Innovative Routes for Enhanced Methane Production Using Microwave Pre-treatment of Food Waste in Anaerobic Digestion

by

Mariana Vieira Turnell Suruagy

Submitted in accordance with the requirements for the degree of
Doctor of Philosophy

The University of Leeds
School of Civil Engineering

September 2020

The candidate confirms that the work submitted is her own, except where the work has formed part of a jointly-authored publication and has been included. Also, appropriate credit has been given within the thesis where reference was made to the work of others.

I. Okoro-Shekwaga, C. K., **Suruagy, M.**, Ross, A. B. and Camargo Valero, M. A. Particle size, inoculum-to-substrate ratio and nutrient media effects on biomethane yield from food waste, published in Renewable Energy, Vol. 151, pages 311-321. DOI: 10.1016/j.renene.2019.11.028 (Appendix A).

II. **M.V.T. Suruagy**, A.B. Ross and M.A. Camargo-Valero. Influence of microwave pre-treatment on the biomethanation of food waste under mesophilic anaerobic conditions. *In Proceedings* of the 10th International Forum of Solid Waste, João Pessoa, Paraíba, Brazil. 2019. ISSN: 2527-1725 (Appendix B).

In Publication I, the candidate contributed to lab experimentation, data analysis and draft of the manuscript. Okoro-Shekwaga, C. K. designed the experiments, collected data, analysed the results and drafted the manuscripts. Dr. Ross, A. B. and Dr Camargo-Valero, M. A. reviewed and provided suggestions to all manuscript versions. In Publication II the candidate designed the experiments, collected data, analysed the results and drafted the manuscripts. Both papers have been reproduced in some sections of Chapter 4 and 5.

DEDICATION

I dedicate this thesis to my family, including my loving husband Edgar Suruagy and my beloved children Edward and Gabriel. Cannot forget to mention my parents Maria Vieira and David Turnell (in memoriam), who always stimulated my curiosity for life and science.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my parents Maria de Fatima Vieira and David Turnell (in memoriam) for instigating my curiosity for life and science since my young age. The stimulation for knowledge would not have been fructiferous if it wasn't for your investment and sacrifice towards giving me and my siblings access to the best education. Thank you for always believing in me.

I am also thankful to my siblings, in-laws, uncles and aunts for their love and encouragement during this process.

My sincere gratitude to my husband Edgar Suruagy who believed in my potential as a researcher long before I did. For taking good care of our precious sons Edward and Gabriel during these four years I was away. Thank you Edward and Gabriel for reenergizing my strength with a smile and love via Skype® and during the few moments we could be together during this time.

My appreciation to the Brazilian Government through CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior for funding my PhD research.

I would like to thank my fellow laboratory colleagues Christian, Vicky, Cynthia, Cigdem, Dorian, Saleh and Fika, for their support and friendship during my PhD journey. I would also like to express my appreciation to the staff members of the IPHEE laboratory: Dr. David Elliot, Emma Tidswell and Morgan McGowan, to the staff from the Energy Building: Dr. Adrian Cunliffe and Karine Alves, as well as Dr. Andrew Robson and Dr. Andrew Connelly technicians from Earth and Environment building, who were extremely helpful with the microwave equipment.

My gratitude to Dr. Miller Alonso Camargo-Valero for his support during my PhD studies. For his constant patience and encouragement of my work. For the important and pertinent advice on my research project, which without a doubt has made me a better researcher. I would also like to acknowledge my co-supervisor Dr. Andrew Ross for the granted access to the microwave equipment.

My deepest gratitude to Dr. Ian Andrew Glover for the help towards proof reading my thesis and the microwave energy balance analysis. Truly grateful for the valuable discussions.

Thank you GOD, for giving me strength and health to complete such an important task in my life, for the one more blessing.

ABSTRACT

Increasing amounts of food waste (FW) are being generated around the globe. The most common disposal route for this FW is landfill, contributing to the emission of greenhouse gases (GHG), soil and groundwater pollution and undesirable leachates. Recovery of energy from FW (e.g. in combined heat and power systems and methane production) is generally achieved through anaerobic digestion (AD). The consequent increase of AD technology used worldwide has resulted in a significant growth in the amount of digestate produced, increasingly used for soil amendment. Strict land regulations, land shortages and rising disposal costs (including gate fees) requires digestate management to be re-thought. This study addresses strategies to optimise biomethane production from FW under mesophilic anaerobic conditions (MAD).

The first strategy identifies the best combination of particle size and inoculum-to-substrate ratio (I/S) for biomethane production without nutrient supplementation. An I/S ratio of 3 combined with a 1 mm particle size promotes a stable digestion process and increases methane yield by 95% with respect to yield for and I/S of 1.

The second strategy addresses the effects on AD of pre-treating the FW with microwave (MW) irradiation. Improved hydrolysis rate, reduced T80 and higher methane yields are obtained when compared with untreated samples. Optimum MW heating parameters (final temperature 85°C, heating rate 7.8˚C/min) are identified from a range of four final temperatures and three heating rates. A higher final temperature regardless of the heating rate, resulted in poor process performance due to bacterial inhibition, probably related to the formation of Maillard compounds (phenols and melanoidins). Solid fraction removal resulted in a 65% increase in methane yield compared to the digestion of raw FW, and up to a 74% increase in methane yield obtained when compared to the digestion of MW FW whole fraction.

The third strategy addresses the effects of re-circulating MW pre-treated digestate into the MAD of FW in a proposed new cascade process. This novel approach has several advantages over digestion of raw FW alone including: a) avoidance of system acidification; b) amelioration of TVFAs/alkalinity ratio and c) increased biodegradability. Energy recovery from digestate was successfully achieved for all of the conditions tested. This strategy resulted in increases of 109% and 132% in methane yield in relation to when MW FW treated at a final temperature of 85°C and a heating rate of 7.8˚C/min, and in relation to raw food waste digested alone, respectively.

Combining AD with MW pre-treatment has a lower environmental impact (reducing the carbon footprint by 98%) than food waste disposed to landfill and thus represents an attractive strategy for food waste and digestate management. There is a positive net energy balance for selected scenarios suggesting that residual energy in the form of biomethane can be recovered from digestate and FW using the cascaded process proposed here transforming these substrates from a cost to a source of revenue.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS/ABBREVIATIONS

AD	Anaerobic Digestion
ASBR	Anaerobic Sequencing Batch Reactors
AFW	Autoclaved Food Waste
AW	Abattoir Wastewater
BEA	Back to Earth
BMW	Biodegradable Municipal Waste
CFW	Composite Food Waste
CH	Conventional Heating
CHP	Combined heat and power
CSTRs	Continuous stirred-tank reactor
CV	Coefficient of Variance
DEFRA	Department for Environment, Food and Rural Affairs
DIG	Potential of digestate
DmFnt	Dual stage system treating liquid fraction of FW without pre-treatment
DmWnt	Dual stage system treating whole fraction waste without pre-treatment
DmWt	Two-stage system treating microwaved whole fraction
EPS	Extracellular Polymeric Substances
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FCC	Federal Communications Commission
FSC	Food Supply Chain
FVW	Fruit and Vegetable Waste
fw	Fish Waste
FW	Food Waste
GHG	Greenhouse Gas
GNI	Gross National Income
HaFS	Households, Hospitality and Food Service
HM	Hydrogenotrophic Methanogenesis
HRT	Hydraulic Retention Time
IEA	International Energy Agency
IPHEE	Institute of Public Health and Environmental Engineering
ISM	Industrial, Scientific, and Medical
KW	Kitchen Waste
kWh	Kilowatt hour
LATS	Landfill Allowance Trading Scheme
LCFA	Long-chain Fatty Acids
LFD	Liquid Fraction Digestate
LOF	Labile Organic Fraction
MAD	Mesophilic Anaerobic Digestion
MC	Moisture Content
Mpa	Mega Pascal
MSW	Municipal Solid Waste
MWSS	Microwaves sewage Sludge
MWW	Municipal WasteWater

