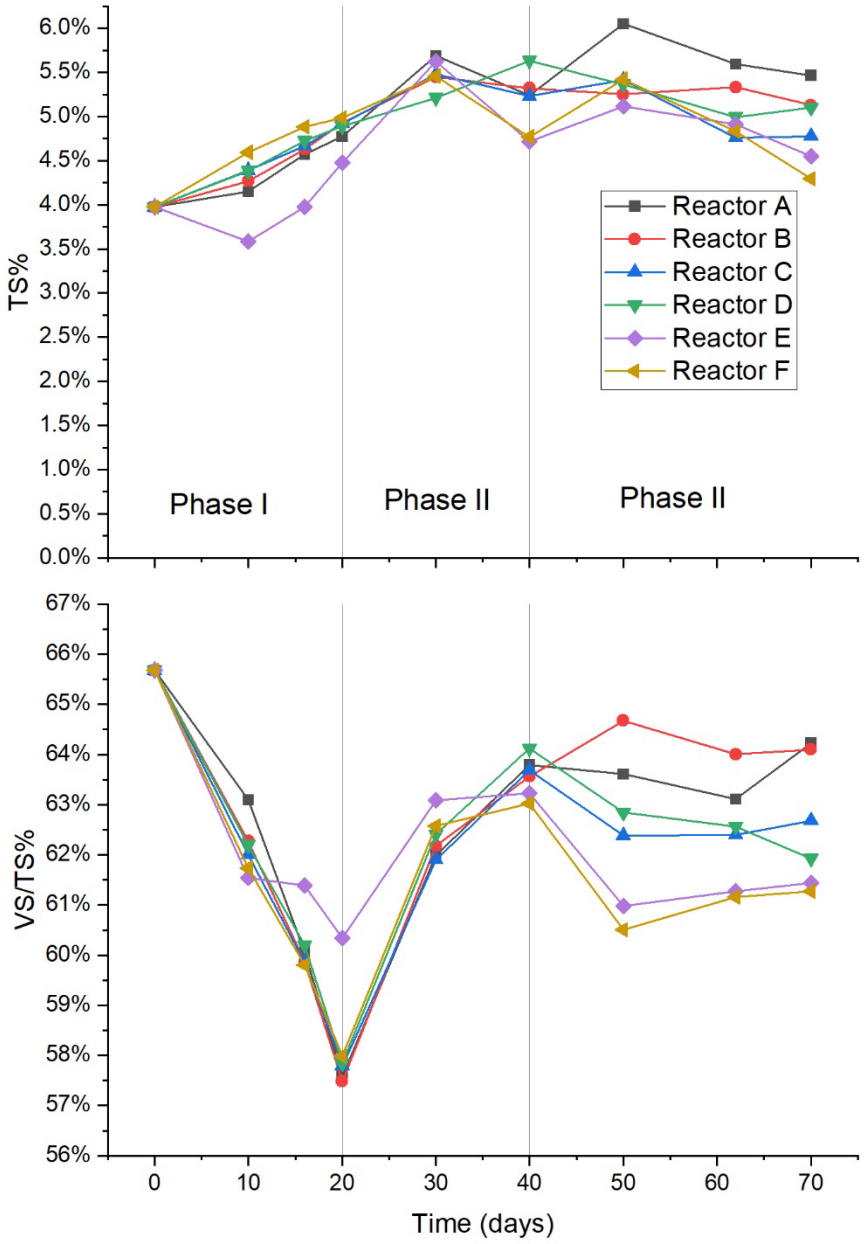

Figure 58. Total solids (top) and volatile solids on a dry basis (bottom) of semi-continuous reactors

Figure 59 shows the methane production for each reactor. A similar trend to pH values is observed for methane production, where three clear phases can be distinguished within this experiment. Up until day 14, the behaviour of all reactors was similar, with a significant increase in methane production. This is as might be expected since OLR and HRT are still within comfortable values for methanogenesis (5 g VS L^{-1} day^{-1} and 11 days, respectively). On day 16, a maximum methane production value of 177 L CH_4 kg^{-1} VS was observed, except for reactor E which started declining. From thereon, methane production in all reactors started declining as a result of increasing OLR, reaching yields lower than 14 L CH_4 kg^{-1} VS on day 28. At this point, pH was below 5.5 which is considered too acidic for methanogensis. On day 42, a significant increase

in methane production was observed for reactors C-D, and E-F, and to a lesser extent reactors A-B. The observed peaks in methane yield coincided with the peaks in pH. This can be explained by the consumption of VFA, which decreases the acid content, and typically results in pH increase. This pH increase further benefits methanogens. It is unclear what triggered methane production; however, it is possible that further methanogens potentially adapted to lower pH were introduced by the substrate. Not only it is common to find methanogens naturally growing in MSW, but also the ORS is a product of washing OFMSW with water treated in an AD system. After a few days, reactors C-D, and E-F appeared to approach stable gas production between 50-80 L CH_4 kg^{-1} VS. Reactors A-B reached stable gas operation at 23 L CH_4 kg^{-1} VS. Therefore, it can be concluded that methanogens were successfully inhibited by the high OLR of 8-9.5 g VS L^{-1} day^{-1}. Although not shown in Figure 59, the CO_2 yields followed a very similar pattern to that of CH_4 yields. It is possible that methanogenic activity could have been further supressed by higher OLR, however, this would affect the HRT too. It was concluded from batch experiments that a minimum of 4-5 days is desired for maximum VFA yields. Alternatively, to reach higher OLR while maintaining a HRT of 4-5 days, the feed concentration could be increased. Due to the experimental interruption, this could not be investigated in this study.

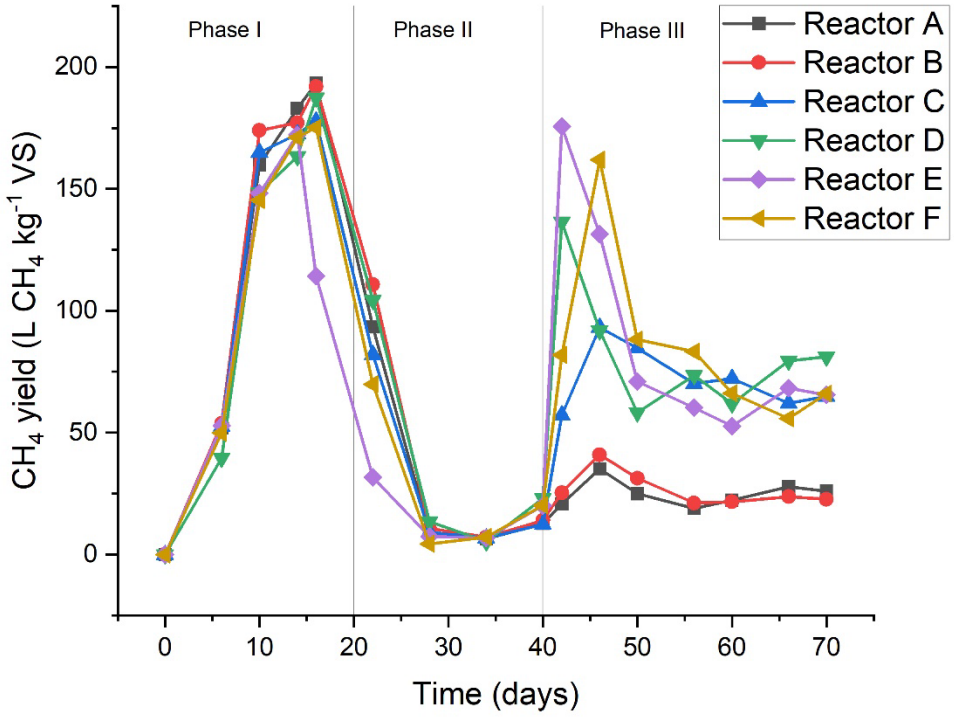

Figure 59. Methane yield for semi-continuous reactors

Figure 60 shows the VFA yields and concentrations. Up until day 16-18, VFA yields were negligible due to methane production. VFA yields rapidly increased in the next 12-14 days. Maximum VFA

concentration (11.1 g COD L^{-1}) and yield (0.25 g COD g^{-1} VS or 0.18 g g^{-1} VS) was observed on day 32 by reactor C. This yield is significantly smaller (roughly half) than those in previous studies using food waste [176], but similar to that estimated from the OFMSW study (0.23 g g^{-1} VS or 0.34 g COD g^{-1} VS) [175]. Food waste is more soluble than ORS and contains a significantly higher portion of VS on a dry basis, so this difference is expected.

During phase III, VFA yields of reactors A-B were significantly higher than those of reactors C-D, and E-F. This was mostly due to acetic acid being consumed for biogas production in the latter reactors. However, butyric acid concentrations were also higher in reactors A and B. The productivity of reactors A-B varied between 1 and 1.55 g VFA L^{-1} day^{-1}, similar to the productivity of AF of food waste at 4 days HRT observed in another study [176]. The productivities of C-D and E-F were 0.25-0.68 and 0.08-0.40 g VFA L^{-1} day^{-1}, respectively. The production of VFA was not stable due to variations in propionic acid and acetic acid concentrations. This instability could be due to the intrinsic variability of the experimental setup (substrate moisture content, pH changes, sampling method, aerobic/anaerobic atmosphere, etc.), which are much more difficult to control in CSTR systems than in batch systems, especially at bench scale. These results are in agreement with previous studies using food waste-CSTR systems which confirm that increasing OLR lead to an increase in relative abundance of acetic acid and a decrease in propionic [176, 178]. However, discrepancies are observed in terms of butyric acid relative abundance as the same studies report increasing OLR led to a decrease in butyric. This discrepancy can be simply explained by differences in substrate composition, as food waste is different to ORS.

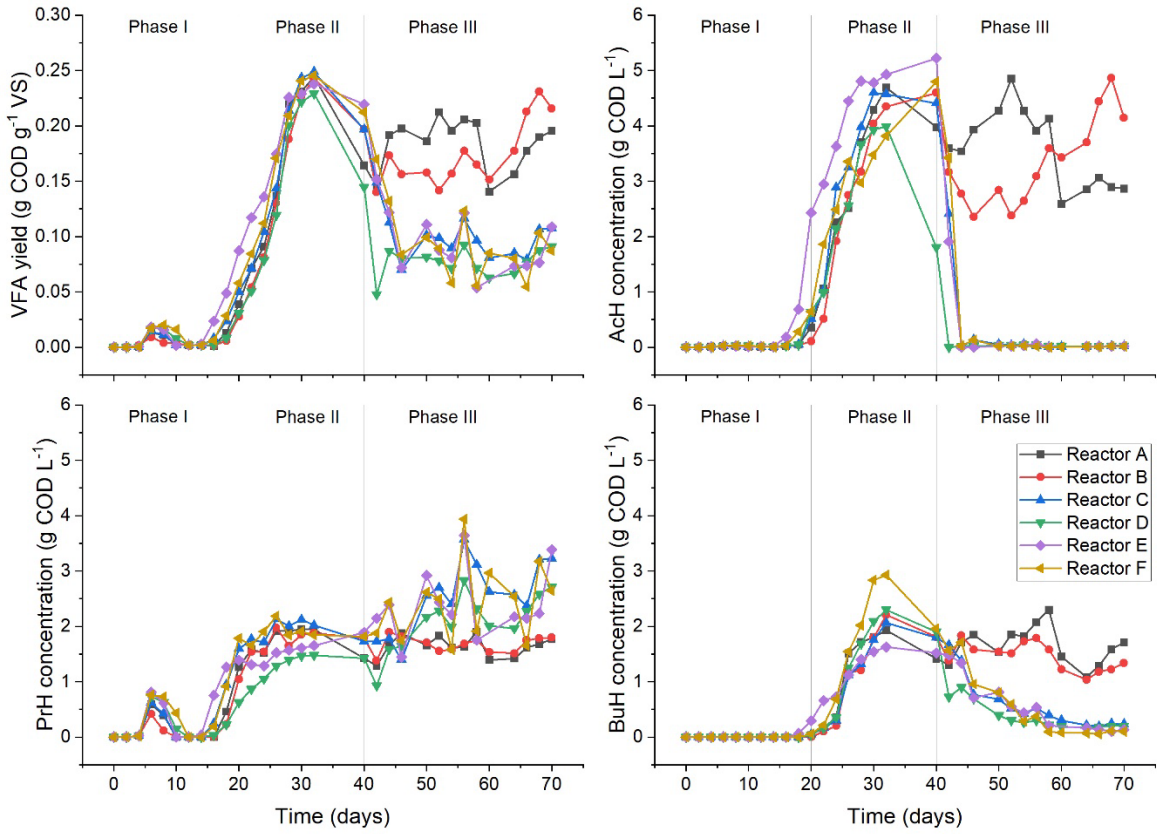

Figure 60. VFA yields, AcH, PrH and BuH concentrations from the semi-continuous experiment

The soluble VS as a proportion of total VS on day 70 was 12.4%, 16.5%, 10.1%, 7.9%, 10.2% and 9.7% for reactors A, B, C, D, E and F, respectively. Higher soluble VS percentages in reactors A and B are explained by lower methane yields. Soluble VS is still relatively low despite enzyme addition, suggesting that the process could benefit from further pretreatments.

Figure 61 depicts the DoA and soluble TOC for each reactor on day 70. As expected, soluble TOC was lower in reactors C-D, and E-F compared to A-B. This is likely to be due to the consumption of carbon (VFA) for biogas production. Interestingly, the DoA was lower in these reactors, meaning that low VFA yields were also due to by-product formation. It was originally hypothesised that shorter HRT would lead to more by-product formation, however this was not the case as reactor A and B had the largest DoA (>85%).

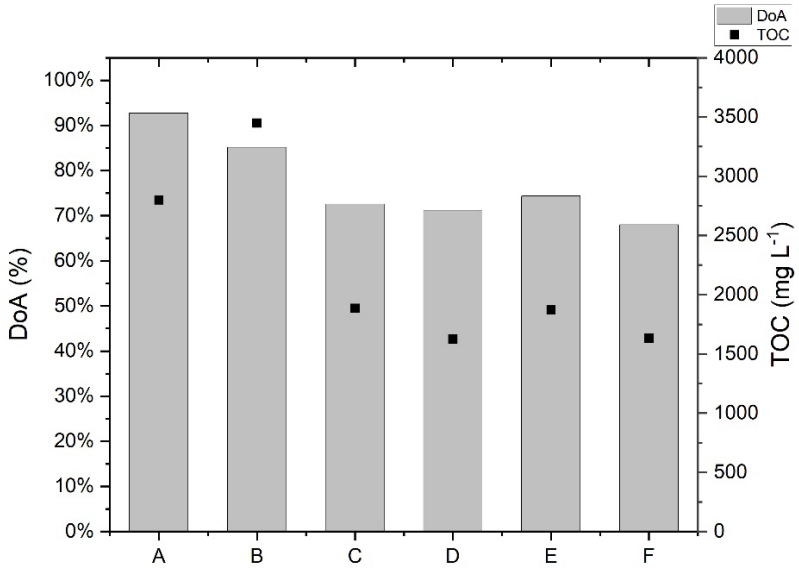

Figure 61. DoA and soluble TOC of semi-continuous reactors on day 70

5.3 Fed-batch experiment

In this work, it was demonstrated that optimum enzyme hydrolysis time is 24 hours. In batch fermentation experiments, maximum VFA concentrations were reached after 4-5 days of fermentation in most cases. Therefore, for fed-batch mode, it was suggested that feeding should occur at least every 4 days.

The aim of this experiment was to attain higher concentrations of VFA than the maximum VFA concentrations achieved in previous batch and semi-continuous experiments. In addition, the effect of different feeding modes was studied. In this experiment, six stirred tank reactors (named as A to F), each with a 0.8 L working volume were used. At the start, the working volume of all reactors was 0.4 L comprising the same mixture of ORS, inoculum, enzyme, and water: 130 g, 120 g, 6 mL and 144 mL, respectively. Under these conditions, the S/I was 5 g TS g^{-1} TS (5.2 g VS g^{-1} VS). Feeding details for each reactor are summarised in Table 19. Enzyme loading was 2% as in previous experiments. 15 mL samples were taken every 8 days for analysis and measurement. This was taken into consideration in the mass balance calculations.

Table 19. Feeding breakdown for fed-bath experiment

Conditions	**A-B**	**C-D**	**E-F**
Part 1 (day 0-18)	30 g ORS every 2 days (15 g ORS day^{-1})	30 g ORS every 4 days (7.5 g ORS day^{-1})	60 g ORS every 4 days (15 g ORS day^{-1})
Part 2 (day 19-28)	60 g ORS every 2 days (30 g ORS day^{-1})	30 g ORS every 4 days (7.5 g ORS day^{-1})	-
Part 3 (day 29-36)	-	30 g ORS every 4 days (7.5 g ORS day^{-1})	-
Total ORS fed (g)	670	370	370
Equivalent TS%	16%	13%	13%

Figure 62 shows the VFA concentrations and VFA yields at different stages of the experiment. VFA concentrations and yields of reactors A-B and C-D followed an increasing trend with time. For reactors E-F, VFA concentrations and yields increased until day 32. From day 32 to 36, VFA concentration and yield decreased slightly for reactor E and stayed approximately the same for reactor F. The maximum VFA concentration of 44 g COD L^{-1} was reached in reactors A-B on day 36. However, higher concentrations did not translate to higher yields. Reactors A-B's yield was 0.38 g COD g^{-1} VS on day 36, whereas reactors E-F reached 0.43 g COD g^{-1} VS on day 32. A compromise between concentration and yield should be found in the process optimisation depending on the aims of the process.