NEB	Net Energy Balance
NVZ	Nitrate Vulnerable Zones
OFMSW	Organic Fraction of Municipal Solid Waste
OLR	Organic Loading Rate
PAS	Publicly Available Specification
PHA	Polyhydroxyalkanoates
PS	Primary Sludge
RF	Radio Frequency
RNM	Retention of Nutrients and Microorganisms
RSN	Retention of Soluble Nutrients
SAO	Syntrophic Acetate Oxidation
SBR	Sequencing Batch Reactors
SCOD	Soluble Chemical Organic Demand
SFt	Single-stage System Treating Liquid Fraction
SKW	Synthetic Kitchen Waste
SS-DIG	Solid Separated Digestate
STP	Standard Temperature and Pressure
SWA	Supplemental Water Additions
SWA20	Supplemental Water Additions 20%
SWA30	Supplemental Water Additions 30%
TBP	Total Biogas Productions
TCOD	Total Chemical Oxygen Demand
TKN	Total Kjeldahl Nitrogen
TMP	Theoretical Methane Potential
TWAS	Thickened Waste Activated Sludge
US	Ultrasound
VFA	Volatile Fatty Acid
VSS	Volatile Suspended Solids
WAO	Wet Air Oxidation
WAS	Waste Activated Sludge
WRAP	Waste & Resources Action Programme
WWTP	Waste Water Treatment Plant

CHAPTER 1

1. INTRODUCTION

1.1 Justification

Recent population growth together with increasing urbanization has led to increased generation of organic waste including Municipal Solid Waste (MSW). As of 2011, the world generated an estimated two billion tonnes of MSW (Amoo and Fagbenle, 2013). It is predicted that, globally, approximately $2.5 \cdot 10^9$ tonnes of Food Waste (FW) will be generated by 2025 (Karthikeyan *et al.*, 2018). According to the Intergovernmental Panel on Climate Change (IPCC, 2006), MSW (which excludes industrial waste) comprises plastic, metal, glass, textile, wood, rubber, leather, paper, food and others. FW is a mixture of organic materials that can originate from the processing, sorting, cooking, preparation and handling of food, and makes a dominant contribution to MSW (25–70%) (Ariunbaatar *et al.*, 2015). From the moment food is produced, to its packaging, distribution and storage, it requires the use of a range of environmental resources such as water, land, fertilizer, fuel and energy. It also requires human labour and capital. Most of these stages emit Greenhouse Gases (GHG) thereby contributing to climate change (Xu *et al.*, 2015).

According to the Waste Resources Action Programme (WRAP, 2020), the annual FW arising within UK households, hospitality and food service (HaFS), food manufacture, retail and wholesale sectors in 2018 was around 9.5 million tonnes, representing a 5% increase in relation to 2012. Of this total, 70% was classified as avoidable waste and 30% (the ‘inedible fraction’) was classified as unavoidable waste. This represented a financial drain of £19 billion, and was responsible for the emission of more than 25 million tonnes of GHG (WRAP, 2020).

FW management is a challenging task due its high moisture content and propensity to decay rapidly under ambient conditions. Conventional disposal methods such as landfilling are not desirable due to (a) methane emissions, a gas 20 – 25 times more powerful in its greenhouse effect than CO_2, (b) soil and groundwater pollution, (c) undesirable leachates, and (d) utilization of large areas of land (Zhang and Jahng, 2012).

In Europe, the European Landfill Directive is one of the legislations applied for solid waste management. According to the Department for Environment, Food and Rural Affairs DEFRA (2015), the UK’s Biodegradable fraction of the municipal waste (BMW) sent to landfill has reduced monotonically over the past years. In 2013, BMW sent to landfill was 9.2 million tonnes and in 2010 it was 13 million tonnes. The 2013 figure represents 26% of the 1995 baseline value, which comfortably met the 2013 EU target (which was set as no greater than 50% of the 1995 baseline). However, there is a further EU target to restrict the proportion of BMW going to landfill to 10% by 2035.

Nevertheless, Landfill (European Parliament and Council, 2018a),the Waste Framework (European Parliament and Council, 2018b), and Packaging Waste (European Parliament and

Council, 2018c) Directives were amended in 2018 to include new targets and actions beyond 2020 as follows:

- targets for reuse and recycling of municipal waste to be at least 55% by 2025, 60% by 2030 and 65% by 2035;
- targets for recycling of packaging waste to be at least 65% by 31 December 2025 and 70% by 31 December 2030;
- a target to reduce the proportion of municipal waste going to landfill to a maximum of 10% by 2035;
- a ban on landfilling of waste suitable for recycling effective from 2030;
- mandatory separate collection of bio-waste by 31 December 2023 and of textiles and hazardous waste from households by 1 January 2025;
- minimum requirements for all extended producer responsibility schemes;
- simplified and improved definitions and harmonised calculation methods for recycling rates throughout the EU.

Despite Brexit, the United Kingdom (UK) has already adopted EU legislation into UK law and currently the same targets remain for the UK. Continuing effort will therefore be needed to divert BMW fraction from the landfill disposal route assuming the UK own regulatory targets in this area do not diverge significantly from those of the EU in the coming years (House of Commons, 2016).

As well as a reduction in the amount of biodegradable waste going to landfill, the UK government committed to achieving a 60% reduction in GHG emissions by 2050 (BERR and DEFRA, 2003). Over the coming years, the UK must overcome the energy gap resulting from the closure of coal and nuclear power plants, which in 2006 represented 30% of the electricity supply (Shah, 2006). It will also have to find ways of mitigating the fact that the fraction of energy consumption represented by gas is rising (from 32% in 2009 to an expected 50% in 2020), and that by 2020 up to 90% of the UK gas requirements will rely on imports from the Soviet Union, North Africa and the Middle East triangle (Remme *et al*., 2008).

In this context, FW needs to be avoided, reduced or recovered. If recovered, it needs to be processed and recycled with a view to developing a sustainable circular economy (Karthikeyan *et al*., 2018). Moreover, if large amounts of organic waste are to be diverted from landfill, sustainable strategies for treating this waste and recovering valuable products including energy from it must be investigated. The shift towards sustainable energy systems offers a promising solution to reduce environmental pollution and provide an alternative fuel supply (Ramanathan *et al*., 2016; Elsayed *et al*., 2019). In this respect, it has been said that FW management can make an important contribution (Lee and Willis, 2010).

Recovery of energy and valuable compounds from organic wastes, including FW, is now common throughout Europe. The most established technology for bioenergy production is Anaerobic Digestion (AD). This is a microbiologically mediated process during which organic carbon present in biopolymers and other degradable compounds is converted to its most reduced form (methane) and its most oxidised form (carbon dioxide) in the absence of oxygen. Trace amounts of other gases such as nitrogen (N_2), hydrogen (H_2) and hydrogen sulphide (H_2S) are also formed (Pind *et al*., 2003; Zhu *et al*., 2009; Madsen *et al*., 2011). This is a promising approach due to its capacity to treat and stabilize organic matter, as well as producing renewable energy in the form of biomethane (Pullen, 2015). It has therefore, been regarded as an attractive option for FW management (Veeken and Hamelers, 1999).

AD of FW is a complex process. FW is converted into biogas or biomethane by the sequential actions of fermentative bacteria and methanogenic archaea, while minerals are retained in the digestate as nutrients. The organic carbon conversion rate is directly proportional to the biogas yield under well-operated conditions (Khalid *et al*., 2011; Karthikeyan and Visvanathan, 2013). AD in the United Kingdom is already well established. New reactor plants have been installed in the UK since 2012 at an average rate of 25 to 30 reactors/year (WRAP, 2013). In 2019, there were a total of 611 AD treatment plants, most of them operating on a commercial scale and processing different types of material including FW, manure, slurries, crop residues and purpose-grown crops; of this total, 111 treat agricultural waste and FW (WRAP, 2020). An increase in the number of AD plants, ultimately leads to an increase in the amount of digestate being produced.

On the other hand, the UK's recent regulations regarding digestate application to agricultural land and crops has become stricter. Digestate originating from mixed waste materials is now limited to use in land restoration projects only (WRAP, 2013). Additionally, an increased digestate volume will require a substantial area of land to provide a secure and suitable market for this product (WRAP, 2013). Not all available land, however, is suitable for digestate application. Reasons for this include: a) the spreading window being limited to certain times of the year (e.g. not being allowed in winter) and/or the land not requiring nutrients, b) the agriculture demand for nutrient supply fluctuating due to the crop cycle and c) land already being saturated with phosphorus and nitrates (Sharpley, 2016; WRAP, 2016).

With increasing amounts of digestate being produced, the costs associated with its transportation and spreading has become a challenge for operators. A supplier of digested fibre in the earlier 1990s could command up to £20/tonne (£26/tonne, 2007 prices) when selling it as a fertilizer for agriculture application. Digestate was therefore a profitable business at that time. However, since 1996, the price of digestate has decreased to between £0 and £13/tonne (including delivery and spreading costs) in 2015. Therefore, the value of digestate to the producer is now low, and in some cases may be negative (WRAP, 2009; Horan *et al*., 2015). With third party contractors willing to take the material away for around £10 per cubic metre, a 30 kt AD plant could incur digestate disposal costs in excess of £250,000 a year (Lewens, 2011; Horan *et al*., 2015). At the same time, relying solely on the agricultural route for the digestate, leaves the AD industry exposed to a significant risk since capital-intensive enhancement methods do not favour the development of optional solid markets for the digestate,

Many attempts to address these problems have been made over the past decade, including various innovative approaches to digestate valorisation. Most of them involve dewatering the digestate resulting in fibre and liquor. This has the advantage of reducing the initial volume of digestate for subsequent storage and off-site transportation, as well as expanding its potential markets. However, dewatering represents a substantial investment (high capital costs) and typically comes also with high maintenance costs (Horan, 2012).

Several strategies to recover value from digestate are available once the digestate is separated into liquor and fibre (also known as digestate cake). The most common techniques for enhancing the fibre are enzymatic hydrolysis, composting, alkaline stabilization land application. All these techniques are available in the United Kingdom. Most of these processes are used for converting the cake into a soil improver or act as a pathogen killing mechanism, and none of them results in energy recovery or elimination of the digestate. An exception is the enzymatic/thermal hydrolysis process, which reduces the volume of solid digestate by converting it into a bio-liquid that can be used to produce bio-fuels (WRAP, 2013).

Recovery of energy from the fibre is possible, through process like incineration, gasification and pyrolysis. However, to take advantage of this, an additional drying step would have to be added to the system, implying more investment, and the cost-benefit analysis may not always be positive. In the case of food-based digestate, the dewatering process can be even more complex. The surface charge properties of food-based digestates make it challenging to dewater using standard approaches. While dewatering of food-based digestates has proven possible by selection of the correct polymer, polymer dose and dosing conditions remain economically challenging (Horan *et al*., 2015).

With respect to the liquid fraction of digestate, several nutrient recovery processes can be adopted to produce refined end products (i.e., concentrated nutrients and purified water) including biological oxidation (i.e. Algal pond/ Photo Bio reactor) in which the liquor is used as a feedstock for algae growth, and ammonia striping, and evaporation (Peng and Pivato, 2017). The liquid fraction of digestate from OFMSW and FW cannot be directly used for agricultural purposes without further treatment, following Article 6 of the EU Waste Framework Directive EEC, 2000 (Malamis *et al*., 2016). This is because FW, as a complex waste (with broad origin, composition, etc.) makes the quality of its digestate and input materials difficult to stablish, hence limiting its application and use (Saveyn and Eder, 2014).

It is necessary, therefore, to study alternative approaches for digestate management and utilization options when considering AD as an option for FW processing and valorisation into biomethane (Wellinger *et al*., 2013; Neumann *et al*., 2016).

When treating FW, low methane yields are obtained (50 - 60% of the theoretical maximum value). This is related to the complex structure and composition of its components (lignocellulosic materials, fats and proteins), which make hydrolysis the limiting step for anaerobic processing (Marin *et al*., 2010). This limitation can be circumvented by adopting different feedstock pre-treatments. Thermal processing, with or without mechanical pre-treatment (e.g., grinding) has been shown to achieve significantly higher methane (CH_4) yields and it is thus considered by many to be the most reliable pre-treatment option for FW to enhance biomethane production in AD reactors (Naran *et al*., 2016).

Microwave irradiation (MW) or Radio Frequency (RF) heating, a particular form of thermal treatment, takes place in nonconductive materials at frequencies between 3 and 300 GHz. Microwaves (MWs) belong to that part of the electromagnetic spectrum with wavelengths from 1 mm to 1 m with corresponding frequencies between 300 MHz and 300 GHz and constitute a subset found at the higher frequency end of the radio spectrum. The electromagnetic spectrum is not used only (or even primarily) for heating, but also for cellular phones, radar, and satellite communications (including satellite TV). Nevertheless, the Federal Communications Commission (FCC) for industrial, scientific, and medical (ISM) purposes reserves two common frequency bands for RF and MW heating centred on 0.915 and 2.45 GHz (Lauf *et al*., 2013).

Although the main use of microwave heating over the past 40 years has been to serve as a kitchen-cooking appliance, especially in western countries, new applications have recently arisen. Its application in the processing of materials and, more specifically, the pre-treatment for organic wastes such as sludge, are relatively new developments (Thostenson and Chou, 1999). Due to its high moisture content and suspended organic solids content, FW (and its variations, including Kitchen Waste (KW), fruit and vegetables wastes, etc.) also seems to be suitable candidates for MW irradiation pre-treatment.