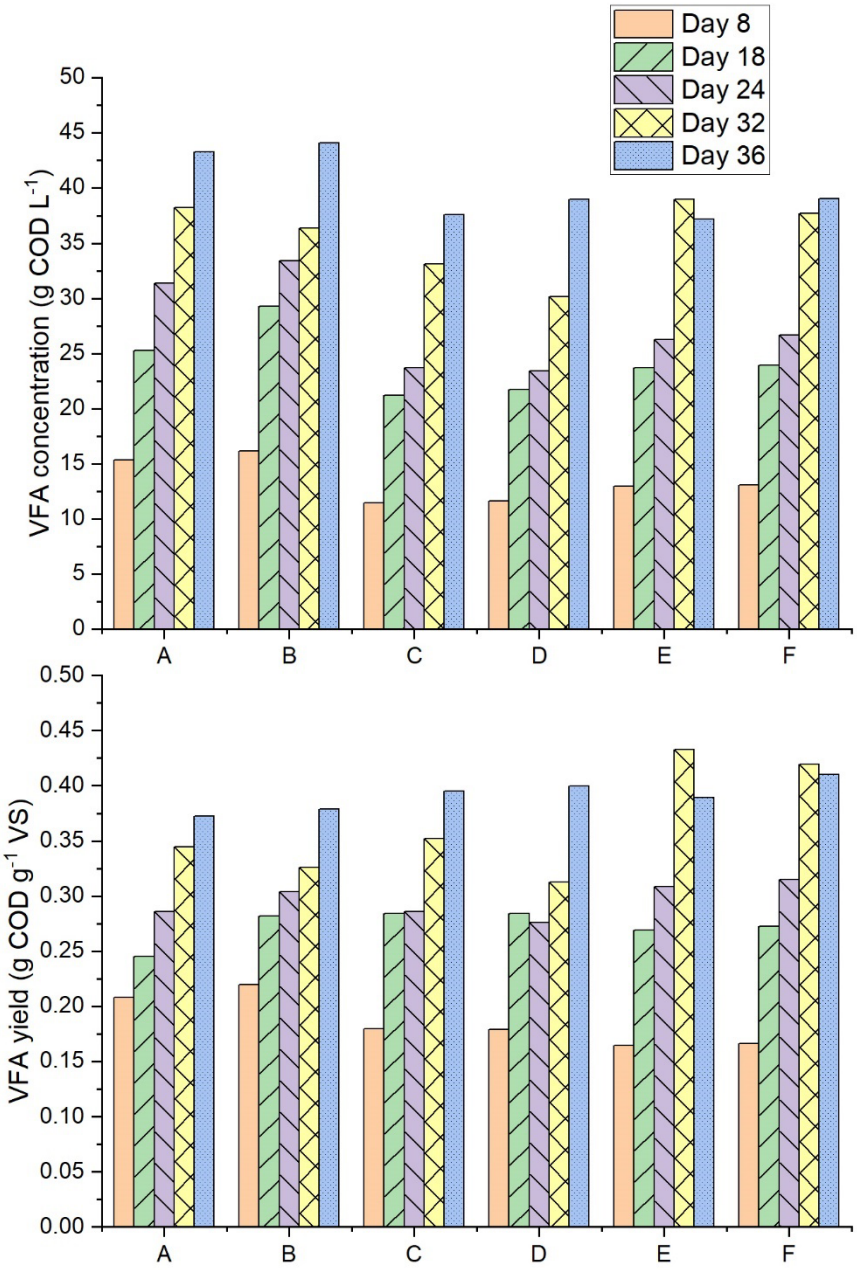

Figure 62. VFA concentrations (top) and VFA yields (bottom) of fed-batch experiment

Figure 63 shows the pH values during this experiment. pH followed a similar pattern for all reactors despite differences in VFA concentration. Initially, pH rapidly dropped from 7.8 to approximately 5.5 within one day. pH in reactors C, D, E and F fluctuated slightly during the first few days, but eventually settled at around pH 5 as in reactors A and B. As pH was always above the average pKa value (4.8) and VFA production seemed not to be affected (i.e. VFA yields did not plateau), the pH was not adjusted at any point in this experiment.

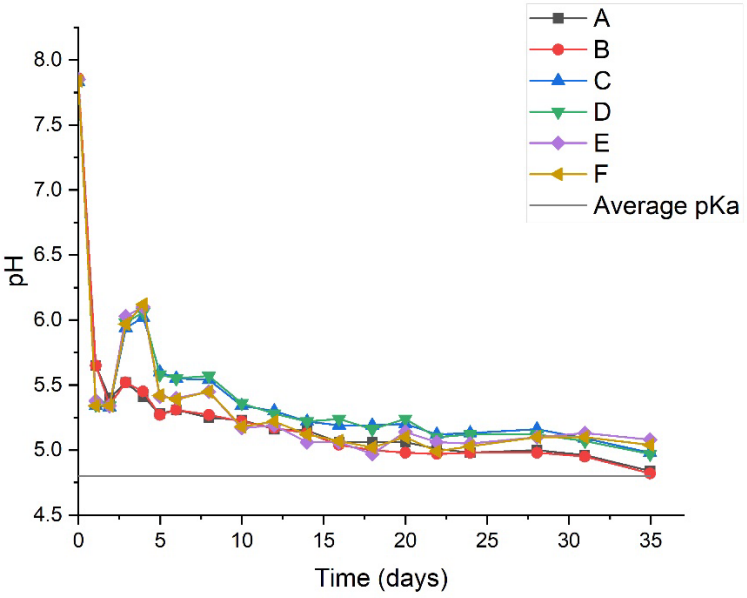

Figure 63. pH measurements of the fed-batch experiment

The TS% for each reactor at different fermentation times are plotted in Figure 64. As expected, reactors A, B, C and D had an increasing trend in TS% as they were fed during all stages of the experiment. The TS% of reactors E and F decreased slightly and plateaued after day 18. The small fluctuations can be explained by the heterogeneity of the reactor contents in terms of solids distribution, which led to variability within samples.

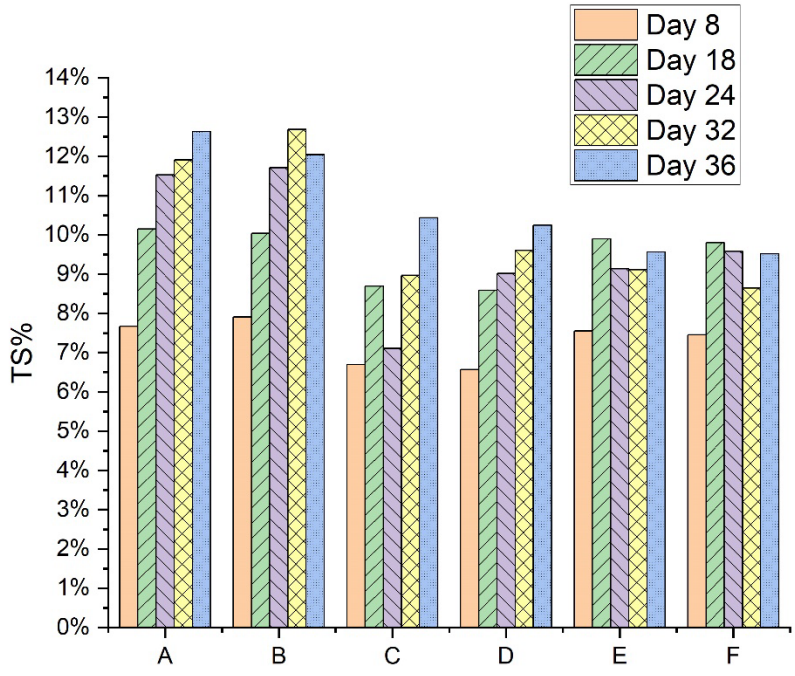

Figure 64. TS% of fed-batch experiment

The soluble VS as a proportion of total VS on day 36 was 22.07%, 21.97%, 15.62%, 19.50%, 22.98% and 22.01% for reactors A, B, C, D, E and F, respectively. As values in reactors C and D were considerably lower than the rest, it appears that low feeding rates and the absence of a non-feeding period towards the end of the experiment negatively affected the hydrolysis.

Figure 65 shows the cumulative gas production after 36 days of operation. The most noticeable aspect of these results is that accumulated biogas (CO_2 + CH_4) was negligible for reactors E and F. The low methane yield in all reactors, but particularly in reactors E and F, indicate methanogenic inhibition, probably caused by high VFA concentrations and low pH. The low CO_2 accumulation in reactors E and F is more challenging to explain, since typically, CO_2 generation is proportional to VFA concentrations. The VFA concentrations in reactors E and F are similar to those in reactors C and D in the final stages, yet the differences between CO_2 accumulations are significant. Despite the match of VFA production in reactors A and B, the CO_2 accumulation in reactor A is roughly half that of reactor B. It is worth highlighting that most biogas production in all reactors occurred after day 28, which might indicate a change in fermentation pathways.

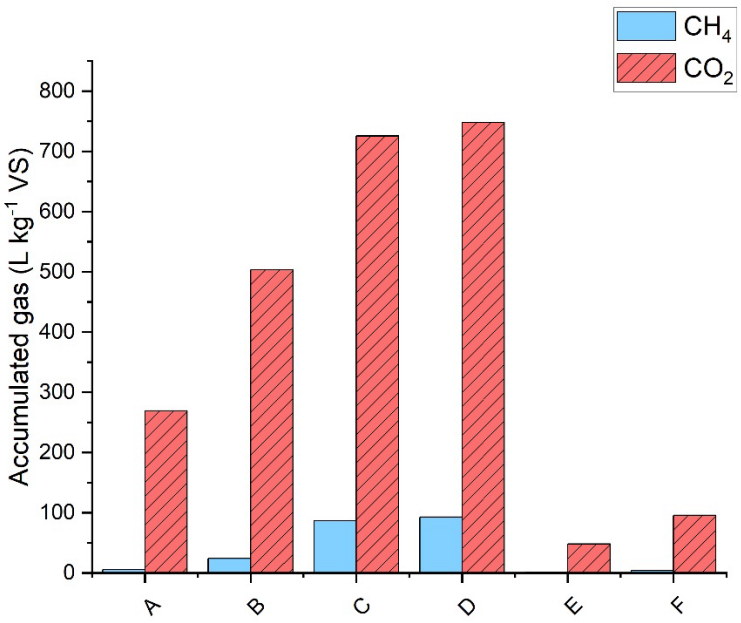

Figure 65. Accumulated gas of fed-batch experiment after 36 days of operation

A clearer idea of the pathways can be established by analysing Figure 66. On day 8, the VFA profile favoured butyric and acetic for all reactors, followed by propionic and valeric. On day 18, butyric acid was the most predominant VFA in all reactors. At this point, small but significant concentrations of hexanoic were also observed in all reactors. On day 24, butyric acid concentrations slightly increased for all reactors, and hexanoic acid concentrations almost doubled for reactors A and B. On day 36, butyric acid and hexanoic acid concentrations

significantly increased in all reactors, particularly reactors A and B which had the maximum concentration of both acids. Butyric acid and hexanoic acid concentrations reached approximately 12 g L^{-1} (20 g COD L^{-1}) and 6 g L^{-1} (13 g COD L^{-1}), respectively. The hexanoic acid relative abundance is much higher compared to that in previous batch and semi-continuous experiments. This is an indication that the reactor mode can influence VFA profile. Often the presence of hexanoic acid is a result of chain elongation (i.e. conversion of short chain fatty acids to hexanoic). It is not entirely certain, but the reduction of acetic and propionic as hexanoic is generated suggests that at least part of the hexanoic production might be a result of chain elongation. Although the pathways of hexanoic generation are not clear, it could be said that fed-batch mode is beneficial for the production of medium chain fatty acids. This may be a result of long retention times and high short chain fatty acids concentrations. Based on relative abundances, the feeding mode did not seem to have significant effect on VFA profile.

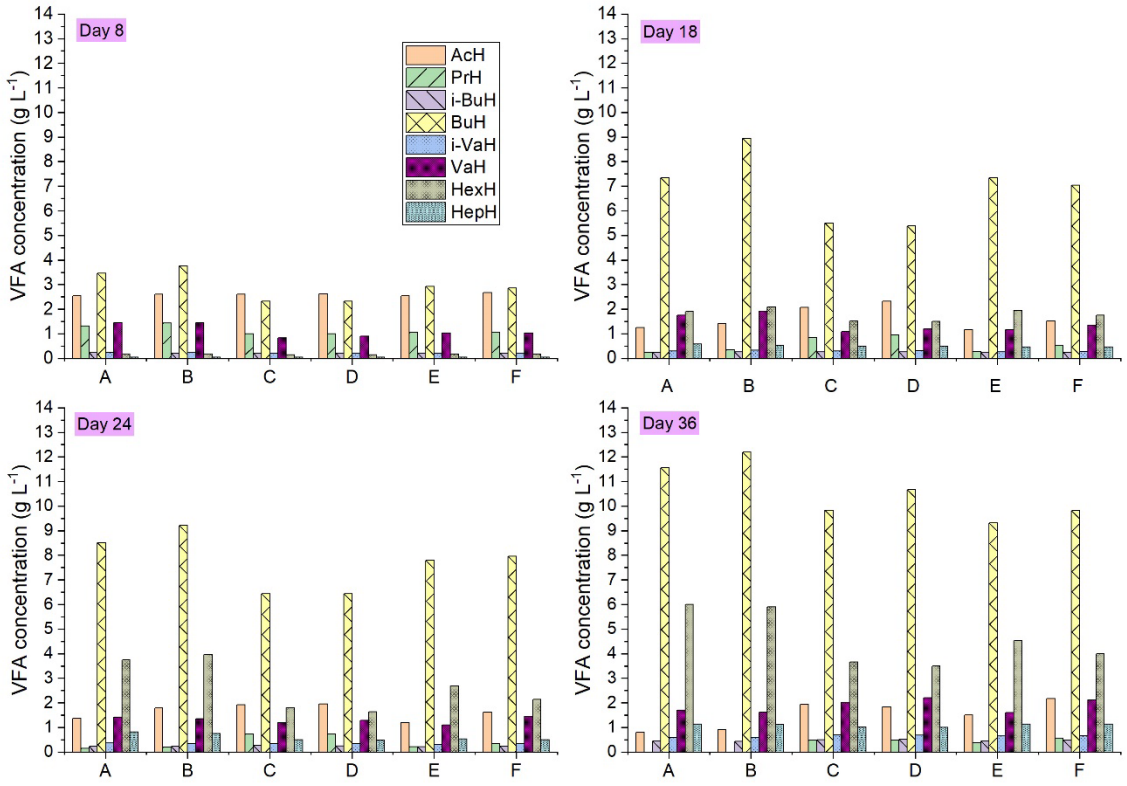

Figure 66. VFA composition at different stages of the fed-batch experiment

Figure 67 shows the DoA of each reactor on day 36. The highest DoA was achieved in reactors C-D followed by E-F and A-B. This is an indication that slow feeding (lower g VS day^{-1}) is preferable to avoid the formation of by-products. The DoA values of A-B reactors were also lower than those of

E-F reactors, despite the long retention time (10 days) after the last feeding.

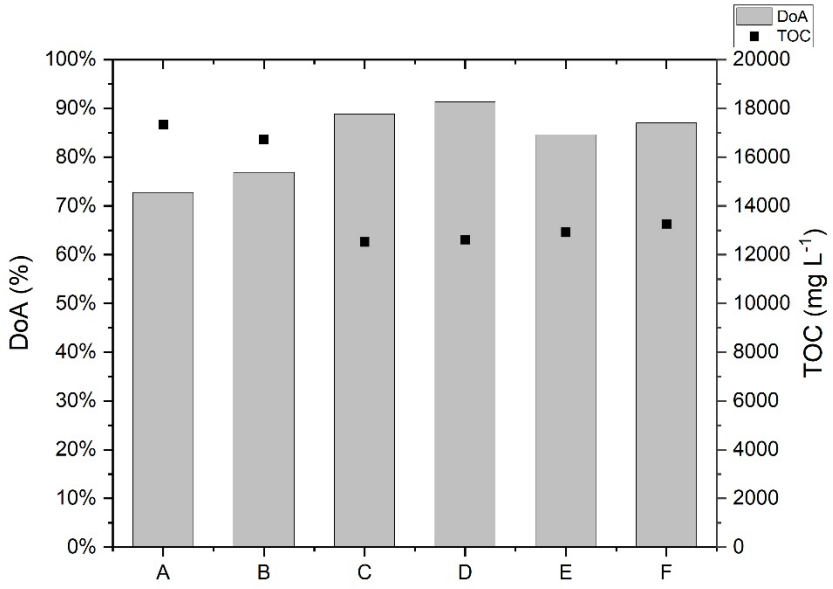

Figure 67. DoA of fed-batch experiment on day 36

5.4 Conclusions

In industry, continuous production systems are generally preferred over batch systems. Studies on continuous (or semi-continuous) AF of OFMSW-like residues, however, are scarce. In this chapter, the effect of HRT and ORL in semi-CSTR on methanogenic inhibition and VFA yields was shown. Results show that long-term stability of semi-CSTR reactors is challenging, particularly at bench scale (<1 L), potentially due to the introduction of exogenous microbes and the pH instability.

This chapter demonstrated the potential of fed-batch feeding system to improve VFA concentration and yields, providing a novel strategy to inhibit methanogenic activity and increase VFA concentrations. It was also found that fed-batch mode could lead to chain elongation, facilitating the production of longer chain fatty acids with higher economic value.

Chapter 6 Economic assessment

This chapter covers an economic assessment of the acidogenic fermentation towards volatile fatty acid production using organic residuals solids (ORS). All calculations and assumptions are included in the chapter: it is not necessary to refer to the methodology chapter to understand the results and discussion from this economic assessment. In order to include capital costs, a selection of equipment and equipment sizing has been included in this study.

6.1 Introduction

The typical configuration in wastewater treatment plants is: preliminary treatment, primary settlement, aeration, secondary settlement and disinfection. Primary and secondary settlement stages produce waste raw sludges which are normally sent to anaerobic digestion (AD). Like the other stages of the process, AD is typically carried out in continuous mode. Normally, AD takes place in large gas-tight tanks, with fixed or floating roof tops. Different mixing strategies within the digester have been used for AD in wastewater and other applications. In this work, a tank with a fixed cone-shaped top is suggested for the acidogenic fermentation of ORS (see Figure 68). The ORS feedstock and the recirculated digestate (fermented mixture) are transported using screw pumps (positive displacement) which also help with mixing and breaking down flocs. Screw pumps are appropriate for sludge-like 'fluids' and therefore suitable for this application. The digestate inside the fermenter is recirculated from the bottom of the fermenter, pumped through an external heat exchanger, and returned to the fermenter through the top. This serves two purposes: maintaining design temperature and providing turbulence for appropriate mixing inside the fermenter. This type of mixing configuration is known as 'jet mixing'. The chosen heat exchanger is a concentrical double pipe heat exchanger using hot water as heating agent. This heat exchanger configuration is common in wastewater treatment plants. It has been assumed that both the feedstock and the recirculated digestate are mixed before entering the heat exchanger, although optimum design should include a form of heat recovery (which has not been included in this work). The feedstock is first prepared in a mixer where ORS, water and enzyme solution are added and mixed. In a detailed design, a dosing pump and solid conveyor would be necessary, but these were ignored as they are considered to constitute a small proportion of the capital cost. An additional pump (discharge pump) is necessary to pump digestate from fermenter to the next treatment unit. The requirements of this pump are assumed to be the same as the feed pump, as roughly the same flowrate would be required to maintain a mass balance. In a

detailed design, friction losses through the pipework and other rheological properties should be considered for accurate size and duty pump selection. In this study, capital costs of solid separation and VFA purification have not been included.