Overall, MW irradiation has proven to be effective in solubilising organic matter, improving biodegradability and the production of biogas and biomethane from both FW and sludge (Qiao *et al*., 2008; Toreci *et al*., 2009; Marin *et al*., 2010; Zhang *et al*., 2016).

Like sewage sludge, digestate is as an end product of a biological treatment process, and has similar properties such as an incomplete mineralization stage (20 - 60%) at the end of the AD (Park and Ahn, 2011). It still, therefore, has organic matter available post AD for microbial actuation and degradation. Several pre-treatment methods to enhance methane recovery from this substrate have been tested, including mechanical, thermal and chemical treatments (Menardo *et al*., 2011; Biswas *et al*., 2012; Lindner *et al*., 2015). However, most have not been widely adopted due to limitations related to high-energy requirements. A chemical-free and environmentally friendly pre-treatment method is therefore desired and MW irradiation has been identified as a possible candidate (Liu *et al*., 2019).

Microwave irradiation of digestate has not yet been widely tested/adopted, despite several studies which have demonstrated its efficacy in improving solubilisation of organic matter, VS removal and biogas production in the context of sewage sludge processing (Kaparaju and Rintala, 2005; Menardo *et al*., 2011; Biswas *et al*., 2012; Lindner *et al*., 2015). A further option for digestate management is to re-circulate the material into AD after it has been pre-treated. This particular strategy is not new and the overall effects of digestate re-circulation have been reported to be positive varying from enhanced VS removal to methane yield. Nevertheless, no previous work on the re-circulation of pre-treated digestate on the AD of FW was found at the time of conducting this study.

In this research project, microwave irradiation use is considered not only a suitable option to effectively pre-treat FW and digestate, but it can also be an important tool for the recovery of energy (biomethane) and nutrient recycling from these underutilized substrates when coupled with AD.

1.2 Aim, objectives and scope

1.2.1 Aim and objectives

The aim of this project is to explore alternative paths for the enhancement of energy recovery from FW via anaerobic digestion by integration with microwave irradiation pre-treatment of FW and digestate. The cascaded processes intended to eliminate the environmental impacts of FW discarded in landfills and transform digestate from a cost to a revenue centre. In that context, this project comprises a series of experimental work using food waste generated at the refectory of the University of Leeds to determine the most suitable process conditions that allow the integration of microwave pre-treatment to enhance biomethane production from AD reactors.

The overall aim was achieved by the following objectives:

Objective 1: To evaluate the full potential of FW as a feedstock for anaerobic digestion by assessing its anaerobic biodegradability (Biomethane Potential – BMP) with and without balancing essential nutrients (Carbon, Nitrogen and Phosphorous) and by optimizing BMP test conditions (particle size, inoculum/substrate (I/S) ratio).

Objective 2: To evaluate the impact of MW irradiation process conditions (final temperature and heating rate/power) on the characteristics of MW FW and its potential for biomethane production under Mesophilic Anaerobic Digestion (MAD) conditions.

Objective 3: To evaluate the impacts of MW process conditions (final temperature and set heating rate/power conditions) on the characteristics of MW treated digestate from FW processing, and the potential for digestate recycling into MAD reactors processing raw FW to enhance biomethane production.

Objective 4: To assess the technical feasibility of integrating MW pre-treatment and AD for selected scenarios processing FW and digestate, through mass and energy balances, based on the results obtained from experimental analyses.

1.2.2 Scope

The integration process concept was developed and tested using lab-scale Anaerobic Batch reactors, that provided suitable data for conducting mass and energy balances to model process conditions and to predict biomethane yields.

1.3 Thesis structure

The thesis is organized in nine chapters with the introduction section comprising the first chapter, which summarises the research problem and gaps, and presents the aim, objectives and scope of the study.

Chapter 2 provides the rationale for the study. It includes a thorough literature review of the FW problematics, (including its generation and environmental impacts) and current waste to energy conversions, focusing on AD. The impacts of increasing digestate volumes being produced over the last decades (as a result of AD becoming a feasible and well-established technology for energy recovery and waste stabilization) are also discussed, focusing on the current waste to land limitations. The technological approaches developed to treat this substrate for energy recovery and by product generation are briefly described, with positive and negative aspects of each, highlighted. Microwave heating as a recent technology to treat organic waste including FW, is described and the benefits of a cascade process with AD presented. The novel aspect of MW irradiation on digestate management is also highlighted.

Chapter 3 describes the material and methods used. An overall description can be found at the beginning of the chapter (e.g. FW collection, characterization, digestate characterization and BMP tests), followed by a detailed explanation of each experimental phase.

In chapters 4 to 7, the results arising from each stage of the project are reported and discussed. These chapters have been written in a paper format and, therefore, contain appropriate sections including introduction, results, discussions and summary.

Chapter 4: "Characterization of FW and its potential use as a feedstock for methane (CH_4) production via mesophilic anaerobic digestion", is related to Objective 1, and focusses on the effect of nutrient supplementation, particle size and inoculum to substrate ratio (I/S) on the biomethanation of FW. The overall suitability of the source of FW used as a substrate for the AD experiments is addressed. The results for optimum particle size and I/S ratio are used as a reference for the further research objectives. Material in Chapter 4 draws form and/or has been presented in the following abstracts and publications. Turnell, Suruagy. M.V, Camargo-Valero, M. A. **Alternative route to digestate stabilization and recovering residual value from food waste**, presented at AD Network - Early Career Researcher Event (oral presentation), Birmingham, United Kingdom, 2016. Okoro-Shekwaga, C. K., Suruagy, M., Ross, A. B. and Camargo-Valero, M. A. **Particle size, inoculum-to-substrate ratio and nutrient media effects on biomethane yield from food waste**, published in Renewable Energy, Vol. 151, pages 311-321. DOI: 10.1016/j.renene.2019.11.028.

Chapter 5: “Influence of microwave temperature and power on the biomethanation of food waste under mesophilic anaerobic conditions” focuses on Objective 2 and discusses how the final temperature and heating rates/power change the characteristics of FW and MAD process performance, including SCOD removal, Volatile Fatty Acids (VFAs) behaviour, solubilization and biodegradability. The amount of microwaved food waste to inoculum on each reactor followed the optimum conditions established in Chapter 4. The main factor influencing process performance and the optimum microwave operating parameters for energy recovery from food waste are established. Part of the results from this chapter were presented orally at the following conferences:

M. V. T. Suruagy, A. B. Ross and M. A. Camargo-Valero. **Influence of microwave irradiation pre-treatment on the biodegradability of food waste under mesophilic anaerobic conditions**. In XIII Taller Y Simposio Latinoamericano de Digestión Anaerobica, Medellin, Colombia. 2018.

M.V.T. Suruagy, A.B. Ross and M.A. Camargo-Valero. **Influence of microwave pre-treatment on the biomethanation of food waste under mesophilic anaerobic conditions.** Published in the event proceedings- Anais do 10ª Fórum Internacional de Resíduos Sólidos, João Pessoa, Paraíba, Brazil. 2019. ISSN: 2527-1725.

Chapter 6: “Influence of microwave irradiation on the biomethanation of the soluble fraction of food waste under mesophilic anaerobic conditions” also addresses Objective 2. This chapter was added after performing BMP tests on the microwave pre-processed food waste under various temperatures and heating rates. It includes a detailed analysis of the biomethane potential originating from the soluble fraction of microwaved samples processed with a final temperature of 175°C. Important changes in the characterization of the microwaved waste after phase separation are discussed. Improvements in process performance as a result of solid fraction removal is highlighted, with emphasis on the resulting methane yields.

Chapter 7: Effects of the re-circulation of microwave pre-treated digestate on the mesophilic anaerobic digestion of food waste” addresses Objective 3 and presents the results of BMP tests performed for different volumes of MW pre-treated digestate together with raw food waste. This constitutes a novel approach for a co-digestion process using FW and digestate. The effects of MW process conditions on the digestate’s characteristics are discussed, as well as its influence on BMP test performance. The process performance improvement, including VFAs behaviour, biodegradability, technical digestion time and process kinetics, is discussed in detail. These results are compared both to each other and to the previous tests reported in Chapter 5 and Chapter 6. The energy recovery of the re-circulated digestate relative to raw food waste digested alone is addressed in this chapter. This, together with the final methane yield, is evidence that the co-digestion of this substrate is capable of producing more biomethane than the digestion of raw food waste alone. At the end of the chapter, a statistical relationship between methane yield obtained from the co-digestion process and the previously tested conditions with high methane yields is established, and the optimum condition for energy recovery, identified.

Chapter 8: “Mass and energy balance study for the integration of microwave irradiation and anaerobic digestion of food waste and digestate” is related to Objective 4. Total and Volatile Solids removal, together with Total and Soluble Chemical Oxygen Demand removal constitutes the mass balance analysis of the scenarios tested. The energy balance is shown from a Net Energy Production perspective and the process feasibility based on this parameter for all the conditions tested is discussed. The net energy together with the mass balance is an important consideration when deciding on the optimum strategy for energy generation from FW and digestate on an industrial scale.

Chapter 9: General discussions, discusses the main findings regarding the use of microwaved FW and microwaved digestate on the mesophilic anaerobic digestion on batch tests and its implications when applied to a large-scale system (e.g. CSTR reactors).

Chapter 10: Conclusions and recommendations, presents the overall conclusions of the thesis and makes recommendations for future work.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Research problem – theoretical context and motivation

2.1.1 Food waste generation

The definition of FW is not simple and it can be analysed from many different perspectives. The more "traditional" definition of food waste is related to discarded household food either because was not eaten by the due date or because it was left on plates after a meal. A broader definition takes account of food discarded at any point in the Food Supply Chain (FSC). Table 2.1 shows the various stages of the FSC and their respective food waste generation. Therefore, food waste includes any waste generated during production, processing, distribution, consumption and/or disposal. During harvesting, for example, food can be lost to consumption by animals such as birds or rodents. Harvesting losses can also be incurred (for example due to inclement weather) if the timing of harvest is not optimal. However, for most people, FW is more obvious at the final stages of the supply chain: retail and consumer stages (Table 2.1) (Parfitt *et al.*, 2010).

According to Lin *et al*. (2013), food waste can be defined as:

> *The end-product of various food processing industries that has not been recycled or used for other purposes. It is the non-product flows of raw materials whose economic value is less than the cost of collection and recovery for reuse; therefore, discarded as waste.*

Lipinski *et al*., (2013) on the other hand, defines food waste as:

> *Food that is of good quality and fit for human consumption but that does not get consumed because it is discarded either before or after it spoils. Food waste typically, but not exclusively, occurs at the retail and consumption stages in the food value chain and is the result of negligence or a conscious decision to throw food away.*

Although several attempts have been made to quantify global FW production, they are, typically, underestimated. This is because of the variety of definitions of FW and the fact that waste can arise at any stage of the FSC, thus making it difficult to scrutinize. However, the most often quoted estimate of food lost or wasted throughout the supply chain, from production to consumption, is about 1.3 billion tonnes per year – i.e. one third of the food produced in the world for human consumption annually (FAO, 2011).

Table 2.1 Food Waste Generation at different stages in the supply chain (Parfitt *et al*. 2010).

	Stage	**Examples of food waste/loss characteristics**
(1)	Harvesting – handling at harvest	edible crops left in field, ploughed into soil, eaten by birds, rodents, timing of harvest not optimal: loss in food quality crop damaged during harvesting/poor harvesting technique out-grades at farm to improve quality of produce
(2)	Threshing	loss through poor technique
(3)	Drying – transport and distribution	poor transport infrastructure, loss owing to spoiling/bruising
(4)	Storage	pests, disease, spillage, contamination, natural drying out of food
(5)	Primary processing – cleaning, classification, de-hulling, pounding, grinding, packing, soaking, winnowing, drying, sieving, milling	process losses contamination in process causing loss of quality
(6)	Secondary processing – mixing, cooking, frying, moulding, cutting, extrusion	process losses contamination in process causing loss of quality
(7)	Product evaluation – quality control: standard recipes	product discarded/out-grades in supply chain
(8)	Packaging – weighing, labelling, sealing	inappropriate packaging damages produce grain spillage from sacks attack by rodents
(9)	Marketing – publicity, selling, distribution	damage during transport: spoilage poor handling in wet market losses caused by lack of cooling/cold storage
(10)	Post-consumer – recipes elaboration: traditional dishes, new dishes product evaluation, consumer education, discards	plate scrapings poor food preparation technique: edible food discarded with inedible food discarded in packaging: confusion over ‘best before’ and ‘use by’ dates
(11)	End of life – disposal of food waste/loss at different stages of supply chain	food waste discarded may be separately treated, fed to livestock/poultry, mixed with other wastes and landfilled

Thi *et al*. (2015), reported that the per capita FW productions in industrialised and developing countries is 107kg/year and 56kg/year, respectively, suggesting that in industrialised countries where the standard of living standard is higher there is greater FW generation (Lipinski *et al*., 2013). Perhaps more surprisingly, according to data gathered by the Bureau Population Reference (2014), there is no marked difference between the aggregate FW generated in industrialized (670 million tonnes) and developing (630 million tonnes) countries. This is because although FW in developing countries is low and there is both less food demand and less per capita consumption, net population in the Global South is higher – i.e., China and India, contribute with 37% of the total worldwide population (Figure 2.1).