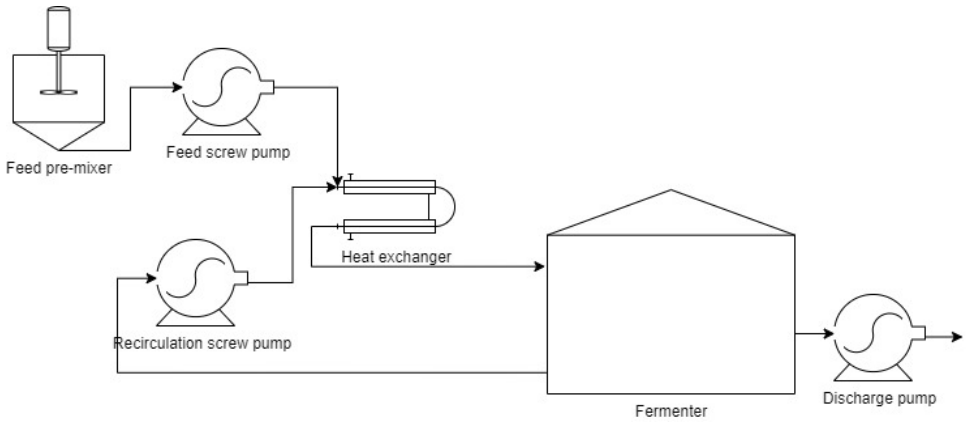

Figure 68. Large-scale process configuration flow diagram for the AF of ORS

6.2 Methodology

6.2.1 Equipment sizing

Table 20 shows the input data assumed for the design of the different process units. The data is a close round estimate based on of previously obtained results from the experimental work carried out in this thesis.

Table 20. Process input data

Variable	**Value**	**Units**
ORS flowrate (F_{ORS})	18,000	tonne $year^{-1}$
ORS total solids (TS_{ORS})	30% (w/w)	-
ORS VS/TS	70% (w/w)	-
ORS density (ρ_{ORS})	1	tonne m^{-3}
Total solids reduction	15%	-
NaOH load	0.7	kg m^{-3} feed
Hydraulic retention time (HRT)	5	day
Feedstock total solids (TS_{feed})	10% (w/w)	-
Enzyme loading	2%	g g^{-1} TS

Enzyme VS (VS_{enz})	42% (w/w)	-

The working volume of the fermenter was calculated using the HRT from Table 20 and the feed flowrate (F_{feed}):

$$F_{feed}\left(\frac{m^3}{day}\right) = \frac{F_{ORS}\left(\frac{t}{year}\right) \cdot TS_{ORS}}{\rho_{ORS}\left(\frac{t}{m^3}\right) \cdot TS_{feed} \cdot 365\left(\frac{day}{year}\right)} \quad \text{Equation 6.1}$$

$$Fermenter\ working\ volume\ (m^3) = F_{feed}\left(\frac{m^3}{day}\right) \cdot HRT\ (day) \quad \text{Equation 6.2}$$

To calculate the total volume of the fermenter (V), an extra 5% volume was added for headspace necessary for small fluctuations in volume and possible foam formation. A height to diameter ratio (H/D) of 0.8 was chosen following the rule of thumb for the design of tanks. To simplify, the diameter was calculated assuming a cylindrical shape as follows:

$$V\ (m^3) = \pi\left(\frac{D}{2}\right)^2 H \quad \text{Equation 6.3}$$

$$V\ (m^3) = \pi\left(\frac{D}{2}\right)^2 0.8D \quad \text{Equation 6.4}$$

Isolating D from equation 4, D was calculated using Equation 6.5:

$$D\ (m) = \left(\frac{4V}{0.8\pi}\right)^{1/3} \quad \text{Equation 6.5}$$

The general equation for heat transfer in a heat exchanger is as follows:

$$Q = UA\Delta T_{lm} \quad \text{Equation 6.6}$$

Where:

U = heat transfer coefficient (W m-2 K-1)

A = area of heat exchange (m2)

ΔT_{lm} = logarithmic mean temperature difference (K)

However, the area of the heat exchanger is unknown, therefore, to estimate the heat exchanger dimensions, both the heat loss in the fermenter and the heat necessary to heat up the feedstock were calculated. The assumptions made for these calculations are summarised in Table 21. Convection coefficients are dependent on a number of variables including physical properties of the fluid (viscosity, density, temperature, conductivity, heat capacity, etc.) as well as flow properties (velocity and turbulent/laminar flow) [292]. These variables should be considered in a detailed design. For the purpose of equipment sizing for capital cost estimation, reasonable

values within ranges found in the literature have been selected for the convection and conductivity coefficients.

Table 21. Heat exchange parameters

Variable	**Value**	**Units**	**Ref.**
Water convection coefficient (h_{water})	3000	W m^{-2} K^{-1}	[293, 294]
Air convection coefficient (h_{air})	35	W m^{-2} K^{-1}	[293, 294]
Wall thickness (L_{wall})	10	mm	-
Insulation thickness ($L_{insulation}$)	13	mm	-
Stainless steel conductivity coefficient (λ_{steel})	14.4	W m^{-1} K^{-1}	[295]
Vacuum Insulation panels conductivity coefficient ($\lambda_{insulation}$)	0.005	W m^{-1} K^{-1}	[296]
Temperature of reactor ($T_{reactor}$)	35	°C	-
Temperature of air (T_{air})*	-10	°C	-
Soil conductivity coefficient (λ_{soil}) - clay loam at 18% moisture content	0.6	W m^{-1} K^{-1}	[297]
Soil temperature (T_{soil})	5	°C	-
Feedstock temperature (T_f)	5	°C	-
Feedstock heat capacity (C_{feed})**	4200	J kg^{-1} K^{-1}	-
Maximum hot water temperature ($T_{hotwater}$)	75	°C	-
Return water temperature ($T_{outwater}$)	60	°C	-
Digestate max temperature drop ($\Delta T_{digestate}$)	3	°C	-

* Assuming worst case scenario for temperature as wind speed is not included in calculations

** Assuming same as water heat capacity

The heat transfer coefficient for the tank walls was then calculated using Equation 6.7 [298] and appropriate unit conversions:

$$\frac{1}{U} = \frac{1}{h_{water}} + \frac{L_{wall}}{\lambda_{steel}} + \frac{L_{insulation}}{\lambda_{insulation}} + \frac{1}{h_{air}}$$ Equation 6.7

Where:

h = convection coefficient of fluid (W m^{-2} K^{-1})

L = thickness of solid material (m)

λ = Conductivity coefficient (W m^{-1} K^{-1})

The heat loss via the fermenters' walls was obtained as follows:

$$Q_{wall} = U_{wall}\, A_{wall} \left((T_{reactor}) - (T_{air})\right) \quad \text{Equation 6.8}$$

The heat transfer coefficient for the tank floor and the heat loss via the floor were calculated using Equation 6.9 and Equation 6.10:

$$\frac{1}{U_{bottom}} = \frac{L_{wall}}{\lambda_{steel}} + \frac{L_{soil}^{*}}{\lambda_{soil}} \quad \text{Equation 6.9}$$

$$Q_{bottom} = U_{bottom}\, A_{bottom} \left((T_{reactor}) - (T_{soil})\right) \quad \text{Equation 6.10}$$

*L_{soil} was assumed one meter, beyond which conductivity effects are considered negligible.

Heat required to heat up the feedstock was calculated using Equation 6.11:

$$Q_{feed} = F_{feed}\rho_{feed}C_{feed} \left((T_{reactor}) - (T_{feed})\right) \quad \text{Equation 6.11}$$

Where ρ_{feed} was assumed one tonne m^{-3}.

Total heat requirement was therefore calculated as the sum of all the heat requirements:

$$Q_{TOTAL} = Q_{wall} + Q_{bottom} + Q_{feed} \quad \text{Equation 6.12}$$

In wastewater treatment plants, the recirculated digestate of anaerobic digesters is typically heated in a concentric double pipe heat exchanger. In this thesis, the material chosen for this heat exchanger is stainless teel 316 which is corrosion and heat resistant. This type of heat exchanger requires a heating agent. For the operating temperatures in which the fermenter needs to be, water is the safest and most available heating agent. The water would be heated by a conventional boiler, with a set 'hot' temperature (75°C), then transferred to the heat exchanger, and returned to the boiler at set 'cold' temperature (60°C).

In the proposed design, the heat exchanger is used to heat up both the recirculated digestate and the fresh feedstock. The output temperature is the fermenter's set temperature (35°C). The resulting temperature ($T_{digestate}$) has been assumed to be proportional to the streams being mixed and their flowrates, according to Equation 6.13:

$$T_{digestate} = \frac{T_{feed}F_{feed} + T_{reactor}F_{recirc}}{F_{feed} + F_{recir}} \quad \text{Equation 6.13}$$

$T_{digestate}$ can be any value between 32°C and 35°C (as maximum $\Delta T_{digestate}$ is 3°C). Lower $T_{digestate}$ allows for lower F_{recirc}, translating in lower pumping requirements. Therefore, for $T_{digestate}$=32°C, the F_{recirc} is 15.4 kg s^{-1}. The F_{recirc} to F_{feed} is consequently 9, which seems reasonable value for good mixing inside the fermenter. In a detailed design, the nozzle design (number of nozzles, type and position) and determination of hydraulic properties would be required [299]. The logarithmic mean temperature difference in the heat exchanger was calculated using Equation 6.14 [298]:

$$\Delta T_{lm} = \frac{(T_{hotwater} - T_{reactor}) - (T_{outwater} - T_{sludge})}{\ln \frac{(T_{hotwater} - T_{reactor})}{(T_{outwater} - T_{sludge})}} \quad \text{Equation 6.14}$$

The overall U of the heat exchanger was obtained using Figure 69. It was assumed the heated fluid is an aqueous solution and the heating agent is boiling water (lower range). Once the value of U was obtained, the total area of the heat exchanger was calculated using Equation 6.6.

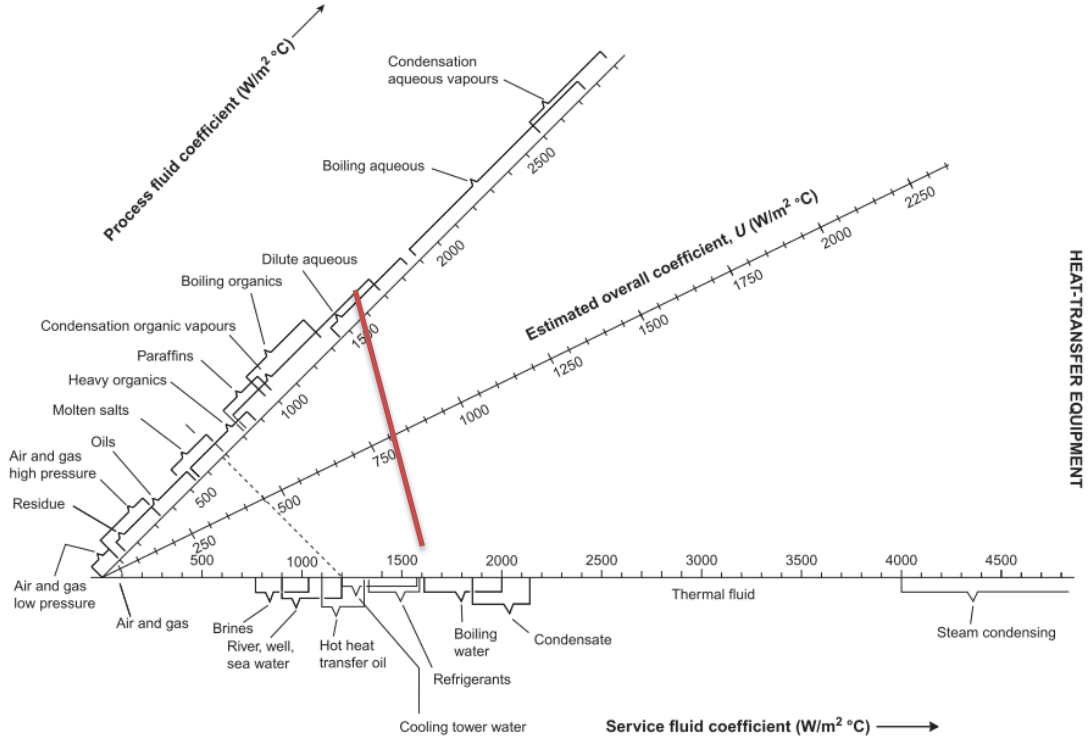

Figure 69. Graphic method for the calculation of overall heat transfer coefficient for different heat transfer equipment [292]

For the pre-mixer, it was assumed that a 0.5 h HRT is sufficient to assure good mixing of the ORS, water and enzymes. Using the fixed value F_{feed}, the volume of the pre-mixer (V_{pm}) was obtained using Equation 6.15 and appropriate unit conversions:

$$V_{pm} = \frac{F_f}{HRT} \quad \text{Equation 6.15}$$

The specific power input (SPI) estimated by US EPA as typical value to mix AD digestate ranges between 5-8 W m^{-3} [300]. As ORS at 10% TS concentration is relatively more viscous than AD digestate, a value of 10 W m^{-3} was assumed. Assuming 75% electrical conversion efficiency (ε), the power requirement for the pre-mixer (P_{pm}) was calculated according to Equation 6.16:

$$P_{pm} = \frac{SPI \cdot V_{pm}}{\varepsilon}$$ Equation 6.16

The specific work requirement (SWR) for the pumps was calculated based on the height of the fermenter (head) plus 10% addition to allow for pressure losses due to friction. A detailed design of the pipelines would be required to obtain a more accurate work requirement. The specific work was therefore calculated as follows:

$$SWR = g \cdot H \cdot 110\%$$ Equation 6.17

Where g is the acceleration due to gravity (9.8 m s^{-2}).

Based on the flowrates and assuming 75% efficiency, the power requirement for each pump was calculated as follows:

$$P_{pump} = \frac{SWR \cdot F}{\varepsilon}$$ Equation 6.18

Where F was F_{feed} for the feedstock and discharge pumps; and F_{recirc} for the recirculation pump.

6.2.2 Capital costs

The economic assessment presented in this chapter was carried out following the methodology described in *Biorefineries and Chemical Processes: Design, Integration and Sustainability Analysis* [301]. The capital cost of standard design equipment was obtained from different sources [302–304]. Matche.com provides with html software to calculate the cost of equipment based on its capacity or characteristic size parameter [303]. Coulson and Richardson's book contains the factors to calculate the cost of various equipment using the following equation [302]:

$$C_e = CS^n$$ Equation 6.19

Where:

C_e = purchased equipment cost

C = cost constant

S = c haracteristic size parameter

n = index for that type of equipment

The capital cost of equipment is subject to changes over time due to inflation and other economic factors. A present cost can be calculated using a past cost and the corresponding cost indexes [301, 302]:

$$C_f = C_o \left(\frac{I_f}{I_o}\right) \quad \text{Equation 6.20}$$

Where:

C_f = cost in final year

C_0= cost in initial year

I_f= cost index of final year

I_0= cost index of initial year

The cost indexes (Chemical Engineering Plant Cost Index, CEPCI) were obtained from chemengonline.com [305]. The most recent CEPCI value available (year 2020) is 596. This is the CEPCI value assumed for years 2020 and 2021 in this work.

The capital costs were calculated as the sum of direct capital costs (TDCC) and total indirect capital costs (TICC) plus a 12% contingency, see Table 22 for a detailed breakdown of the calculations. TDCC and TICC were estimated as the sum of established percentages of the total purchased equipment cost (TPEC) for each category [301]. TPEC was calculated based on worst case scenario, i.e. as the sum of all most expensive options.

Table 22. Calculation of total capital costs. Based on BAAQMD Mariposa Energy Project [306]

Installation costs	
Foundation & supports	8%*TPEC
Erection & handling	14%*TPEC
Electrical	4%*TPEC
Piping	2%*TPEC
Insulation	1%*TPEC
Painting	1%*TPEC
TOTAL DIRECT CAPITAL COSTS (TDCC)	**TPEC+Instalation costs**
Indirect capital costs	
Engineering and supervision	10%*TPEC
Construction and field expenses	5%*TPEC
Contractor fees	10%*TPEC
Start-up	2%*TPEC
Performance testing	1%*TPEC
TOTAL INDIRECT CAPITAL COSTS (TICC)	**Sum of all indirect capital costs**
TOTAL CAPITAL COSTS (TCC)	**TDCC+TICC+12%*(TDCC+TICC)**

6.2.3 Operating costs

The operating costs are comprised of the sum of total fixed operating costs (TFOC) and total variable operating costs (TVOC). TFOC were calculated as the sum of all fixed operating costs, for which each category was calculated differently, as shown in Table 23. Each variable operating cost was calculated using different assumption and values found in the literature. TVOC was calculated as the sum of all variable operating costs. Personnel operating costs (POC) was calculated following the rules of thumb proposed by Sadhukhan and Hernandez [301]. As they suggest, two people would be required for a continuous solid-liquid system. The salary proposed is for an average salary of chemical engineer in the US (2008). Although this is probably an overestimation, as operators do not require high skills, it is based on a worst-case scenario.

Table 23. Breakdown of total operating costs

Fixed operating costs	
Maintenance[1]	5%*TICC
Personnel (POC)[1]	(2 people)*40h/week*52week*40$/h
Laboratory costs[1]	20%*POC
Supervision[1]	20%*POC
Plant overheads[1]	50%*POC
Capital charges[1]	10%*TICC
Insurance[1]	1%*TICC
Local taxes[1]	2%*TICC
Royalties[1]	1%*TICC
TOTAL FIXED OPERATING COSTS (TFOC)	**Sum of all fixed operating costs**
Variable operating costs	
Landfill tax[2]	133.69$ $tonne^{-1}$
Feedstock	0$ $tonne^{-1}$ (as feedstock is generated on site)
Enzymes[3]	6.27$ kg $protein^{-1}$*VS_{enz}*TS_{ORS}*enzyme loading*F_{ORS}
NaOH	350$ $tonne^{-1}$*NaOH load*F_{feed}
Natural gas[2]	0.03$ kWh^{-1}*Q_{TOTAL}
Electricity for pumps and mixer[2]	0.2059$ kWh^{-1}*(sum of power requirements)
Water[2] (90% recycled)	6.106$ m^{-3}*(100%-TS_{ORS})*F_{feed}*10%
Purification cost[4]	3 kWh^{-1} kg VFA*0.2059$ kWh^{-1}*$F_{effluent}$*C_{VFA}
TOTAL VARIABLE OPERATING COSTS (TVOC)	**Sum of all variable operating costs (except landfill tax)***
DIRECT PRODUCTION COSTS (DPC)	**TFOC+TVOC**
TOTAL OPERATING COSTS	**1.2xDPC**

1. Based on Sadhukhan and Hernandez (2014) [301]

2. Based on UK prices – converted from GBP to USD 2021

3. Value of enzymes based on Liu et al. (2016) [144]

4. 3 kWh^{-1} kg VFA based on Veluswamy et al. (2021) [307]. Effluent flowrate and VFA concentration are 54,000m^3 $year^{-1}$ and 20 Kg m^{-3} respectively.

*Landfill tax would be charged for ORS regardless of AF treatment. AF treatment converts part of the ORS (total solids), introducing landfill tax savings. These savings are included as profit assuming 15% TS conversion.