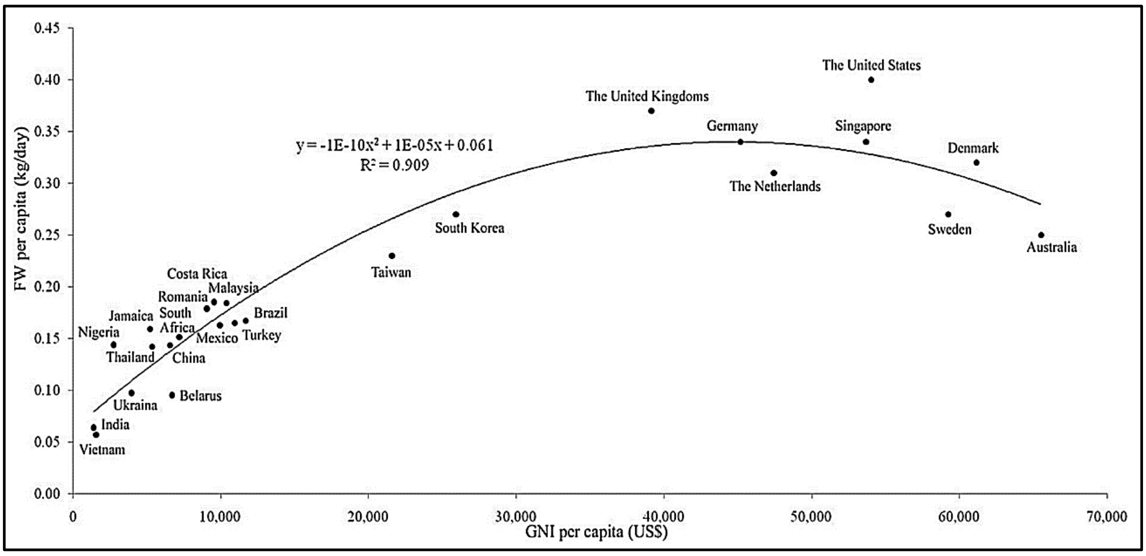


Figure 2.1 Relationship between Gross National Income per capita (GNI) and FW per capita in various countries (Thi *et al*., 2015).

Table 2.2 shows the difference in food waste generation scenarios in industrialized and developing countries. The United States, for example showed the highest FW per capita, equivalent to 0.52kg/day, which could be related to its bigger population size (316,128,839). The United Kingdom, on the other hand, exhibited a higher daily food waste generation (14,257,000) and FW per capita (0.37) than Germany (12,257,998 and 0.34 daily food waste generation and FW per capita, respectively), despite a lower population. The daily food waste generation values of industrialized countries are on average 43.82% higher than in developing ones.

Moreover, the proportion of FW in the MSW varies across the globe. Data gathered by Thi *et al.* (2015), showed that in countries such as the United Kingdom, Sweden, Germany and Nigeria FW represents a higher percentage of their MSW: 50, 67, 61 and 60% respectively. Conversely, in the Ukraine and Belarus there is a lower proportion of FW in their MSW: 37 and 27% respectively.

Table 2.2 Scenarios of food waste generation in different countries (Thi et al., 2015).

Country	GNI (US$)	Population	Total FW (tonne/year)	FW per capita (kg/day)	FW percentage in entire MSW (%)	Reference – year
Industrialised countries						
Australia	65,520	23,130,900	2,261,061	0.25	40.00	Liu, (2014)
Denmark	61,160	5,613,706	790,502	0.32	NA	Véronique *et al.* (2010), Barbara *et al.* (2012)
Sweden	59,240	9,592,552	1,915,460	0.27	67.00	Véronique *et al.* (2010), Naturvårdsverket (2010)
Singapore	54,040	5,399,200	796,000	0.40	NA	NEA (2013)
The United States	53,670	316,128,839	60,849,145	0.52	NA	Grocery Manufacturers Association (2012), Buzby and Hyman, (2012)
The Netherlands	47,440	16,804,224	8,841,307	0.31	NA	Véronique *et al.* (2010), Barbara *et al.* (2012)
Germany	45,170	80,621,788	12,257,998	0.34	61.00	Véronique *et al.* (2010), Barbara *et al.* (2012)
The United Kingdom	39,140	64,097,085	14,257,000	0.37	50.00	WRAP (2013a), WRAP (2013b)
South Korea	25,920	50,219,669	6,241,500	0.27	NA	Hou (2013)
Taiwan	21,592	23,268,087	2,318,169	0.23	NA	Taiwan EPA (2018)
Developing countries						
Brazil	11,690	200,361,925	33,489,000	0.17	54.90	Corsten *et al.* (2012)
Turkey	10,950	74,932,641	12,375,000	0.17	49.50	Sezer and Arikan, (2011), Ioannis *et al.* (2013)
Malaysia	10,400	29,716,965	5,477,263	0.18	55.00	Mohamad and Keng, (2013)
Mexico	9940	122,332,399	19,916,000	0.16	52.00	Alonso and Themelis, (2011)
Costa Rica	9550	4,872,166	903,375	0.19	NA	Dhia *et al.*, (2011)
Romania	9060	19,963,581	3,573,481	0.18	NA	Romania (2018)
South Africa	7190	59,590,000	9,040,000	0.15	NA	Wahlen and Winkel, (2017)
Belarus	6720	9,466,000	903,690	0.10	27.00	RECO Baltic Tech project (2012)
China	6560	1,357,380,000	195,000,000	0.14	56.60	Yang *et al.* (2012)
Thailand	5370	65,479,453	9,312,788	0.14	44.43	Alice and Janya, (2012)
Jamaica	5220	2,715,000	433,333	0.16	53.70	Meghan (2014)
The Ukraine	3960	45,489,600	4,440,000	0.10	37.00	IFC (2013)
Nigeria	2760	173,615,345	25,000,000	0.14	60.00	Ogwueleka, (2009)
India	1570	1,252,139,596	71,952,838	0.06	51.00	Ranjith, (2012), Jena (2013)
Vietnam	1407	89,708,900	5,743,056	0.06	60.00	Ministry of Natural Resources and Environment of Vietnam (2011)

Source Thi *et al.* (2015)

Regarding the impact of consumer's behaviour on FW generation at the consumption phase, it is found that in a population with high education level (industrialised countries), FW is mostly generated "before the meal," while for populations with low and middle education levels (developing countries), FW occurs "after the meal," due to populations in developing countries generally paying less attention to how their FW will be disposed (Maaike, 2014). This suggests that additional factors determine the amount of FW in the MSW, not just the level of development of the county ().

So, the amount of FW generated in a specific location is a multifaceted issue dependent on factors such as: the market economy, climate, legislation, cultural differences, urbanization, supply chain technology as well as dietary habits (Parfitt *et al.*, 2010). In addition, different locations imply lifestyle and cultural differences which impacts both recycling practices and the type of FW produced (Ward *et al.*, 2008).

FW composition is region specific which affects its bio-chemical characteristics. For instance, in China vegetables, cereals and fruit represent approximately 80% of the FW, whilst in Turkey vegetable and fruit represents approximately 70% of the total FW volume. Bakery and dairy products account for approximately 50% of the FW in western countries, with meat representing 3–5% of the FW in all regions. Consequently, there is a need for different management/disposal plans in different regions of the world (Karthikeyan *et al.*, 2018).

2.2 Food waste in the United Kingdom - an expensive business

In 2012, approximately 9 million tonnes of food and drink waste was generated in the UK each year. This figure includes streams such as agricultural production, food manufacture, retail/wholesale – including distribution and from stores, hospitality/'profit' catering – restaurants, pubs, hotels, and 'cost' catering – schools, hospitals, services (WRAP, 2013). It does not, however, include FW from households, which according to WRAP (2012a) was responsible for adding another 7 million tonnes of food and drink waste to the picture. Of this total, 82% was FW. Household FW is any food or drink that is consumed within the home, including retail and contributions from homegrown food and takeaways but excluding any food or drink consumed 'on-the-go', for example, in the workplace or in catering establishments (WRAP, 2013).

For research purposes, WRAP adopted the following FW categories: a) **Avoidable** – food and drink thrown away that was, at some point prior to disposal, edible (e.g. slice of bread, apples, meat); b) **Possibly avoidable** – food and drink that some people eat and others do not (e.g. bread crusts), or that can be eaten when a food is prepared in one way but not in another (e.g. potato skins), and c) **Unavoidable** – waste arising from food or drink preparation that is not, and has not been, edible under normal circumstances (e.g. meat bones, egg shells, pineapple skin, tea bags).

When considering these categories, 4.0 million tonnes per year of the total food and drink waste arising from households in the UK in 2012 was **avoidable**. The remaining 3 million tonnes was split equally between unavoidable and possibly avoidable waste (WRAP, 2013).

Just under half of avoidable food and drink waste, in 2012, was classified as ‘not used in time’ and thrown away because it had either gone off or passed the ‘consume before’ date on the packaging. A further 31% was classified as ‘cooked, prepared or served too much’: this included food and drink that had been left over after preparation or serving. Around 14% was linked to personal preferences including health reasons and not liking certain foods. Accidents – including food dropped on the floor and failure of a freezer – accounted for 4% (Figure 2.2) (WRAP, 2013). These results suggest that FW in the UK is strongly related to behaviour and habits.

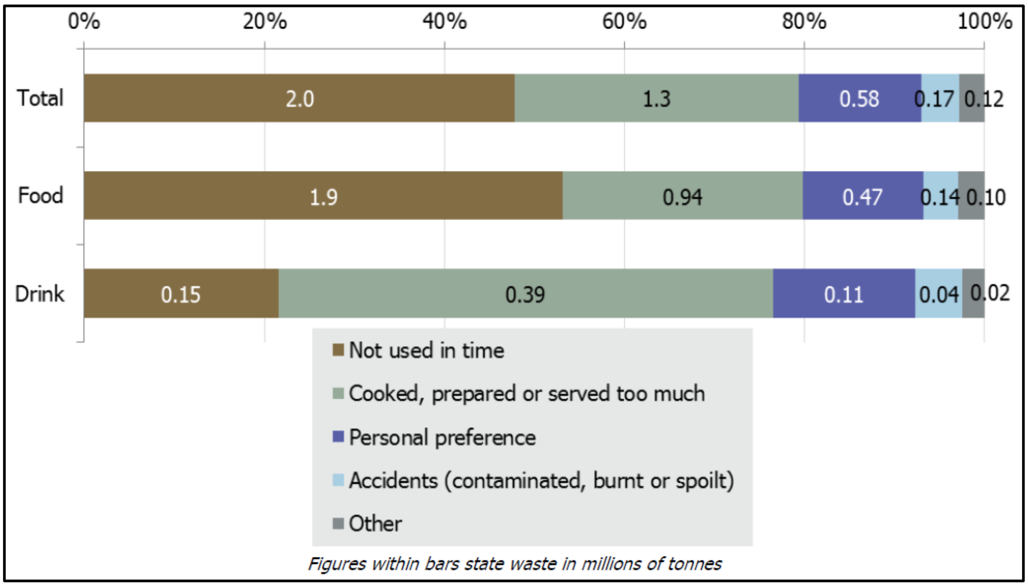


Figure 2.2 Weight of avoidable household waste (food and drink in 2012), split by reason for disposal (WRAP, 2013).

According to WRAP (2012), food and drink not used in time and cooked; prepared or served too much was estimated to be worth £9.7 billion pounds.

A later study conducted by DEFRA, (2015), estimated that 47% of the food consumed in the UK is imported, originating from various countries such as EU (28%), Africa (4%), Asia (4%) and North America (4%). In total, 23 countries accounted for 90% of UK food supply in 2013. The results showed that vegetables and fruits, as well as meat and meat products are the two largest import categories. Interestingly, these items are the ones which have the highest disposable rates on the UK’s waste stream, meaning that the government is subsiding a very expensive financial drain (Mena *et al*., 2011). In 2014, imports cost £8.7 billion, while exports were worth £0.9 billion, giving a trade gap of £7.8 billion (Figure 2.3). The latter was responsible for £6 billion of investment.

Between 2007 and 2012, the UK achieved a 15% reduction in the generation of food and drink waste despite a 4% increase in household numbers. The amount of waste prevented is considerable, and would be enough to fill 2,600 Olympic swimming pools, as well as a saving of £85 million in landfill tax and gate fees for the local authorities (WRAP, 2013). However, the UK still has a long way to reach a zero-waste economy (WRAP, 2013). Furthermore, it has been estimated that all the FW in the UK would be enough to feed 250,000 people. This raises moral questions, since in 2011 one billion people worldwide were considered malnourished (Naylor, 2011).

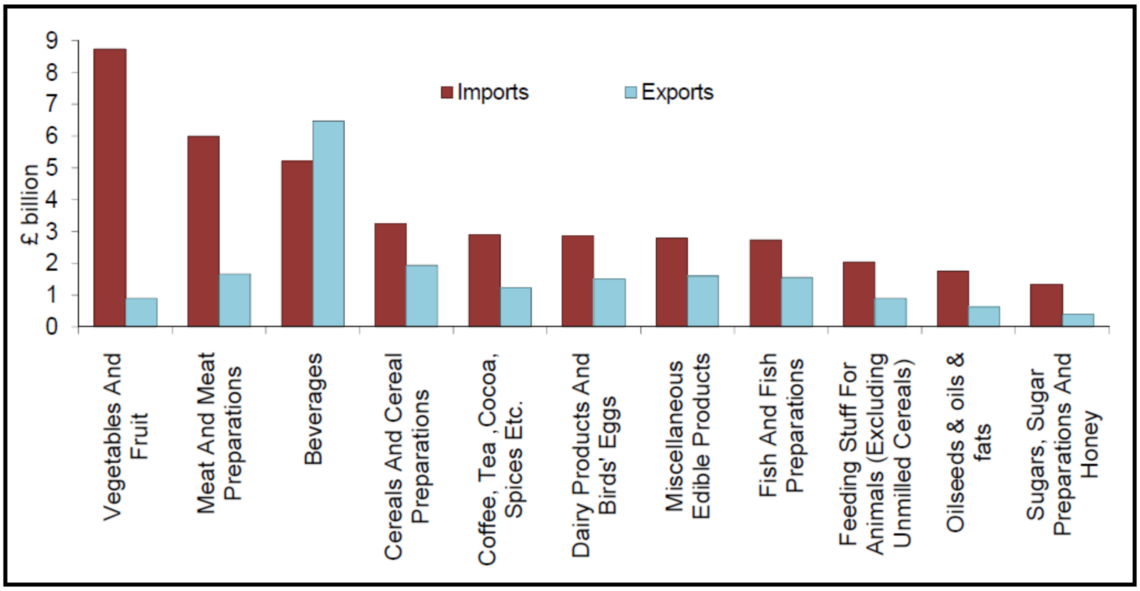


Figure 2.3 UK trade in different food groups in 2014. HM Revenue and Customs. DEFRA (2014).