6.2.4 EBITDA & Net Present value

Table 24 shows the prices of the main individual VFA and the earnings before interest, taxes, depreciation, and amortization (EBITDA) resulting from VFA sales. In this process, EBITDA is a result of the VFA sales and the landfill tax discount resulting from the solids destruction, i.e. a reduction in total solids sent to landfill. No other profits have been considered as CH_4 production should be minimised and avoided for maximised VFA production. Fertilizer has not been considered as products/profits, as it is unlikely that the remaining solids from unconverted ORS can be used as fertilizer. Additionally, no significant H_2 production was observed during the experimental work, so H_2 is not considered for profit calculations. The cashflow was calculated as the difference between EBITDA and TOC. A positive cashflow is a good indication of possible viability, but typically not a sufficient proof of viability as it does not include capital costs, devaluation, etc.

Table 24. Breakdown of assumed values for the calculation of EBITDA

VFA mixture price*	1.88	\$ kg^{-1}
VFA concentration in effluent (C_{VFA})	20	Kg m^{-3}
Effluent flowrate ($F_{effluent}$)	54,000	m^3 $year^{-1}$
VFA sales	= 1.88*20*54,000	
Landfill tax discount	=15%*133.69*18,000	
EBITDA	**= VFA sales + landfill tax discount**	
CASH FLOW	**= EBITDA – TOC**	

*Average of the three main acids (acetic, propionic and butyric) prices. Prices obtained from Table 1

A common index for assessment of economic viability is the net present value (NPV), calculated as follows:

$$NVP = \sum_{n=0}^{n=T_{PL}} \frac{CF}{(1+r)^n}$$ Equation 6.21

Where:

CF = Cash flow

TPL = plant life

r = discount rate

The capital investment was assumed to be distributed over year -1 and 0, at 40% and 60% of the TCC, respectively. The discount rate was assumed as 15% and the plant life as 25 years.

6.3 Results and discussion

The calculated dimensions of the fermenter are summarised in Table 25 and the dimensions and requirements of the heat exchanger are summarised in Table 26. The pumps and mixer requirements are shown in Table 27.

Table 25. Fermenter's dimensions for a AF process treating 18,000 tonne ORS year^{-1}

Variable	**Value**	**Units**
Working volume	740	m^3
Headspace	37	m^3
Total volume*	777	m^3
Diameter	10.73	m
Total height*	8.59	m
Headspace height	0.41	m
Area of cylinder walls (A_{wall})	289	m^2
Area of bottom (A_{bottom})	90	m^2

*of cylindrical shape, not including volume or height of cone roof

Table 26. Heat exchanger's dimensions and requirements

Variable	**Value**	**Units**
U_{wall}	0.38	W m^{-2} K^{-1}
Q_{wall}	4,954	W
U_{bottom}	0.60	W m^{-2} K^{-1}
Q_{bottom}	1,628	W
Q_{feed}	215,753	W
Q_{TOTAL}	247,704	W
ΔT_{lm}	33.64°	°C
$U_{heat\text{-}exchanger}$	800	W m^{-2} K^{-1}
$A_{heat\text{-}exchanger}$	8.3	m^2

Table 27. Pumps and mixer requirements

Variable	**Value**	**Units**
Volume of the pre-mixer (V_{pm})	3.08	m^3
Pre-mixer power requirements	41	W
SWR for pumps	92.55	J kg^{-1}
Recirculation pump power requirements	1902	W
Feedstock pump power requirements	211	W
Discharge pump power requirements	211	W

Based on these results, the most significant energy requirement of the fermentation stage is for heating, representing 99% of the total energy requirement. It is therefore paramount to integrate and optimise the heating resources within the process. In this study, using effective vacuum insulation, most of the heat demand is due to the low temperature of the feed. A conservative approach was followed when choosing feedstock temperature. If the water obtained after VFA recovery is recirculated and reused in the feedstock preparation, the temperature of the feedstock will likely be higher than 5°C. The ORS is also likely to be at warmer temperature from previous washing process, since water utilised for this purpose is obtained from a mesophilic anaerobic digestion system. For example, if feedstock temperature can be maintained at 15°C it would result in 32% natural gas savings. Based on these estimations, it can be said that there is potential for heat integration within the process, however, the process heat data was not readily available, therefore the integration has not been considered.

The price of each piece of equipment was calculated using different methods from different sources. The results are summarised in Table 28. As expected, due to its large volume, the most expensive item is the fermenter tank, followed by the pre-mixer. Although a detailed design would be required to have an accurate estimation of the equipment cost, these estimations are generally accepted in the early stages of projects.

Table 28. Equipment prices using different methods from various references

Equipment/ Reference	**Reference 1** [302]*****	**Reference 2** [304]*****	**Reference 3 (Matches, 2014)**
Cone roof tank	243,788$ (SS)	-	321,385$ (API, shop, SS 304)
Heat exchanger	26,847$ (SS-shell and SS-tube) **	22,142$ (Double pipe)	35,905$ (Double pipe, large, SS 316)
Recirculation pump	-	10,886$ (Single stage centrifugal)	49,874$ (Progressive cavity, medium, SS, mechanical seal)
Feedstock & discharge pump	-	(2x) 8,454$ (Single stage centrifugal)	(2x) 23,799$ (Progressive cavity, medium, SS, mechanical seal)
Pre-mixer	-	142,461$ (Jacketed, agitated, SS 304)	122,408$ (Reactor, fermenter, SS 316)

SS= stainless steel

*Calculated using equation 16

** Calculated using graphical method in Sinnott RK (2010) [302]

Total capital costs are summarised in Table 32, for a calculated TPEC of 597,221$. It must be noted that TPEC is dependent on the mass of ORS treated, which in this work has been assumed to be 18,000 tonne year^{-1}. The profitability of the process discussed in this chapter is therefore dependent on this assumption.

Table 29. Installation costs, indirect capital costs and total capital costs in US$

TPEC	597,221
Instalation costs	
Foundation & supports	47,778
Erection & handling	83,611
Electrical	23,889
Piping	11,944
Insulation	5,972
Painting	5,972
TOTAL DIRECT CAPITAL COSTS (TDCC)	**776,387**
Indirect capital costs	
Engineering and supervision	59,722
Construction and field expenses	29,861
contractor fees	59,722
start-up	11,944
performance testing	5,972
TOTAL INDIRECT CAPITAL COSTS (TICC)	**167,222**
TOTAL CAPITAL COSTS (12% contingency)	**1,056,842**

Table 30 shows the results for fixed operating costs, variable operating costs and total operating costs. The most significant fixed operating cost is represented by personnel. Personnel cost can highly vary depending on location and qualifications. A conservative approach was followed in this study by assuming the two required workers are chemical/process engineers in the USA.

Most of the variable operating cost is due to cost of enzymes (26%) and the cost of VFA purification (62%). Other authors previously reported that the VFA purification stage is the most expensive part of the process [18, 169], followed closely by pretreatment [17, 308]. It is important to highlight that the price of chemicals is highly dependent on market fluctuations and specific agreements with suppliers. As the demand for enzymes is growing due to the expansion of biotechnological processes, particularly those linked to biomass conversion to biofuels, enzyme prices are expected to drop. Further optimisation studies might also help reduce the amount of enzyme mixture required, to some extent. In addition, enzyme prices reported in other references are significantly lower [301] than the value assumed here.

Table 30. Breakdown of total operating costs

Fixed operating costs	**$ year^{-1}**	**% of TFOC**
Maintenance	8,361	2.4%
Personnel (POC)	166,400	47.8%
Laboratory costs	33,280	9.6%
Supervision	33,280	9.6%
Plant overheads	83,200	23.9%
Capital charges	16,722	4.8%
Insurance	1,672	0.5%
Local taxes	3,344	1.0%
Royalties	1,672	0.5%
TOTAL FIXED OPERATING COSTS (TFOC)	**347,932**	
Variable operating costs		**% of TVOC**
Landfill tax	2,406,474*	N/A
Feedstock	0.00	0%
Enzymes	284,407	26.3%
NaOH	13,230	1.2%
Natural gas	82,970	7.7%
Electricity	4,267	0.4%
Water	29,675	2.7%
Purification cost	667,116	61.7%
TOTAL VARIABLE OPERATING COSTS (TVOC)	**1,081,665**	
DIRECT PRODUCTION COSTS (DPC)	**1,429,597**	
TOTAL OPERATING COSTS	**1,715,517**	

*Landfill tax not included in TVOC as the fee is applicable to the Fiberight process without including the herein proposed AF of ORS

Table 31 summarises the results from EBITDA and cashflow calculations. The total earnings (or EBITDA) are higher than the total operating costs, presenting a promising result. However, this

difference (cash flow) is a good indication of viability, but typically not a sufficient proof of viability as it does not include capital costs, devaluation, etc.

Table 31. VFA sales, landfill discount, EBITDA and cashflow values

VFA sales	2,030,400	$ year^{-1}
Landfill tax discount	360,971	$ year^{-1}
EBITDA	**2,391,371**	**$ year^{-1}**
CASH FLOW	**675,854**	**$ year^{-1}**

The calculated NPV is plotted in Figure 70. At year 0, the process starts in negative values below minus 1M$. Between year 0 and 2, the process runs solely for the purpose of recovering the initial capital investment. From year 2, the process becomes profitable and by the end of the plant life, the NPV is about 3.3M$. As the original capital investment was approximately 1M$, it can be said, in simple words, that this process turns 1M$ into 4.3M$ over 25 years. Although the capital cost is significant (776,387$ direct capital cost) there is potential for savings. For example, if the fermentation was optimised for a shorter retention time, the volume of the fermenter (most expensive piece of equipment) could be reduced.

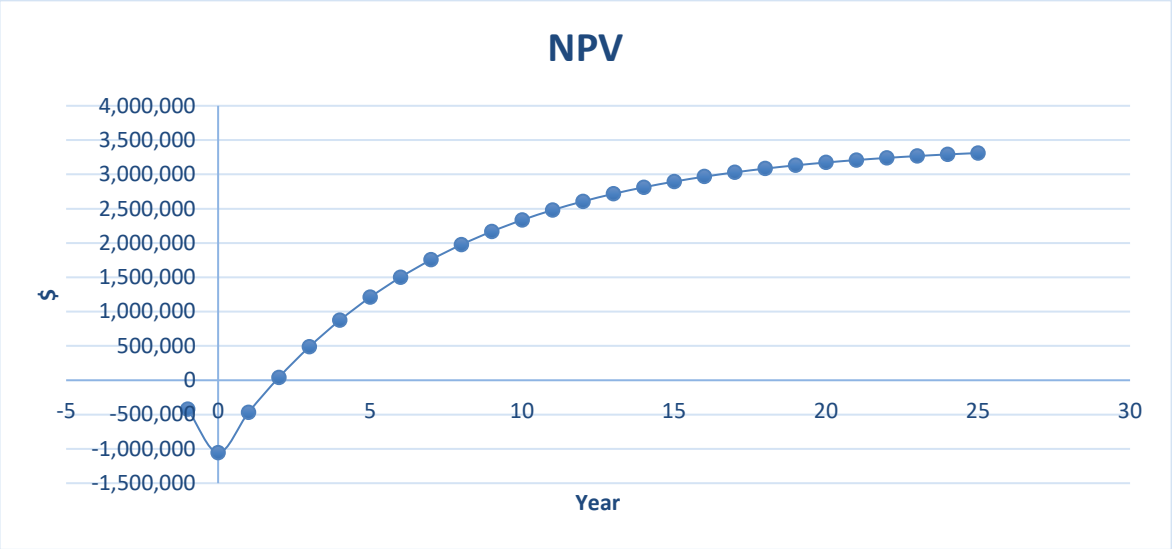

Figure 70. Net present value for an AF process treating 18,000 tonnes ORS year^{-1}

The process proposed in this thesis, however, does present some limitations. Prior VFA purification, a solid-liquid separation system is required (e.g. clarification and filtration). In this economic assessment, solid-liquid separation has not been considered, as it is assumed to be a small proportion of the total purification cost.

On the other hand, the calculated cost of mixed VFA purification, which was based on conventional electrodialysis proposed by Veluswamy et al. (2021) [307] for VFA concentrations of 0.1-1 mol L^{-1} (0.1-88 g L^{-1}), is 12.35 \$ m^{-3} of effluent for a VFA concentration of 20 g L^{-1} (~30 g COD L^{-1}). This is lower than the value proposed by Bonk et al (2015), who calculated that, to achieve profitability using OFMSW as substrate, the maximum value of purification should be 15 \$ m^{-3} of effluent [31]. Using the value proposed by Bonk et al., the purification cost in this process would be 810,000 \$ $year^{-1}$, resulting in an 2.2M\$ NPV after 25 years. This suggests that degree of feasibility is highly dependent on the cost of purification assumed. It should also be noted that electrodyalisis technology for VFA recovery is a membrane separation method. Membranes are prone to fouling and, therefore, require replacements. This could result in higher operational costs, which have not been considered in this thesis as data of membrane fouling for this application is not available.

Additionally, the price of a VFA mixture product is unknown due to the lack of market data, and the value of it was assumed as the average price of the main acids. If the price of the VFA mixture assumed here is reduced to 1.4\$ kg^{-1} VFA, the feasibility of the process is lost resulting in negative NPV across the 25 year life of the plant, see Figure 71.

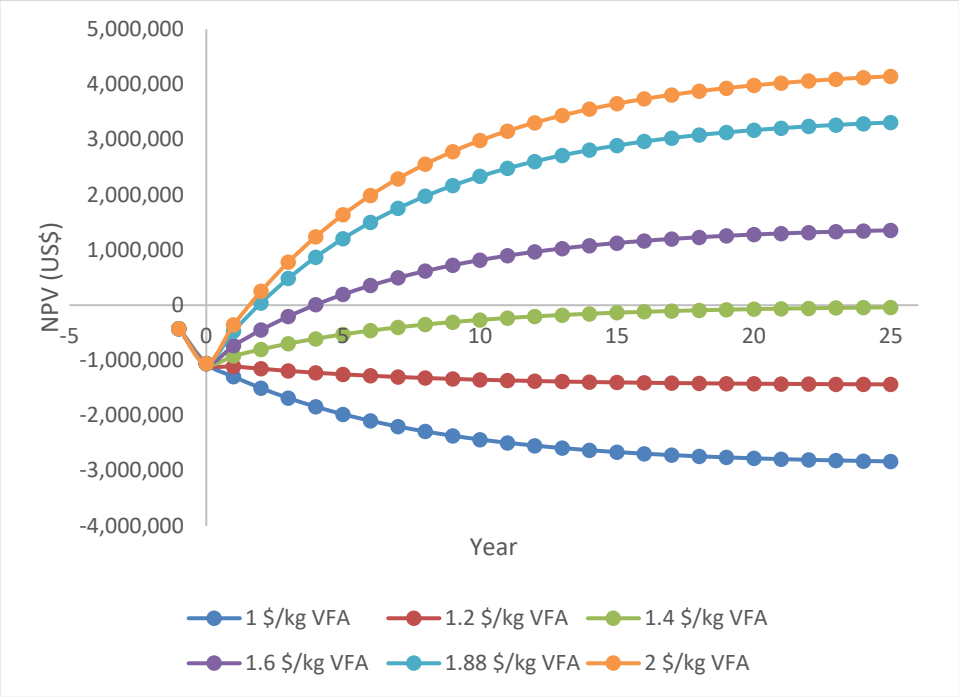

Figure 71. Sensitivity analysis of NPV for different mixed VFA prices of an AF process treating 18,000 tonnes ORS $year^{-1}$

6.4 Conclusions

VFA production from wastes might be economically advantageous in comparison to biogas production [26]. This is primarily due to the fact that current VFA market values and those of VFA derivatives are higher than that of methane [23]. However, economic studies looking at all aspects of VFA production are scarce [31, 307]. Large scale VFA extraction from dirty fermentation systems is still in its early stages, therefore, there is limited information on process performance and capital/operating costs. The economic viability of VFA production from wastes, particularly those derived from MSW, is therefore not fully demonstrated.

In this thesis, an economic assessment of the acidogenic fermentation of ORS derived from MSW determined that biological VFA production has economic potential towards viability under current market values and assumptions made. Significant reductions in feedstock pretreatment and VFA recovery costs can still be made in order to improve economic attractiveness, as these represent 26 and 62% of the total variable costs, respectively. For this reason, future research should focus on optimisation of these two stages of the AF process. Recent pilot scale research demonstrated that VFA recovery via electrodialysis is effective in obtaining a separate VFA-rich solution from the fermentation broth and can significantly improve VFA yields [309]. However, considerable improvement of the recovery process is required to obtain higher VFA concentrations in the final product.

In the fermentation stage, significant energy demand is required to reach mesophilic temperatures. However, efficient heat integration could result in significant energy savings. If all the heating required was internally generated, it would result in 5.8% savings in DPC. Future AF research should focus on improving AF under psychrophilic temperatures in order to make the process more energy-efficient.

Although the economic drive of the AF process could be improved, it is important to highlight the original motivations for bio-based VFA production from wastes: reduction in CO_2 emissions, reduction in waste volume sent to landfill, and providing a cost-effective alternative to petroleum-derived VFA. These might become income streams, depending on government policies, in form of incentives, such is the case of bio-methane processes. Ultimately, the feasibility of bio-based VFA will depend on policies, VFA market value and the desire of consumers to purchase 'environmentally friendly' products.

Chapter 7 Conclusions

This chapter discusses the main findings of this thesis; examines its contribution to the field of acidogenic fermentation and to society; and proposes the best line of future work.

7.1 Key findings and limitations

In this thesis, a series of bench scale experiments were carried out to elucidate the effect of different fermentation conditions.

The results show that AF of ORS was possible without any pretreatment. Higher TS concentration led to higher VFA concentrations, as a higher concentration of feedstock was available; however TS% did not have a significant influence on VFA yields.

As demonstrated, in batch AF, removing fermentation broth could be used as strategy to control the VFA profile. This is because removing the liquid fraction and replacing it with fresh water helps to restore pH. Effectively, this system simulates a batch system with a VFA/soluble components recovery system, which suggests in situ VFA recovery system and its interactions with AF should be investigated. High removal rates (equivalent to HRT of 3.6 days), however, had a detrimental effect on DoA due to by-product formation. The results also showed that in batch AF, the limiting factor was hydrolysis and not product inhibition.

ORS high lignin content also suggested that ORS needed to be subjected to pretreatment for a more efficient AF. In this thesis, the use of exogenous enzymes contributed to the improvement of the ORS biodegradability and their conversion to VFA. A mixture of 81% cellulase/beta-glucosidase/hemicellulase and 19% amylase was identified by the model as the optimum recipe to treat ORS. Enzyme loading had the most significant effect on enzymatic pretreatment performance, followed by incubation time. The optimum conditions of 7.5-10% TS, 2% enzyme loading and 24 hours of hydrolysis led to final hydrolysis yield of 360 mg sCOD g^{-1} VS. The experimental yield under these conditions, however, was 330 mg COD g^{-1} VS, due to the generation of CO_2 and other by-products. Despite the improvement in solubility achieved by the use of this enzyme mixture, the investigation of other forms of pretreatment, in addition to the enzymatic hydrolysis, is recommended to further increase yields and reduce solid waste.

Even with short incubation time of 24 hours, enzymatic pretreatment at 50°C led to growth of the microbial population introduced by the ORS, which, presumably, contains mostly lactic acid

producers. This resulted in a significant pH drop attributed to high lactic acid concentrations which is likely to hinder VFA production.

Alkaline addition after pretreatment to maintain a 'healthy' pH above 5.5 can result in improved VFA yields. Pretreatment in combination with alkaline addition resulted in 128% increase in VFA concentrations, but a minimum of 3 days lag phase had to be overcome to observe a significant difference compared to control replicates. Maximum concentration was reached after 6 days. Results confirmed that conversion of lactic acid to butyric acid took place under slightly acidic pH values. In contrast, butyric to acetic conversion was promoted under neutral pH.

Urea addition and effects of pH on AF were also studied. The optimum pH range for ammonia release from urea by AD species was between 6 and 8. For that reason, the use of urea as buffering agent after enzymatic pretreatment was considered an ineffective strategy due to the low pH resulting from undesired lactic acid production. In contrast, when AF is undertaken within optimum urea hydrolysis pH range, ammonia (or urea) is a better buffering agent compared to NaOH, leading to a more stable pH and higher VFA yields. Maximum ammonia release also takes a minimum of 3-4 days. Therefore, urea should only be used when fermentation conditions have been optimised around neutral pH (6-8) and with a minimum retention time of 3-4 days.

Urea addition was used to modify the C/N ratio. It was found that C/N ratio had a significant effect on VFA profile. Decreasing the C/N ratio from 40 to 10 by urea addition resulted in yield increase and a switch in VFA profile. A C/N of 10 for untreated ORS resulted in a mix of predominantly acetic and propionic acids, whereas acetic and butyric were the predominant VFA for simultaneous hydrolysis and fermentation, i.e. when enzymes were added at practically the same time of the inoculation. This difference is partly explained by lower pH reached at higher hydrolysis rate (i.e. higher acidification), which promotes butyric acid production.

The literature suggested that subjecting the inoculum to thermal pretreatment would kill methanogens and the acidogens would form spores which would be re-activated during fermentation. In fact, this work demonstrated that this strategy was detrimental for VFA production from ORS. It was found that VFA production was low, probably due to AD species being inactive and microbial population present in the ORS thriving, resulting in low DoA (i.e. high by-product formation). A microbial population analysis would be necessary to understand this phenomenon. The literature also suggested that using an adapted inoculum could result in improved VFA yield as a means of improved methanogenic activity inhibition and hydrolysis rates. In contrast to this suggestion, when using 'ORS-adapted' inoculum; the adapted inoculum did not perform better or worse than the original AD inoculum in terms of VFA yield, hydrolysis rate or DoA. Methanogenic activity was inhibited in both cases due to other fermentation conditions (e.g.

low pH). It was also found that similar VFA profile was developed despite using different inoculum types, confirming that VFA profile is highly influenced by substrate composition, as suggested in the literature. These results confirm the significant influence of substrate composition and fermentation conditions on the performance of AF, including methanogenic inhibition.

In this thesis, it was demonstrated that S/I ratio had a significant effect on VFA yield and DoA. A S/I ratio of 5 g TS g^{-1} TS at retention times shorter than 6 days led to high yields due to high DoA. In contrast, the same S/I ratio was not effective in inhibiting methanogenic activity at longer retention times (12 days), which resulted in VFA degradation.

In the literature, $CaCO_3$ has been used to increase the buffering capacity of AF and AD systems. In the AF of ORS, differences in alkalinity, modified by NaOH and $CaCO_3$, did not have a significant effect on VFA yields at the same pH levels. Although overall yields were not affected, the presence of $CaCO_3$ resulted in an increase in hexanoic acid production at pH 7. $CaCO_3$ addition did not help to stabilise pH under alkaline pH values, with similar quantities of NaOH needed to maintain desired pH values for replicates both with and without $CaCO_3$. Highly alkaline pH (pH 10) has been proven beneficial for the AF of different substrates in the literature, however, this high pH can have adverse effects when relying on biological hydrolytic agents to reach maximum VFA yields. In this case, pH 10 resulted in low ORS solubilisation in SHF, presumably due to enzymatic activity being inhibited, resulting in much lower yields than pH 8.5 or 7. Neutral (pH 7) and slightly alkaline (pH 8.5) led to a switch from butyric acid to acetic and propionic. It was concluded that, although $CaCO_3$ can be used as buffering agent in slightly acidic conditions, it would not help maintain alkaline pH values. Careful consideration of benefits and impacts must be taken when designing the AF process, as the addition of chemicals, even those widely available such as $CaCO_3$, can increase production costs.

In the CSTR experiments, it was found that the optimum OLR and HRT for 75% TS feed of ORS is 8-9.5 g VS L^{-1} day^{-1} and 4-5 days respectively. Under these conditions, a maximum VFA yield of 0.25 g COD g^{-1} VS was achieved. At lower OLR, methanogenic activity was not successfully inhibited and therefore VFA were consumed, resulting in lower yields. This OLR is only recommended for wastes that have been pretreated or when additional enzymes are added. It is likely that higher OLR would give an increase in VFA yields; however, a minimum of 2 days HRT should be maintained for the enzymes to offer maximum performance and a minimum of 4 days is required to obtain maximum VFA concentrations. At shorter HRT more reactor instability is observed, therefore, higher OLR should be achieved by increasing the concentration of the ORS feed. OLR also affected by-product formation.

Fed-batch mode proved to be a good strategy to reach higher VFA concentrations from ORS, achieving a maximum value of 44 g COD L^{-1} (22 g VFA L^{-1}). Fed-batch mode was also beneficial with regards to yield giving a maximum of 0.43 g COD g^{-1} VS (0.19 g VFA g^{-1} VS), which was considerably a higher yield compared to batch and semi-continuous experiments. The explanation for higher yield was partly the increased feedstock concentration, as observed earlier in batch modes. Another reason for the higher yield (higher COD per g of VS) is due to the formation of a larger amount of hexanoic which has a higher COD value. However, the formation of longer chain VFA does not account for all the yield improvement. The increased proportion of hexanoic acid indicated that reactor mode can have a significant impact on the type of VFA produced. It is therefore possible that fed-batch promotes chain elongation for the production of longer chain fatty acids. Fed batch was also a very successful strategy to inhibit methanogenic activity due to high VFA concentrations despite long retention times (>30 days). The feeding mode had a significant effect on by-product formation and, therefore, it should be optimised accordingly to maximise the target product, with lower feeding rates leading to higher DoA.

A sizing of the equipment needed for a large-scale AF process was carried out. This allowed an economic assessment which included capital costs. Based on the net present value (NPV), the AF of ORS using enzymes would turn profitable after 3 years. The final NPV would be 4.3 million USD after 25 years. The assessment also highlighted that future research should focus on reducing the cost of pretreatment and VFA recovery to improve the profitability of bio-VFA processes.

7.2 Scientific contribution

An extensive literature review identified the knowledge gaps on the field of mixed culture acidogenic fermentation. A data comparison across the literature was possible by converting the published results to the same units. This helped shape the rest of the work and influenced decision making in experimental design, in addition to providing guidance to future researchers.

Multiple substrates have been reported in the literature for VFA production. In this thesis, a novel substrate, the ORS, derived from a real industrial MSW treatment, was studied and showed a great potential for VFA production. The complex composition of this substrate equates to studies focusing on food waste or the organic fraction of MSW.

A model was created to estimate the optimum conditions for enzymatic pretreatment of ORS. Although this model is only applicable for the ORS feedstock and the studied enzymes, the same methodology could be followed for other feedstocks/enzymes.

A detailed systematic study where experiments were carefully designed to consider interacting variables, where possible, was carried out in batch mode. Although considerable amount of work was done on AF from different wastes, it is not always clear why some conditions were chosen as optimum or preferred. Results from the batch experiments provided further insight on the effect of different variables such as pH, TS, C/N ratio on the AF in terms of yields, by-product formation, VFA profile. Providing guidance and considerations for future work. The batch experiments demonstrated that some of the strategies proposed in the literature to improve VFA production are substrate dependent; and that fermentations conditions should always be studied in conjunction, and not as independent variables.

Due to the time-consuming nature of experimental work on semi-continuous processes, data on the effect of different continuous variables, such as HRT or OLR, are less readily available in the literature. This thesis provided a further understanding on the effect of HRT, pH, OLR, and methanogenic inhibition on AF of complex substrates.

An innovative approach for increased VFA concentration and yield improvement was also tested by following a fed-batch mode feeding system. It was suggested that a fed-batch system could be beneficial for VFA recovery.

An economic assessment of a large scale continuous acidogenic fermentation process demonstrated the economic potential of bio-VFA generation from ORS. This further supports the argument of economic drive of VFA generation.

Apart from the scientific contributions, this thesis, particularly Chapter 6, could be used as a guidance/proposal to expand the Fiberight process, which would result in improved profitability and availability of a wider range of products. The introduction of the AF process to the Fiberight process would reduce its environmental impact and help with the creation of new jobs.

Despite the significant advances achieved, the integration of the AF with the product recovery is necessary to make this technology work. In addition, not all VFA recovery methods have been fully explored. For that reason, future research work, which includes the integration of AF with a novel VFA recovery process, has been proposed in the following section.

Part of this work was published in “Current perspectives on acidogenic fermentation to produce volatile fatty acids from waste” (2021, doi:10.1007/s11157-021-09566-0). The work carried out in this thesis was presented at several events, the most relevant of which are shown in Appendix F.

Chapter 8 Future work

This chapter presents the proposed future work on an experimental proof of concept testing the integration of a VFA recovery stage with the fermentation stage.

8.1.1 Introduction

The integration of the VFA recovery step with the fermentation step would aid the transition from proof-of-concept stage to commercialisation. Some research has been carried out for the *ex situ* recovery of carboxylic acids from fermentation broths [310–312]. *In situ* recovery, i.e. directly from the fermenter, is often discarded as an option, since the acids need to be in their protonated form, hence requiring acidification of the broth.

Gas stripping with N_2 has shown potential for in situ VFA recovery [239]. The main advantages are: it does not require a membrane, which often leads to membrane fouling; the extractant used (in this case, stripping gas) can be easily recovered, and is non-toxic, non-flammable, widely available and non-polluting (therefore, sustainable). Other advantages of nitrogen gas stripping include: low energy process and, presumably, the ability to operate with high solid concentrations, as gas can passed through porous solids and solid-liquid mixtures.

Other aspects preventing the commercialisation of the AF process are related to pH control. Alkaline or slightly acidic pH can inhibit methanogenic activity, therefore, preventing the metabolisation of VFA into methane. Maintaining alkaline pH requires the addition of an alkaline substance, thus increasing the process cost. For example, to increase the pH from 4.8 to 5.5 in a fermentation broth that contains 0.3 mol L^{-1} of VFA (typical values), 0.1 mol L^{-1} of NaOH is required (see Appendix E for calculations).

In contrast, acidic pH can be achieved by high concentrations of VFA, which can be achieved by using highly concentrated feedstock. At pH<4.8 (average pKa value of C2-C4 VFA), acidogenic bacteria can become inhibited, as the acids in their free form become toxic. Removing the acids *in situ* as they accumulate would help maintain a 'safe' pH and avoid product inhibition [313], while allowing the system to run in the preferred mode of continuous operation.

The proposed study will test a system of acidogenic fermentation with integrated gas stripping recovery. The aim is to demonstrate the technical feasibility of the system for waste substrate.

8.1.2 Gas stripping for in situ recovery

To the best of the author's knowledge, only one study has used gas stripping [239] for VFA recovery; however, glucose was used as substrate. Therefore, the study of the interactions of the gas stripping process with AF using a complex substrate should be studied next. To prove the feasibility of gas stripping for VFA recovery using heterogeneous substrates, the use of solid wastes such as ORS, OFMSW or food waste is proposed herein. Figure 72 shows a schematic of the experimental rig that would be used in this proposed study. The acidogenic fermentation would be carried out in an air-tight reactor with continuous stirring. Nitrogen gas would be injected into the headspace until desired pressure is reached. The stripping gas is then pumped from the headspace of the fermenter to the recovery vessel where it is submerged in a $CaCO_3$ slurry under continuous stirring. A pipe/tube will also connect the headspace in the recovery vessel with the bottom of fermenter for the gas to recirculate and bubble through the solid-liquid fraction.

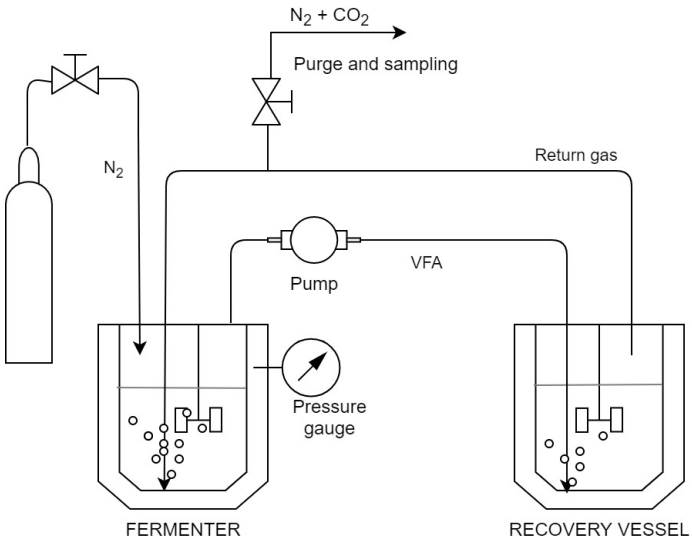

Figure 72. Schematic of carboxylic acid recovery from acidogenic fermenter by gas-stripping. Modified from [239]

The chemical conversion of carboxylic acids into calcium carboxylates is as follows:

$$CaCO_3(s) + 2(CH_3)_xCOOH(aq) \rightarrow Ca((CH_3)_xCOO)_2(aq) + H_2O(l) + CO_2(g) \quad \text{Equation 8.2}$$

$CaCO_3$ has low solubility in water, therefore, the slurry can be filtered to recovered the carboxylate solution and unreacted $CaCO_3$ can be recycled.

The maximum transfer rate of the acids can be defined as follows:

$$TR = Q \cdot C_{VFA}(g) = Q \cdot \frac{C_{VFA}\,(aq)}{K_H \cdot R \cdot T} \quad \text{Equation 8.3}$$

Where:

Q is the volumetric flowrate of the gas;

C_{VFA} is the concentration of the acids, where g indicates gas phase and aq, aqueous phase;

K_H is the Henry's law constant (mol L^{-1} atm^{-1});

R is the gas constant (L atm K^{-1} mol^{-1});

and T is the temperature of the aqueous phase.

Based on the transfer rate equation, it is expected that higher acid concentrations in the fermenter will lead to higher transfer rates. This is true for all recovery processes as this is the driving force of mass transfer (concentration gradient).

8.1.3 By-product carry over and limitations

When using MSW residues or food waste as feedstocks, lactic acid production can occur. The pKa of lactic acid (3.8) is much lower than the C2-C5 VFA (~4.8), and therefore it is less likely to be stripped off. Additionally, lactic acid is less volatile, the Henry's law constant for lactic acid is $6.9x10^5$ mol m^{-3} PA^{-1} compared to 30-100 mol m^{-3} PA^{-1} for C2-C3 VFA [314]. If acidogenic bacteria prevail, lactic acid is likely to be converted to targeted carboxylic acids (VFA).

Ammonia can also be stripped, but the rate of transfer will depend on the concentration, which is generally low, and ammonia is unlikely to be transferred to the saturated $CaCO_3$ solution, unless pH is acidic. A decrease in pH is likely to occur due to CO2 solubilisation, so monitoring the pH in the recovery vessel will be carried out. Contamination of the final product, therefore, is not a major concern; but removal of nitrogen compounds could create a nutrient deficit in the reactor. This is unlikely, however, if the substrate has sufficient protein content.

Foam formation is common in the anaerobic digestion of waste. Foam carry-over in the stripping gas can be avoided by allowing sufficient headspace or by the addition of anti-foaming agent.

8.1.4 Analyses

Total organic carbon (TOC) can be used to estimate the amount of carboxylate salts dissolved in the recovery solution. Ion Chromatography can be used to quantify each VFA type. VFA remaining in the digestate can be quantified using gas chromatography (GC) or IC. Gas compositional analysis of samples collected in gas bags can be carried out using GC.

8.1.5 Commercialisation and economic aspects

In this proposed study, the experimental rig will be operated in batch or fed-batch mode. For commercial use, the process needs to be scaled up into a continuous process. At the end of an experiment, the fermentation broth will still contains acids that cannot be recovered as the system would have reached equilibrium (i.e. the concentration of acids is not high enough to be found in the free form). In a continuous process, this would not happen: another step of solid/liquid separation with liquid recirculation system could be included.

If no additional step is added to the process, the VFA are recovered in the carboxylate form (i.e. calcium acetate, calcium propionate, calcium butyrate, etc.). Applications for these chemicals include food preservative, cosmetics and pharmaceuticals. Calcium acetate can be used as de-icer [315], oral phosphate binder for dialysis patients [316], food additive and viscosity regulator in cosmetics [317]. Calcium propionate is mostly used as antioxidant/preservative in food, particularly processed fruit and vegetables [318] and baked goods [319]. Calcium butyrate is used as supplement in animal nutrition [320]. Although not yet fully studied, calcium butyrate may be of use as anti-inflammatory substance to treat human inflammatory bowel diseases [321]. If the VFA are required in their acid form, this can be achieved through acidification using HCl. Separation of the VFA and the CaCl would be required, making the process more expensive.

The prices of calcium acetate, calcium propionate and calcium butyrate are 900-1500 [322], 1000-2200 [323] and 1000-50000 USD tonne^{-1} [324] (prices for August 2020), respectively.

Appendix A: **Literature data on acidogenic fermentation of organic wastes**

Table 32. Key operating parameters (reactor type, HRT, OLR, temperature, pH, etc.) and performance data (VFA yield and, VFA concentration) for acidogenic fermentation of organic wastes from different experimental studies

Feedstock	**Reactor type**	**HRT (days)**	**OLR (g VS/L/ day)**	**T (°C)**	**pH**	**Other conditions**	**Main acids (% of COD)**	**VFA yield (mg COD/g VS)**	**VFA (g COD/L)**	**VSS reduction**	**Ref.**
Waste activated sludge	Batch 0.5 L	7	9.9 day	60	11 (controlled)	0.02 g Sodium dodecylbenzene sulfonate/g VS	HAc, HPr (No %)	783	7.7	-	[49]
	Batch 5.0 L	9	12.9 day	55	8 (controlled)	-	45% HAc, 20% HVA	368	4.7	-	[202]
	Batch 2.0 L	6	10.8 day	21	6.5 (initial)	0.02 g SDBS/g TS	27% HAc, 23% HPr, 20% iHVa	240	2.6	-	[325]
	Batch 2.0 L	6	9.5 day	21	6.9 (initial)	0.1 g SDS/g TS	53% HAc, 12% HPr, 17% iHVa	235	2.2	-	[326]
Primary sludge	Batch 4.0 L	6	10.4 day	21	6.7 (initial)	-	32% HAc, 26% HPr, 13% iHBu, 16% iHVa	85	0.9	14.60%	[327]
	Batch 3.0 L	5	11.6 day	room	10 (controlled)	-	45% HAc, 25% HPr, 13% Hbu, 10% iHVa	302	3.5	38.00%	[233]
	CSTR 3.0 L + clarifier	1.3	2.9	25	6.9 (initial)	SRT = 10 days	TVFA reported as HAc	278	1.0	45.80%	[162]
Food waste	semi-CSTR 2.0 L	8	9	35	6 (controlled)	-	49% HAc, 24% HPr, 21% HBu	549	39.5	Not reported	[176]

	Batch 0.2 L	9.3	46.1 day	37	5.5 (initial)	Ultrasound + acid pretreatment	HAc, Hbu (No %)	367	16.9	20%	[231]
	Batch 3.0 L	5.0	13.8 day	35	6.5 (initial)	24h enzymatic pretreatment with Viscozyme, Flavourzyme and Palatase (1:2:1 at 0.1% v/v)	40% HAc, 60% HBu	796	11.0	45%	[230]
	Leach bed reactor 3.5 L	14.0	-	50	7 (controlled)	Leachate recirculation	17% HAc, 9% HPr, 63% HBu	330	49.0	72%	[229]
	Semi-CSTR 4.5 L	11.0	7.1	35	6 (controlled)	-	24% HAc, 13% HPr, 53% HBu	496	39.0	Not reported	[167]
	Batch 2 L	4.0	9.5 day	35	5.5 (controlled)	-	37% HAc, 16% HPr, 25% HBu	529	5.0	Not reported	[328]
	Batch 0.35 L	3.0	110 day	-	7 (controlled)	-	46% HAc, 12% HPr, 42% HBu	265	29.2	Not reported	[329]
	Semi-CSTR 2.5 L	3.0	10	-	5.5 (controlled)	-	40% HAc, 22% HPr, 37% HBu	414	12.4	Not reported	[330]
	Biofilm reactor 20 L (fed-batch)	2.3	42 day	30	-	Pre-aeration	HAc, HPr (No %)	76	5.3	55%	[331]
	Batch 0.5 L	20.0	60 day	30	6 (controlled)	-	20% HAc, 70% HBu	855	51.3	60%	[198]
	Batch 0.35 L	3.0	100 day	35	7	-	40% HAc, 40% HBu	443	44.3	Not reported	[332]

	Semi-CSTR	7.0	9	40	unfixed	-	35% HAc, 25% HPr, 15% Hbu	867	54.6	Not reported	[179]
Protein rich food wastes	Batch 0.5 L	9.0	35 day	30	6 (controlled)	Tofu, thermal treatment	50% HAc, 25% HBu	613	21.5	61%	[333]
Kitchen waste	Batch 0.5 L	4	125 day	35	7 (controlled)	-	22% HAc, 73% HBu	462	57.8	82%	[171]
	Semi-CSTR + solid recirculation	3.1	15.52	35	7 (controlled)	SRT = 9.3 days	HAc, HBu (No %)	529	25.5	89%	[171]
OFMSW	Batch	4	40 day	14-22	4-5 (uncontrolled)	-	80% HAc, HPr (No %)	35	1.4	Not reported	[224]
	PFR 80 L	4	42	37	6.2 (initial)	-	76% HAc, 9% HPr, 9% HBu	128	21.4	Not reported	[334]
	PFR 80 L	6	22.4	55	6.7 (initial)	-	23% HAc, 34% HBu, 28% HVa	239	32.1	Not reported	[223]
Cattail	Batch 2.0 L	5	4.1 day	40	6.9 (controlled)	Inoculum: rumen fluid	41% HAc, 35% HPr	570	2.3	75.90%	[335]
Palm oil mill effluent	CSTR 50 L	4	55 day	30	6.5 (controlled)	-	41% HAc, 30% HPr, 29% HVa	368	20.3	63%	[160]
	UASB 12 L	0.9	11.7	35	6 (initial)	-	41% HAc, 59% HPr	490	5.2	Not reported	[336]
Olive oil mill effluent	PBBR 2.5 L	1.41	18.3	25	5.5 (initial)	culture recycling ratio = 0.97	62% HAc, 22% HBu	414	10.7	Not reported	[337]
	Batch 0.2 L	40	50 day	25	6.5 (initial)	Pretreatment = Solid removal by centrifugation	54% HAc, 37% HBu	312	15.6	-	[338]

Wood mill effluent	CSTR 1.6 L + settler	1.5	2	30	5.5 (controlled)	SRT?	51% HAc, 39% HPr	464	1.4	Not reported	[339]
	CSTR 1.6 L + settler	1	4.6	30	5.5 (controlled)	SRT?	62% HAc, 32% HPr	437	2.0	Not reported	[340]
Paper mill effluent	CSTR 1.0 L	2	27.254	37	5.5 (controlled)	-	24% HAc, 48% HBu	96	5.3	Not reported	[164]
	Batch	12	18.5 day	room	6 (controlled)	Ammonia addition (1 g/L)	HAc, HBu (No %)	798	14.8	Not reported	[260]
Cheese whey	CSTR 1.0 L	2	6.5	37	5 (controlled)	-	HAc, HBu (No %)	367	4.8	Not reported	[164]
Dairy wastewater	Upflow reactor 2.8 L	1.0	2.8	55	5.5 (controlled)	P supplement	33% HAc, 22% HPr, 17% Hbu	453	1.3	Not reported	[341]
Gelatin-rich proteinaceous wastewater	Upflow reactor 2.8 L	0.5	2.8	55	5.5 (controlled)	-	14% HAc, 14% HPr, 15% iHBu, 17% HBu, 12% iHVa, 17% HVa	1,568	2.2	Not reported	[204]
Slaughterhouse blood	Semi-CSTR 1.0 L	7	19.0	37	7.5 (initial)	-	21% HAc, 35% Hbu, 27% iHVa	967	128.6	Not reported	[163]
Pharmaceutical wastewater	CSTR	0.5	9.2	35	5.5 (controlled)	-	50% HAc, 19% HPr, 32% Hbu	794	3.6	Not reported	[154]
Primary sludge + waste activated sludge	Batch 4.0 L	6	10.4 day	21	6.7 (initial)	0.02 g SDBS/g TS	21% HAc, 29% HPr, 14% iHVa	174	1.8	23.60%	[327]
Primary sludge + starch-rich wastewater	CSTR 3.0 L + clarifier	1.25	3.6	25	6.2 (initial)	SRT = 10 days	HAc, HBu (No %)	289	1.3	74.60%	[162]

foodwaste + excess sludge	Semi-CSTR 0.5 L	8.9	8.31		7 (controlled)	-	Not reported	393	29.1	Not reported	[342]
foodwaste + waste activated sludge	Batch 5.0 L	4	14.4 day	20	8 (controlled)	-	32% HAc, 52% HPr	576	8.3	61.00%	[199]
Foodwaste + primary sludge	CSTR 5.0 L	5	3.2	room	5.5-5.9 (uncontrolled)	10% food waste + 90% PS	48% HPr, 52% HBu	148	2.4	Not reported	[343]
Sugar industry wastewater + pressed beet pulp	CSTR	2	3.8	35	5.7 (uncontrolled)	1:1 COD mixing ratio, nutrient addition	40% HAc, 51% HPr	511	3.9	Not reported	[156]

Appendix B: **Manuscript on biogas production enhancement**

Enhanced semi-continuous biogas production from organic residual solids using enzymes

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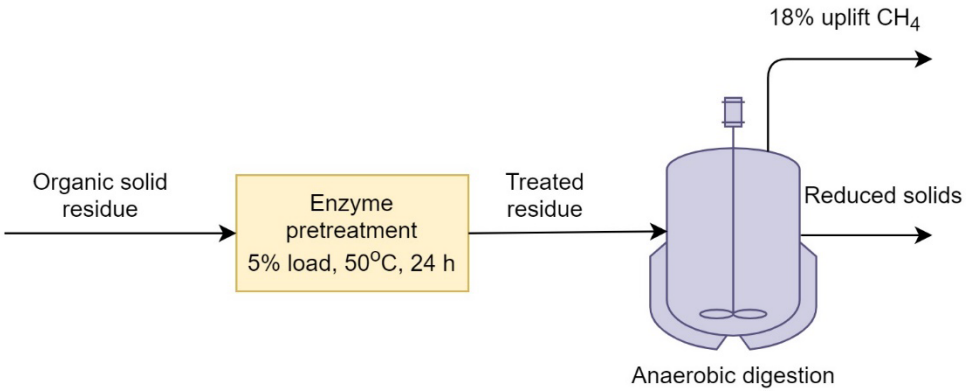
ABSTRACT



PURPOSE: To study the effect of enzyme pretreatment on the anaerobic digestion (AD) of a solid residual stream from the Fiberight process to treat municipal solid waste.

METHODS: The pretreatments efficacies were assessed against their ability to increase feedstock solubility. Cellulase/β-glucosidase/hemicellulase complex (A), arabinase/cellulase/β-glucanase/hemicellulase/xylanase complex (B), pectinase, protease, α-amylase, lignase and lipase were used for this purpose. Optimum conditions were used to pretreat the residue and subject it to semi-continuous AD using 0.5 L stirred tank reactors.

Appendix B

RESULTS: Pretreatment with 5% cellulase/β-glucosidase/hemicellulase enzyme complex loading at 6.75% total solids (TS) for 24 hours at 50°C resulted in 4.3 times increase of the initial soluble chemical oxygen demand (COD). An average methane uplift of 18% was observed with respect to baseline on reactors fed with untreated feedstock. Economic analysis, however, showed that this enzymatic pretreatment would result in a loss of 20 GBP per tonne of OFMSW.

CONCLUSION: Enzymatic pretreatment is a promising option for the improvement of residual solids solubility and biodegradability resulting in enhanced biogas yields during AD. This pre-treatment strategy, however, is not currently economically feasible for enhancing biogas production under current policies. It is proposed that targeting the production of volatile fatty acids (VFA) by inhibiting methanogenic activity could lead to economic feasibility.

Keywords: Biogas, Anaerobic Digestion, Municipal Solid Waste, Enzyme Pretreatment.

Nomenclature

sCOD	Soluble chemical oxygen demand
AD	Anaerobic digestion
COD	Chemical oxygen demand
IA	Intermediate alkalinity
MSW	Municipal solid waste
OLR	Organic loading rate
ORS	Organic residual solids
PA	Partial alkalinity
TS	Total solids
TSS	Total suspended solids
VFA	Volatile fatty acids
VS	Volatile solids
VSS	Volatile suspended solids

B.1 Introduction

Municipal solid waste (MSW) is a biological hazard which needs to be disposed away from residential areas to avoid spread of diseases, pests and parasites. Approximately 2,010 million tonnes of municipal solid waste (MSW) are produced per year by the global population, and it is estimated that it will increase to 3,400 million tonnes by 2050 [1]. The composition of MSW varies across countries and cities, depending mainly on number of inhabitants and food habits [2]. Landfilling and incineration are the conventional choices to manage MSW; however, public opinion, policies such as Zero Waste [7], and current regulations are pushing the agenda towards more environmentally friendly alternatives. The Landfill Directive 1999/31/EC was implemented across European countries in order to achieve zero recyclable waste sent to landfill by 2025 including food waste and other organic wastes [8].

MSW management options include mechanical separation and recovery of recyclable materials and use of the organic fraction (OF) as feedstock for biological processes, such as composting and anaerobic digestion (AD). Both process produce a soil amendment; however, AD has the added benefit of producing methane-containing biogas for energy recovery [2, 67]. Other alternatives like OFMSW conversion into energy through thermal treatments (incineration, gasification and pyrolysis) have some impediments such as the heterogeneity of the OF composition, the high moisture content and the low net calorific value [11]. These characteristics point towards AD as the preferred option for treatment of and energy generation from OFMSW.

Anaerobic digestion is typically characterised as having three main stages: hydrolysis, acidogenesis and methanogenesis [24]. During hydrolysis, the insoluble polymers are broken down into soluble oligomers and monomers by hydrolytic bacteria. The second step known as acidogenesis involves the production of acetic acid and other volatile fatty acids (VFA) such as propionic, butyric, iso-butyric, valeric and iso-valeric acids. As part of this step, acetogens metabolise longer-chain VFA into acetic acid, hydrogen and carbon dioxide. Methanogenesis is the final step when the acetic acid is transformed into methane by methanogenic microorganisms. In the case of some industrial effluents and wastewaters, much of the organic material is dissolved and ready available, and therefore methanogenesis may be the rate limiting step of the process. In contrast, in AD of MSW and other solid wastes, hydrolysis is the rate limiting step due to the complex chemical composition and lower biodegradation rate caused by the presence of lignocellulose [158]. Because of this low biodegradability, the process can be more expensive compared to liquid systems.

The feedstock used in this study is a residue from the Fiberight Ltd. Process [344]. Within this process, the mixed OFMSW is pulped and washed to obtain a clean fibre for further

bioprocessing. As a result, an organic-rich washwater is generated to be treated in a high-rate liquid AD system. This washwater contains a high soluble organic content and a proportion of suspended solids or organic residual solids (ORS) which a volatile solids (VS) content of up to 80% (w/w) depending on the waste source. The ORS solubility is low and a high-rate AD system may not be the most efficient method of treating this material.

Several approaches are possible in order to increase solubilisation of organic solid wastes, including physical, chemical and biological treatments. Enzymatic treatment is a promising option in terms of energy demand, chemical input and environmental factors [21]. Although the use of enzymes to enhance bioconversion of MSW has been proposed since 1980 [22], studies on this subject are scarce. OFMSW is typically comprised of food waste, garden waste, paper and inorganic impurities. Studies using OFMSW or foodwaste demonstrate the efficacy of enzyme pretreatments to increase methane yields and reduce waste volumes [345–347] ; however, little information is available on the economic viability of this technology. Despite the complexity of food waste and OFMSW composition, authors only used carbohydrases (carbohydrate hydrolysing enzymes) [347] or a carbohydrate hydrolytic agent (fungal mash) [345, 346].

This work studied the feasibility of using commercial enzymes for the pretreatment of an OFMSW derived from the Fiberight process, with subsequent continuous biogas production. A variety of enzyme complexes, such as carbohydrases, protease, lignase and lipolase were used to compare efficacies.

B.2 Methods

B.2.1 Formation of ORS

MSW was collected from a waste transfer station in Southampton, UK. This material was pulped and the recyclables were removed from it (processing conditions commercially confidential) at the Fiberight pilot plant in Southampton. The pulped and recyclable free material was washed to remove soluble organics and fine particulate ORS. The ORS was collected by filtering the washwater through nylon filter cloth and then stored at -18°C until used.

B.2.2 Characterisation of ORS

Total solids (TS) and volatile solids (VS) determination was based on Standard Method 2540 G, APHA 2005 [268]. $CaCO_3$ ash was measured by weight difference between material heated at 550°C for 2 hours for VS determination and heated at 900 °C for a further 2 hours. The weight difference is associated with the CO_2 produced in the calcination of $CaCO_3$ into CaO [269]. Determination of carbohydrates and lignin was carried out through acid hydrolysis following a

slightly modified version of the National Renewable Energy Laboratory (NREL) method [272]. The protein content was determined by measuring the total Kjeldahl nitrogen (TKN) and then multiplying this by a nitrogen in protein factor of 6.25 [348]. TKN was determined according to US EPA Standard Method 1687 [271]. Lipid content was determined using Soxhlet extraction with hexane following the US EPA method 9071B [273] with slight modifications. COD was measured by the closed tube reflux method with titrometric determination of the end point [277].

B.2.3 Pretreatments

Batch experiment 1 – Different enzymes pretreatments

Seven enzyme solutions of approximately 2 g L^{-1} of concentrated enzyme liquor were prepared using a buffer solution of pH 5.5 (96% (v/v) of 0.1M KH_2PO_4 and 4% (v/v) of 0.1M Na_2HPO_4) and another of pH 7 (63.3% (v/v) of 0.1M KH_2PO_4 and 36.7% (v/v) of 0.1M NaOH). Cellulase/β-glucosidase/hemicellulase complex (complex A), pectinase, α-amylase, arabinase/cellulase/β-glucanase/hemicellulase/xylanase complex (complex B), lignase and lipolase were mixed separately with pH 5.5. buffer and protease with pH 7 buffer for optimum catalytic conditions. Exact weights of concentrated enzyme liquor and buffer added were recorded. Commercial names of enzymes are not shown for reasons of confidentiality.

Approximately 0.3 g of ORS sample was placed in a sealed test tube with ~4 g of buffer solution. Test tubes were hand shaken to mix the contents, and placed in an incubator at 50°C and 100 rpm for one hour for extraction of soluble materials. Approximately 1.5 mL of liquid was removed using a disposable pipette, avoiding any large solid particles. Liquid was passed through a syringe filter (0.45 µm) to remove solid particles and placed in 2 mL centrifuge tubes. Liquid samples were kept in freezer then thawed at room temperature prior to COD analysis. Approximately 2.5 mL of enzyme solution was added to each solid sample to achieve a 5% enzyme loading based on ORS total solids. The weight of the test tube was recorded after each step. The calculated final total solids content was approximately 1.6%. Tubes were incubated for 17 hours at 50°C. Approximately 2 mL of liquid was removed, filtered and kept frozen as above until COD analysis. A control sample was prepared using buffer solution instead of enzyme solution. All samples were prepared in triplicate. Buffer solutions were tested to ensure they had no effect on COD tests. The COD of the enzyme solutions was also measured and subtracted from the final COD values after hydrolysis.

Soluble COD increase was calculated as follows:

$$sCOD\ increase\ (\%) = \frac{(sCOD\ final\ (mg/gTS) - sCOD\ initial(mg/gTS))}{sCOD\ initial\ (mg/gTS)} \cdot 100$$

Batch experiment 2 – Autoclave pretreatment

All samples were prepared in triplicate. Approximately 1 g of ORS was placed in a PPCO autoclave bottle with ~50 g of DI water, giving a calculated total solids content of approximately 0.6%. The bottles were placed in an incubator at 50°C and 100 rpm for one hour in order to extract soluble material. Sampling for COD analysis was done as described in batch experiment 1. The bottles were autoclaved for one hour at 121°C, then cooled and weighed to check for any water loss. Control replicates were kept refrigerated until the autoclave run finished. Analysis and calculations of sCOD increase were identical to batch experiment 1.

Batch experiment 3 – Cellulase/β-glucosidase/hemicellulase pretreatment at different loadings

Approximately 1 g of sample was placed in a sealed container with ~100 g of buffer solution. Containers were hand shaken to mix the contents, and placed in an incubator at 50°C and 100 rpm for one hour in order to extract soluble material. Sampling for COD analysis was done as described in batch experiment 1. Approximately 25 mL of cellulase/β-glucosidase/hemicellulase complex solution at concentrations of 0.54, 0.22, 0.11 or 0.05 g L^{-1} was added for a 5%, 2%, 1% or 0.5% enzyme loading respectively based on ORS total solids. The weight of the container was recorded after each step. The calculated total solids was approximately 0.2%. Containers were incubated for 17 hours at 50°C. A control sample was prepared using buffer solution instead of enzyme solution. All samples were prepared in triplicate. COD of the enzyme solutions was measured and subtracted from the final COD values after hydrolysis. Soluble COD increase was calculated as in batch experiment 1. In addition to COD analysis, samples were analysed for total suspended solids (TSS) and volatile suspended solids (VSS) by EPA method 160.2 [349].

B.2.4 Semi-continuous anaerobic digestion

Reactor operation

Four stirred reactors with a working volume of 0.5 L were inoculated with mesophilic anaerobic digestate from Milbrook wastewater treatment plant, Southampton (UK). Each reactor had a feed port, through which the ORS was inserted semi-continuously on a daily basis, and a gas outlet connected to a gas-impermeable bag. On day one, reactors were fed with a mixture of deionised (DI) water and ORS to achieve an organic loading rate (OLR) of 2.5 gVS L^{-1} day^{-1}. Each day, the OLR was increased by 0.1 gVS L^{-1} day until the target OLR of 3.5 gVS L^{-1} day was achieved on day 11. The DI water to ORS ratio was kept constant at 3.5 (6.8% TS). All four reactors were fed in this way until day 29. Daily digestate removal to a known liquid level enabled the reactor

working volume to remain relatively constant during the trial. Sampling and removal of reactor contents was performed using a 100 mL syringe connected to a silicone tube long enough to reach the reactor bottom. During this process, stirrers were stopped and reactor contents were briefly exposed to air; the gas bags were closed and disconnected at this time to avoid loss of biogas.

From day 30 onwards, reactors C and D were fed ORS pretreated with 5% cellulase/β-glucosidase/hemicellulase complex (non-buffered). The solid to liquid ratio was kept as before. The pre-treatment was performed by incubating the ORS with enzyme at 50°C and 100 rpm for 24 hours. Centrifuge tubes were laid horizontally for a better mixing. Feedstock for reactors A and B (control) was also kept in the incubator to control for effects caused by incubation.

Gas, alkalinity, pH and VFA measurements

Gas bag volumes were measured using a weight-type water displacement gasometer [274]. All gas volumes were corrected to standard temperature and pressure of 0°C, 101.325 kPa. Methane and carbon dioxide were quantified using a Varian Star 3400 CX gas chromatograph (GC) with a Hayesep C column, a thermal conductivity detector and argon as the carrier gas at a flow of 50 mL/min. pH was measured using a Jenway 3010 meter (Bibby Scientific Ltd, UK). Alkalinity was measured by titration based on Standard Method 2320B [268]. VFA were quantified using a Shimazdu GC-2010 gas chromatograph with a flame ionisation detector and a capillary column type SGE BP-21. Helium was used as carrier gas at a flow of 190.8 mL/min and a split ratio of 100 to give a flow rate of 1.86 mL/min in the column and a 3.0 mL/min in the purge. The GC oven temperature was set to increase from 60 to 210°C in 15 min with a final hold time of 3 min. The temperatures of injector and detector were 200 and 250°C, respectively. Prior to GC injections, samples were clarified by centrifugation (13,000 rpm, 15 min) and acidified using formic acid with a volumetric 9:1 sample to acid ratio.

B.3 Results and discussion

ORS compositionTable 8 summarises the composition of the ORS. The volatile solids (VS) content was lower than average for OFMSW in other studies around the globe (~85%) [2], and therefore, it has a lower methane potential per unit weight. Composition of the organic content will depend on the source of MSW and treatment process used. It is worth noticing that, due to the nature of the ORS generation process, grit and non-biodegradable particles such as plastics are mixed with the degradable material. The presence of plastic can bias the organic content measured as VS, as plastic might degrade when heated at 550°C.

B.3.1 Pretreatments

Figure A1 shows the sCOD results from the first batch experiment. As can be seen, the pretreatment with cellulase/β-glucosidase/hemicellulase complex (complex A) gave a significantly higher sCOD increase compared to the other enzyme complexes. This was as expected, as the ORS has a high cellulose and hemicellulose content and thus the cellulase and hemicellulase cocktail was most effective. Enzyme complex B was expected to give a large sCOD increase as it is also a cellulolytic enzyme mixture; this was not seen, perhaps indicating that the cellulase content in complex B is lower than in complex A. In addition, it should be noted that not all of the enzymes had the same activity (not given by the manufacturer in this case). From these results, it might seem that using complex A individually is the best option, but future experiments are required using a mixture of different enzymes as the enzymes can work in synergy and improve their activity when in the presence of other enzymes [350].

Figure A2 shows the sCOD results from the second batch experiment. As depicted, autoclaving has a significant positive impact on the sCOD; however, the sCOD increase is only 1/4 of that of the 5% complex A pretreatment. Nonetheless, it should be noted that the solid to liquid ratio in the autoclave test was lower than in the first batch experiment and therefore the results are not directly comparable. If the improvement of sCOD by autoclaving is assumed to be linear with respect to the solid to liquid ratio, complex A enzymes still shows more promise as a pretreatment not only for sCOD release but also in relation to the energy input. In addition, it is well known that thermal treatments triggers the production of inhibitory compounds [21].

Figure A3 shows TSS, VSS and sCOD results from the third batch experiment. In terms of sCOD increase, the 2% and 5% enzyme loading are the only loadings that showed a significant difference with respect to the control, with the 5% loading showing the greatest sCOD increase. In terms of TSS and VSS only the 5% loading showed a significant effect with respect to the control. It should be considered that the TS content in the first experiment was 8 times higher than that of the third batch experiment and, as a result, sCOD increase is higher for the 5% enzyme loading in the first batch. It is unclear whether enzymatic pretreatment is improved at higher TS for the same enzyme loading due to a synergy between enzyme and substrate at high TS or between the enzymes themselves when present at higher concentrations. For a fixed enzyme loading rate, effects caused by TS or enzyme concentration cannot be studied separately.

B.3.2 Semi-continuous anaerobic digestion

Reactor data

Figure A4.a shows the specific methane production versus time for all four reactors. Methane production stabilised from day 16 onwards, where values for all reactors fluctuated around 0.15 L/g VS fed (baseline). After pretreated material was fed to reactors C and D from day 30, a significant improvement in specific methane production was observed. In reactors A and B, however, methane production started decreasing almost linearly. This may be an indication that the incubation has a negative effect on the feedstock as methane production was expected to remain constant. It is possible that incubation led to microbial growth, producing an imbalance in the microbial community of the reactors. If this is the case, it may also mean that the enzymatic hydrolysis is not giving the maximum output due to this possible contamination. On average, methane yields from reactors C and D after day 30 were 22.7% and 13.0% higher than the baseline before day 30.

Figure A4.b shows total solids (TS) and volatile solids (VS) of reactors versus time. Total solids remained almost constant for all four reactors, with a slight increasing trend. This could be due to the sampling method used in this experimental setup: The material removed by the syringe may not have been entirely representative of the reactor contents, leading to uncoupling of the liquid and solids retention times, causing an increase in the latter. As can be seen, there were no significant differences in VS or TS content between the reactors fed with control feedstock (A and B) and the reactors fed with complex A pretreated feedstock (C and D). As the volatile solids remained almost constant from day 16 until day 30, it can be said that reactors were stable before pretreated material was fed.

Figure A4.c presents the total alkalinity measurements through time. As observed, alkalinity in all four reactors decreased to a baseline of approximately 4 g $CaCO_3$ L^{-1}. As can be deduced from these results, pretreatment did not have a significant effect on the total alkalinity. Figure A4.d shows the intermediate (IA) to partial alkalinity (PA) ratio. From day 13 onwards, the IA/PA ratios were above the 0.3 value typically recommended for anaerobic digestion [351], which suggests the reactors were not operating under optimum conditions. Reactors C and D showed a significant reduction in the IA/PA ratio after day 30 when pretreated material was fed, whilst the IA/PA ratio in reactors A and B kept increasing. It is possible that the pretreatment with complex A enhanced the release and dissolution of $CaCO_3$ from the ORS, improving the buffering capacity of the reactors. Alternatively, complex A may contain buffering chemicals.

As observed in Figure A4.e, the pH went from slightly alkaline towards neutral within the first 12 days before stabilising at around pH 7. All four reactors maintained approximately the same pH until day 30, when reactors C and D were fed with pretreated feedstock and reactors A and B with control feedstock. The pH did not fall below 6.5 before day 30, which indicates there was a good balance between acetogenic and methanogenic processes. After day 30, reactors C and D maintained a healthy neutral pH of no higher than 7.5, while in the control reactors A and B pH fell below 6.5. The reactors fed with the control feedstock (i.e. treated at 50°C for 24 hours) accumulated volatile fatty acids (VFA) as shown in Figure A4.f, consequently inhibiting the methanogenic microorganisms. This indicates that the ORS may already contain acidogenic bacteria that multiplied during the incubation prior to AD. Main acids produced were acetic and propionic acid. Other acids that have not been accounted for such as succinic acid or lactic acid may also have been generated. This opens the possibility of using the ORS for VFA production instead of methane. VFA are chemical platforms that can be used in a wide range of applications (long chain acids, bioplastics, biofuels, nutrient removal, etc.). In addition to VFA, acidogenic fermentation can produce H_2 as byproduct, which can then be utilised as energy carrier in fuel cell technologies or other applications [15, 17, 18].

Pretreatment data

As the feedstock was diluted with distilled water, the resulting solution had a pH of approximately 6.3. Despite this, it did not have a negative effect on the reactors pH during the first 30 days, which means the reactors had good buffering capacity. After adding complex A, the feedstock pH dropped slightly to 6.1. Control feedstock (i.e. treated at 50°C for 24 hours without enzyme) and pretreated feedstock (i.e. treated at 50°C for 24 hours with complex A) had a pH of approximately 5.2 and 4.7 respectively.

As can be seen from Figure A5, pretreatment with complex A considerably reduced the TSS and VSS of the feedstock solution. This pretreatment also improved the solubility of ORS, giving a final sCOD 4 times higher than that of the control and a TSS destruction of 17.1% compared to 0.9% for the control. Consequently, methane yield increased by 17.8% on average above the baseline value (see Figure A4.e).

Compared to the batch experiment 1 results, sCOD increase due to complex A was not significantly higher under the pretreatment conditions used in the semi-continuous trial, even though the solid contents were much higher in this case. The TS content in the pretreatment was not based on optimum values for enzymatic hydrolysis, but rather set to provide the desired OLR for AD. It is also worth noting that in the pretreatment, no buffer solution was used to avoid a

negative effect on methanogenesis due to low pH. Acidogenic microorganisms can work in a wider range of pH values [178, 181], and therefore, if VFA production is desired, it would be possible to use a buffer solution for optimum ORS pretreatment with complex A.

Economic analysis

Table 34 shows the input data used in the economic analysis. Methane, enzyme and landfill costs are susceptible to change, so this analysis is only valid for the values taken in 2018.

Table 35 summarises the results from the economic analysis. Although anaerobic digestion without pretreatment seems to present savings with respect to landfilling, this would most likely not be the case if operational costs were also included in the study. As landfill taxes are expected to increase for active organic waste in order to meet regulatory requirements, savings with respect to landfill will be higher in the future. In addition, de-activation of waste will be a requirement and therefore economy will not be the driving force to use this technology. Other biological treatments could also be considered, but this is out of the scope of this study. It is clear, on the other hand, that enzyme pretreatment is not economically favourable with respect to untreated AD. Strategies to follow could be further optimisation of the pretreatment using a mixture of enzymes and switching to different biological process with a more valuable product than methane.

Evidence from this study thus suggests that the ORS is better suited for acidogenic fermentation for VFA production. Several arguments favour VFA production over single stage AD for biogas production, including economic feasibility. For example, product revenues for biogas from wastes are significantly lower (5.6 times) than that of PHA from VFA [26].

B.4 Conclusions

Enzymatic pretreatment is a plausible solution to accelerate and optimise biological degradation of solid wastes such as the organic residual solids (ORS) from municipal solid waste (MSW) used in this study. Due to the high carbohydrate content of the ORS, the highest solubilisation by the enzymes tested was achieved by cellulase/β-glucosidase/hemicellulase complex. Pretreatment with this complex improved methane yields by nearly 18%. Testing also demonstrated that enzymatic pretreatment can achieve similar or greater solubilisation rates than autoclaving. After techno-economic analysis, however, it was found that methane uplift due to enzymatic pretreatment did not compensate for the cost of the enzymes due to the low profits from methane and high cost of the enzymes.

In addition, it was found that when the ORS were incubated, there was an accumulation of volatile fatty acids (VFA), mainly acetic and propionic acids, and inhibition of methanogenesis. These results led to the conclusion that the ORS could be a better substrate for VFA production.

Several studies support the advantages of producing VFA from OFMSW over biogas [178, 179, 221, 223, 224, 334]. As with biogas production, pretreatments for VFA production could be carried out prior to fermentation to increase production yields and solids destruction. Both the literature review and the experimental work carried out suggest that VFA production as platform chemicals from MSW may be a more economically viable alternative to biogas production due mainly to the resultant product value. In this case, enzymatic pretreatment could be justified.

B.5 Acknowledgements

The authors acknowledge the financial support of The Engineering and Physical Sciences Research Council and Fiberight Ltd.

B.6 Tables

Table 33. Characterisation of organic residual solids (ORS).

Characteristic	Average value	Error
Total solids	26.64%	±0.05%
Volatile solids	75.94%	±0.96%
CaCO3 ash	5.00%	±0.41%
Glucan	33.33%	±2.94%
Hemicellulose	5.84%	±0.25%
Acid insoluble lignin (AIL)	11.10%	±0.07%
Acid soluble lignin (ASL)	1.40%	±0.10%
Proteins	8.11%	±0.29%
Lipids	4.71%	±0.22%
Total biodegradable content*	64.49%	-

*Total biodegradable content was calculated as the sum of glucan, hemicellulose, AIL, ASL, proteins and lipids. All values based on total solids

Table 34. Input data for economic analysis. Pounds (£) refer to GBP.

Economic data

Landfill cost in the UK (£/tonne) [352]	107
Enzyme price (£/kg), adapted from [353]	2.98
Non-domestic methane price (£/kWh) [354]	0.0375
Methane calorific value (kWh/L)	0.0104

Process data

Methane yield (L/g VS)	0.147
Methane yield after pretreatment (L/g VS)	0.173
TSS reduction after pretreatment	17.08%

Appendix B

Table 35. Results from economic analysis of anaerobic digestion vs. enzymatic pretreatment with anaerobic digestion. Pounds (£) refer to GBP.

	Anaerobic digestion (AD)	Enzyme pretreatment + AD
Cost of landfill (£/tonne ORS)	107.00	88.72
Cost of enzyme (£/tonne ORS)	0.00	40.21
Benefits from CH_4 (£/tonne ORS)	11.63	13.71
Balance (£/tonne ORS)	- 95.37	-115.22
Savings with respect to direct landfill	10.9%	-7.7%

B.7 Figures

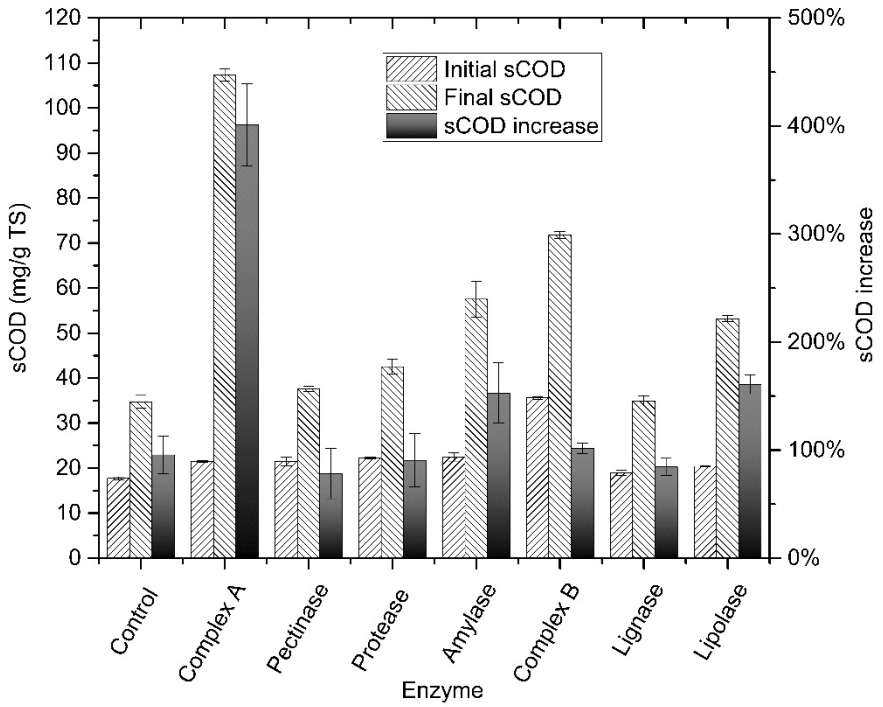


Figure A1. Soluble COD increase for batch experiment 1 (5% enzyme loading, 17 hours at 50°C, 100rpm and 1.6% TS). Complex A and B refer to cellulase/β-glucosidase/hemicellulase complex and arabinase/cellulase/β-glucanase/hemicellulase/xylanase complex respectively

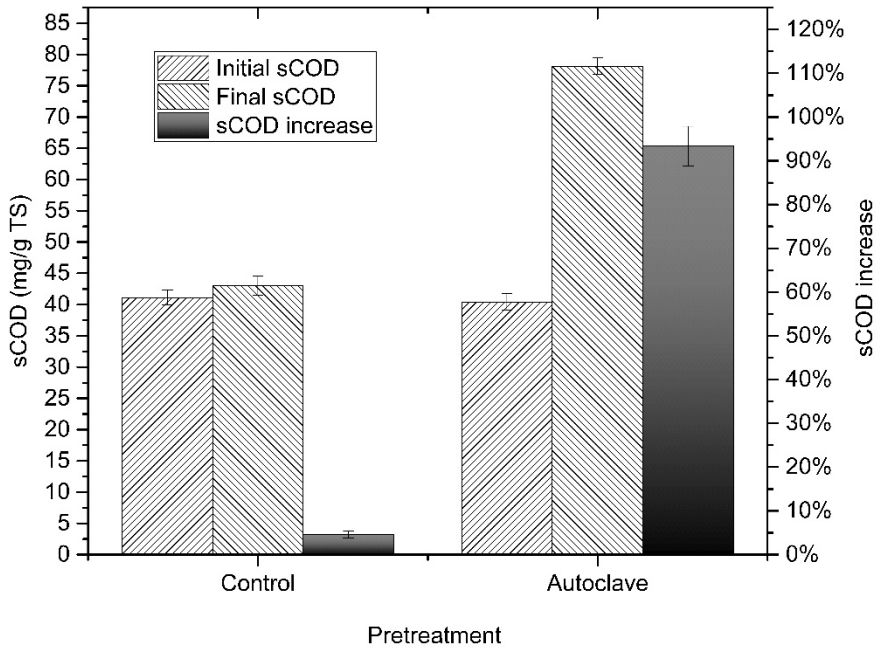


Figure A2. Soluble COD increase for batch experiment 2 (autoclave at 121°C for one hour and 0.6% TS)

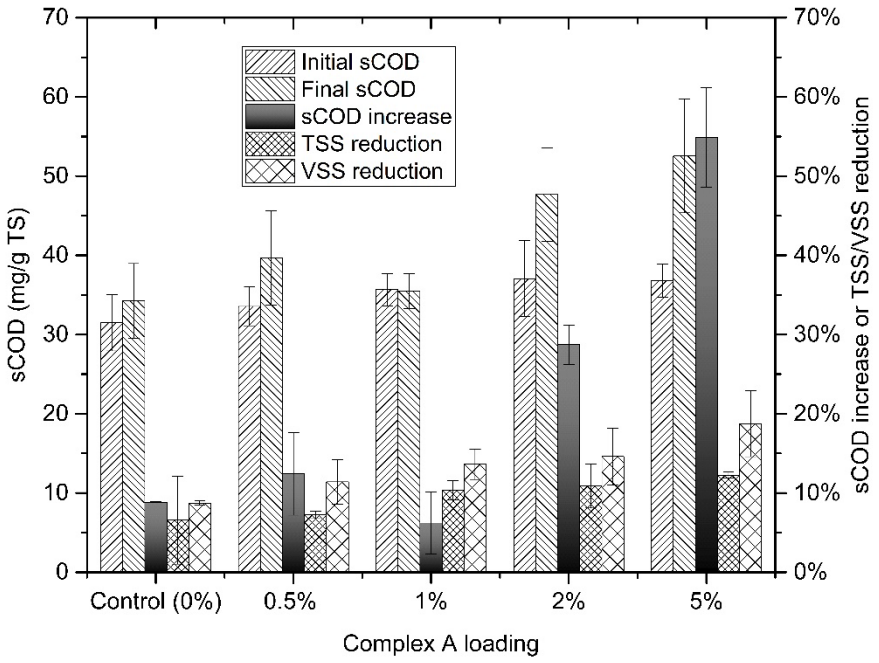


Figure A3. TSS reduction and soluble COD increase for batch experiment 3 (17 hours at 50°C, 100rpm and 0.2% TS). Complex A refers to cellulase/β-glucosidase/hemicellulase complex

Appendix B

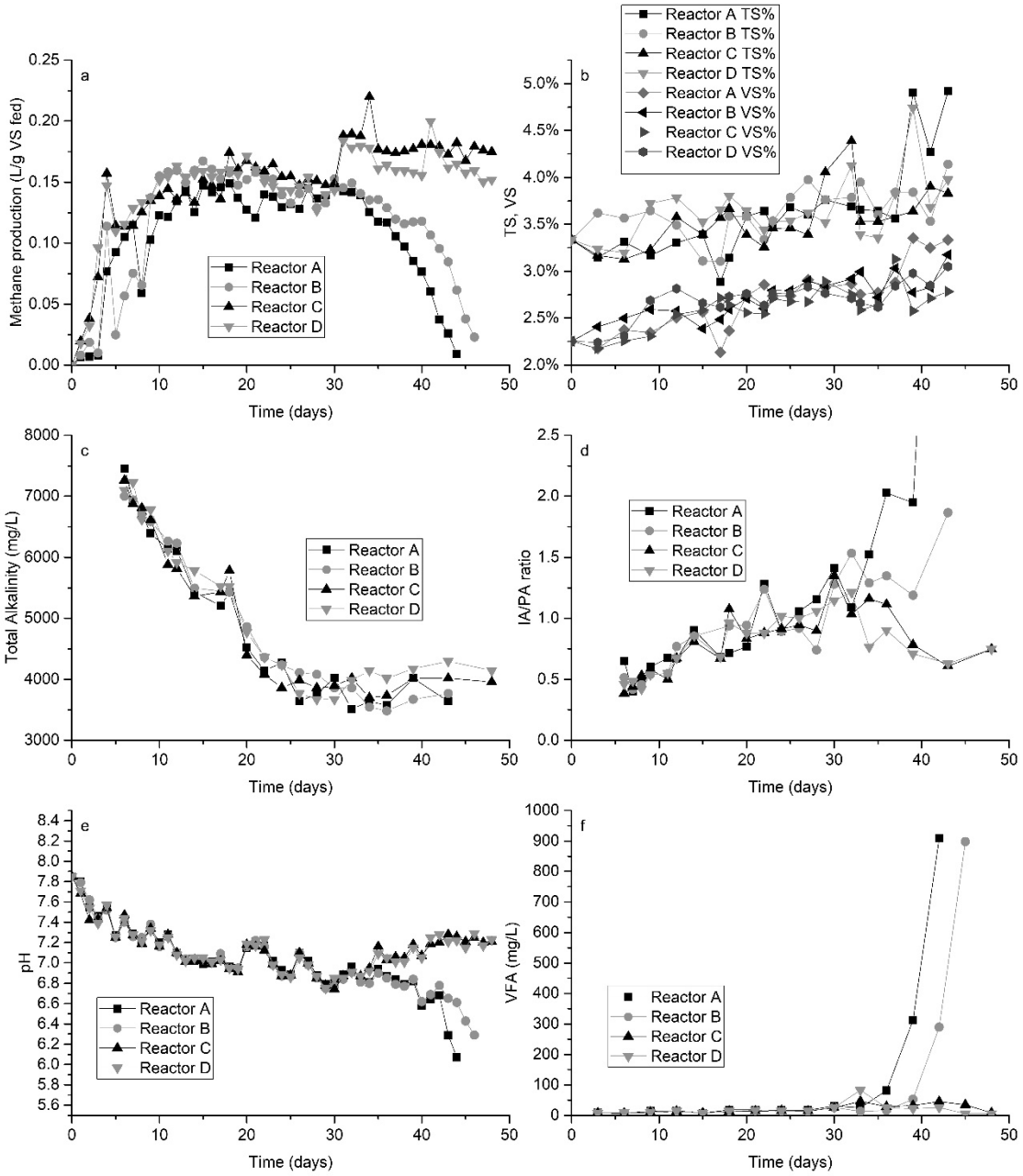


Figure A4. Results from semi-continuous anaerobic digestion. All four reactors fed with fresh ORS until day 30. From day 30, reactors A and B fed with incubated ORS (control) and reactors C and D with enzyme pretreated ORS. Reactor conditions: working volume=0.5 L, HRT=14 days, OLR=3.5 gVS L^{-1} day^{-1}. a) Methane production (L gVS^{-1} fed); b) TS and VS; c) Total alkalinity (mg $CaCO_3$ equivalent L^{-1}); d) IA/PA ratio; e) pH; f) VFA accumulation (mg L^{-1})

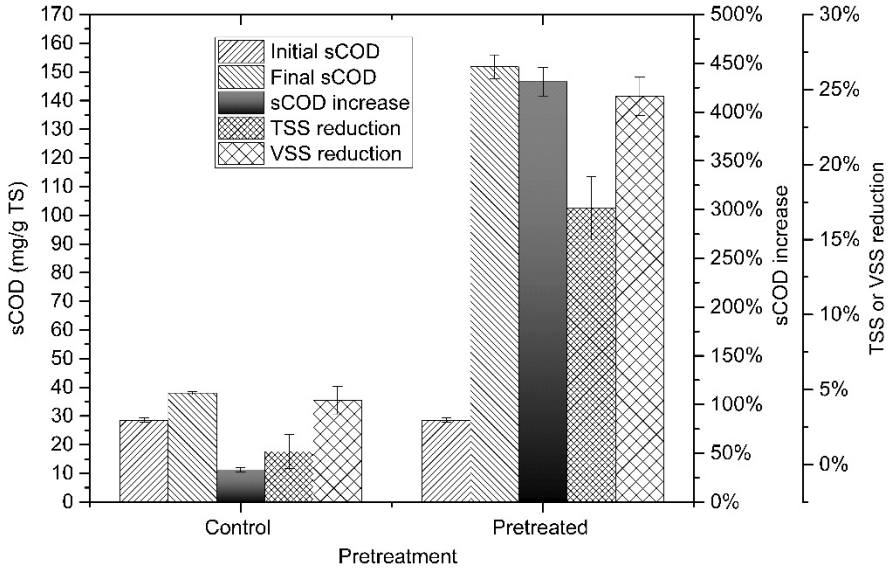


Figure A5. Total suspended solids (TSS) and volatile suspended solids (VSS) reduction of the control feedstock (i.e. treated at 50°C and 100rpm for 24 hours without enzyme) and the pretreated feedstock (i.e. treated at 50°C for 24 hours with complex A at 5% enzyme loading, at 100rpm and 6.8% TS)

Appendix C: **Experimental data**

C.1 Semi-continuous experiment

Table 36. ORS, enzyme solution and tap water fed to CSTR reactors on even days

	Reactors A-B			**Reactors C-D**			**Reactors E-F**		
Day	**ORS (g)**	**Enzyme (mL)**	**Water (mL)**	**ORS (g)**	**Enzyme (mL)**	**Water (mL)**	**ORS (g)**	**Enzyme (mL)**	**Water (mL)**
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-4	19.22	1.38	36.31	19.22	1.38	36.31	19.22	1.38	36.31
6-8	25.62	1.84	48.41	25.62	1.84	48.41	25.62	1.84	48.41
10-12	32.03	2.30	60.51	32.03	2.30	60.51	32.03	2.30	60.51
14-16	38.43	2.77	72.61	38.43	2.77	72.61	38.43	2.77	72.61
18-20	44.84	3.23	84.71	44.84	3.23	84.71	44.84	3.23	84.71
22-36	51.24	3.69	96.82	51.24	3.69	96.82	51.24	3.69	96.82
38-70	76.86	5.53	145.23	51.24	3.69	96.82	32.03	2.30	60.51

C.2 Fed-batch experiment

Table 37. Reactor weight (g) before sampling or feeding during fed-batch experiment. The weight of each empty vessel was approximately 65 g

	Reactor weight (g) before sampling or feeding					
date	A	B	C	D	E	F
01/10/2020	465.3	466.2	464.6	463.4	466.1	464.9
09/10/2020	538.4	540.6	478.9	471	509	509.5
19/10/2020	647.8	643.1	531	519.3	650.8	652.9
25/10/2020	774	774	522	510	607	609
02/11/2020	842	838	549	535	573	574
06/11/2020	804	802	542	529	540	542

Appendix C

Table 38. Sample volume taken during the fed-batch experiment

Date	Sample (mL)					
01/10/2020	A	B	C	D	E	F
10/10/2020	30	30	30	30	30	30
20/10/2020	30	30	30	30	30	30
26/10/2020	30	30	30	30	30	30
03/11/2020	30	30	30	30	30	30
01/10/2020	90	90	90	90	90	90

Table 39. ORS wet weigh (g) added during the fed-batch experiment

Date	ORS (g)					
01/10/2020	A	B	C	D	E	F
02/10/2020	130	130	130	130	130	130
04/10/2020	30	30	0	0	0	0
06/10/2020	30	30	30	30	60	60
08/10/2020	30	30	0	0	0	0
10/10/2020	30	30	30	30	60	60
12/10/2020	30	30	0	0	0	0
14/10/2020	30	30	30	30	60	60
16/10/2020	30	30	0	0	0	0
18/10/2020	30	30	30	30	60	60
20/10/2020	60	60	0	0	0	0
22/10/2020	60	60	30	30	0	0
24/10/2020	60	60	0	0	0	0
26/10/2020	60	60	30	30	0	0
28/10/2020	60	60	0	0	0	0

30/10/2020	0	0	30	30	0	0
03/11/2020	0	0	30	30	0	0

Table 40. Enzyme solution volume added during fed-batch experiment

Date	Enzyme solution (mL)					
01/10/2020	A	B	C	D	E	F
02/10/2020	6	6	6	6	6	6
03/10/2020	1	1	0	0	0	0
04/10/2020	1.4	1.4	0	0	0	0
05/10/2020	0	0	0	0	0	0
06/10/2020	1.4	1.4	1.4	1.4	2.8	2.8
07/10/2020	0	0	0	0	0	0
08/10/2020	1.4	1.4	0	0	0	0
09/10/2020	0	0	0	0	0	0
10/10/2020	1.4	1.4	1.4	1.4	2.8	2.8
11/10/2020	0	0	0	0	0	0
12/10/2020	1.4	1.4	0	0	0	0
13/10/2020	0	0	0	0	0	0
14/10/2020	1.4	1.4	1.4	1.4	2.8	2.8
15/10/2020	0	0	0	0	0	0
16/10/2020	1.4	1.4	0	0	0	0
17/10/2020	0	0	0	0	0	0
18/10/2020	1.4	1.4	1.4	1.4	2.8	2.8
19/10/2020	0	0	0	0	0	0
20/10/2020	2.8	2.8	0	0	0	0
21/10/2020	0	0	0	0	0	0

22/10/2020	2.8	2.8	1.4	1.4	0	0
23/10/2020	0	0	0	0	0	0
24/10/2020	2.8	2.8	0	0	0	0
25/10/2020	0	0	0	0	0	0
26/10/2020	2.8	2.8	1.4	1.4	0	0
27/10/2020	0	0	0	0	0	0
28/10/2020	2.8	2.8	0	0	0	0
29/10/2020	0	0	0	0	0	0
30/10/2020	0	0	1.4	1.4	0	0
31/10/2020	0	0	0	0	0	0
01/11/2020	0	0	0	0	0	0
02/11/2020	0	0	0	0	0	0
03/11/2020	0	0	1.4	1.4	0	0
04/11/2020	0	0	0	0	0	0
05/11/2020	0	0	0	0	0	0
06/11/2020	0	0	0	0	0	0
01/10/2020	0	0	0	0	0	0

Appendix D: **Equations and conversion units**

D.1 COD Conversion Rates

Table 41. Mass to COD, TOC and VS conversion factors for each VFA

Acid	COD factor	TOC factor	VS factor
Acetic	1.07	0.40	1
Propionic	1.51	0.49	1
Iso-butyric	1.82	0.55	1
Butyric	1.82	0.55	1
Iso-Valeric	2.04	0.59	1
Valeric	2.04	0.59	1
Hexanoic	2.20	0.62	1
Heptanoic	2.34	0.65	1
Lactic acid	1.07	0.40	1
Glucose	1.07	0.40	1

D.2 Fermentation Parameter Equations

For batch:

$$VFA\ effluent\ concentration\left(\frac{g\ COD}{L}\right) = VFA\ yield\left(\frac{g\ COD}{g\ VS}\right) \cdot Substrate\ concentration\left(\frac{g\ VS}{L}\right)$$

For semi-continuous:

$$OLR\left(\frac{g\ VS}{L\ day}\right) = \frac{substrate\ concentrion\ \left(\frac{g\ VS}{L}\right) \cdot feeding\ rate\ \left(\frac{L}{day}\right)}{reactor\ volume\ (L)}$$

$$VFA\ effluent\ concentration\left(\frac{g\ COD}{L}\right) = VFA\ yield\left(\frac{g\ COD}{g\ VS}\right) \cdot OLR\left(\frac{g\ VS}{L\ day}\right) \cdot HRT(day)$$

Appendix E **: Derivation of NaOH equation**

Concentration of VFA can be defined as:

$$C_{VFA} = [AH] + [A^-]$$

pH and pKa relation:

$$\mathrm{pH} = \mathrm{pKa} - \log_{10}\left(\frac{[A^-]}{[AH]}\right)$$

Therefore:

$$[A^-] = 10^{pH-pKa}[AH]$$

$$[A^-]_0 = 10^{pH_0-pKa}[AH]_0$$

$$[A^-]_f = 10^{pH_f-pKa}[AH]_f$$

As NaOH is a strong base, we can assume that:

$$[NaOH] = [A^-]_f - [A^-]_0 = [AH]_0 - [AH]_f$$

Therefore:

$$C_{VFA} = [A^-]_f + [AH]_f = [A^-]_0 + [AH]_0$$

$$C_{VFA} = (10^{pH_0-pKa} + 1)[AH]_0$$

$$C_{VFA} = (10^{pH_f-pKa} + 1)[AH]_f$$

Combining:

$$[\mathrm{NaOH}] = \frac{\mathrm{C_{VFA}}}{10^{\mathrm{pH_0-pKa}} + 1} - \frac{\mathrm{C_{VFA}}}{10^{\mathrm{pH_f-pKa}} + 1}$$

Appendix F **: Contributions**

- Presented at the NAXOS 2018 conference (Greece, 6th International Conference on Sustainable Solid Waste Management)
- Presented at the AD network conference in York (2018)
- Presented poster at the Anaerobic Digestion Colloquium (Manchester, 23rd-25th January)
- Won faculty runner up prize – Three minutes thesis (March 2019)
- STEM for Britain poster competition (March 2020)
- Won faculty runner up prize – Three minutes thesis (May 2020)
- ECR online EBnet event – poster presentation (June 2020)

Glossary of Terms

Akaike information criterion (AIC): statistical measurement of model validity.

Biogas: mixture of different gases, primarily methane and carbon dioxide, resulting from the digestion or metabolisation of organic matter by microorganisms.

Biomass: can refer to either mass of microorganisms from a fermentation or organic matter used to feed a biological process.

Bioplastics: plastics derived from renewable sources or produced by microorganisms.

Chemical oxygen demand (COD): measurement of amount of oxygen required to oxidise organic compounds.

Consecutive Hydrolysis and Fermentation (CHF): two-step process involving enzymatic hydrolysis and fermentation in consecutive order.

Continuous stirred tank reactor (CSRT): reactor model that assumes perfect mixing and continuous feed.

Degree of acidification (DoA): COD or TOC due to VFA over total soluble COD or TOC.

Digestate: Effluent or liquid-solid mixture resulting from anaerobic digestion or acidogenic fermentation process. Digestate composition will depend on the type of feedstock.

Hydraulic retention time (HRT): average time that liquid bulk remains inside the reactor.

JMP: computer software for statistical analysis developed by SAS Institute.

Municipal solid waste (MSW): waste collected from municipalities generally containing packaging materials, food waste, garden waste and miscellaneous.

Organic loading rate (OLR): mass of organic substrate fed per volume unit of reactor and time unit.

Simultaneous Hydrolysis and Fermentation (SHF): single-step process involving enzymatic hydrolysis and fermentation at the same time, where exogenous enzymes are added at the beginning or during the fermentation process.

Sludge: biosolids resulting from wastewater processes such as primary sludge, waste activated sludge, sewage sludge, mixed sludge and excess sludge. When sludge is used as feedstock in anaerobic digestion it is referred to as raw sludge. Sludge has also been used in this thesis to refer to the bacterial flocs or suspensions formed inside anaerobic digestors (e.g. granular sludge).

Volatile fatty acids (VFA): herein, this term refers to fatty acids from two to seven carbon atoms.

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