A more recent report from (WRAP, 2020) stated that the annual FW arising within UK households, hospitality and food service (HaFS), food manufacture, retail and wholesale sectors in 2019 was around 9.5 million tonnes, an increase in relation to 2012 (Figure 2.4). Over 85% (by weight) of this wasted food arises in households and food manufacture, demonstrating that this is still is the most challenging sector, as it needs to deal with a change in consumer behaviour.

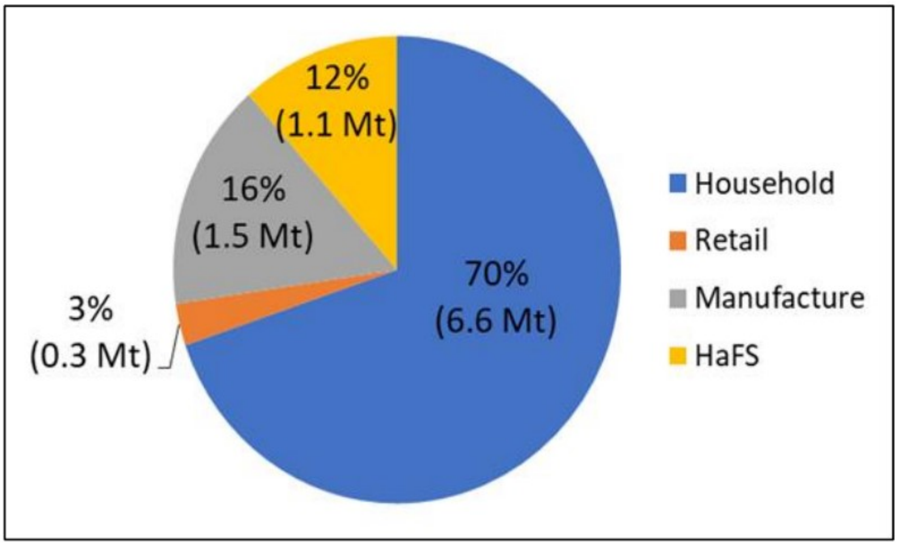


Figure 2.4 Amounts of total food waste arising in the UK in 2019 by sector (total post farm-gate=ca.9.5Mt).

A series of practical measures have been made to reduce the amount of FW generated in the UK, including The Courtauld Commitments. These are a series of voluntary agreement aimed to improve resource efficiency and reduced the carbon and wider environmental impact of the UK grocery sector. The agreements have been funded by the UK Governments and delivered by WRAP.

Table 2.3 details the distinct phases of the commitments, their start and end dates and the main achievement of each one. Launched in 2005 (phase I), this agreement is set to last 20 years. Since then it has achieved a significant reduction in waste generation from households including packaging waste (Table 2.3). As a result of actions by signatories, Love Food Hate Waste, local authorities and charity partners, 670,000 tonnes of FW and 520,000 tonnes of packaging waste was avoided across the UK between 2005 and 2009 (WRAP, 2020).

Phase II (2010-2012) aimed to reduce primary packaging and household food and drink waste, including secondary and tertiary packaging, and supply chain waste. It achieved a 41.6% decrease in the amount of FW and packaging generated in the UK in relation to phase 1, as well as a 45.4% increase in CO_2 savings, which culminated in 72.2% more billions pounds saved. This success was attributed to several factors including the participation of 53 retailers in this process (Table 2.3).

In Phase III (2013-2015) the goal was to achieve a reduction in the weight, and CO_2 emission consequences, of household FW, grocery products and packaging waste. Product and packaging waste was reduced by 3% there was a significant growth in the recovery and recycling rate (from 95% in 2012 to 99% in 2015) and a 7% reduction in the carbon impact of food and packaging was achieved (WRAP, 2020).

Phase IV is currently underway. It comprises a set of ambitious aims and for this reason its set to last over a period of 10 years. Nevertheless, several milestones have been successfully achieved, including a 1.6 Mt of CO_2 saving since 2015, which in fact is lower than the amount of CO_2 savings in Phase II, that only lasted for two years and reached 3-fold higher savings. Nevertheless, because phase IV is currently being performed, it is very likely that good results are also obtained in all targets, due to initiatives such as the implementation of new standards for food packaging design and labelling by retailers and brands, making it easier for people to buy what they need and make better use of what they buy. This strategy could significantly reduce the amount of avoidable waste generated due to lack of knowledge regarding terms such as ‘not used in time’ and the ‘consume before’ date on the packaging, responsible for 4.0 million tonnes per year of the total food and drink waste arising from households in the UK in 2012 (WRAP, 2013).

Table 2.3 The Courtauld Commitments 2025. Adapted from (WRAP, 2020).

Courtland	Aims/Goals	Packaging and Food Waste prevented (Mt)	£ saved (billions)	CO2 savings (Mt)	Remarks
Courtland Phase I (2005-2009)	Less Food Waste and Food packaging in households	1.2 Mt	1.8b	3.3 Mt	Equivalent to filling 128,000wastes lorries
Courtland Phase II (2010-2012)	Idem Phase I added of secondary and tertiary package and supply chain waste	1.7 Mt	3.1b	4.8 Mt	53 retailers worked with Wrap to achieve the targets
Courtland Phase III (2013-2015)	Reduce the weight and CO2 of household food waste, grocery product and packaging waste	–	–	–	● Product and packaging waste reduced by 3%. ● Recovery and recycling rate grew from 95% in 2012 to 99% in 2015. ● 7% reduction in carbon impact of food and package
Courtland Phase IV (2015-2025)	• A 20% per person reduction in food and drink waste associated with production and consumption of food and drink in the UK, post farm gate. • A 20% per person reduction in the greenhouse gas (GHG) emissions associated with production and consumption of food and drink in the UK. • A reduction in impact associated with water use and water stress in the supply chain.	480.000 tonnes (7% progress between 2015-2018)		1.6Mt (since 2015)	Significant increase in recognition of Love Food Hate Waste (Food Waste Trends Survey 2019). A new strategy for citizen food waste prevention, including refocused Love Food Hate Waste campaigns and targeted large-scale citizen behaviour change interventions. Implementation of new standards for food packaging design and labelling by retailers and brands, making it easier for people to buy what they need and make better use of what they buy. The world's first Food Waste Reduction. Roadmap, with more than 120 businesses set to Target their own food waste reduction, Measure consistently, and Act on the evidence. Launch of Guardians of Grub, a national campaign to empower hospitality and food service professionals to reduce the amount of food thrown away in their establishments. Working together to double the amount of food redistributed. Working within high-impact sectors – meat, dairy, fresh produce and bakery – to develop and act on new insights on food waste priorities, including measurement of pre-farm gate waste. Most (over 80%) of the reduction is due to decarbonisation of the UK's electricity grid. The GHG emissions associated with consuming a unit of electricity are 39% lower in 2018.

2.3 Environmental impacts of food waste

As well as representing a threat to global food security and a financial drain for the authorities, FW generates environmental impacts. As an example, 4.2 tonnes of CO_2 are emitted along the food supply chain for every tonne of FW generated (Lin *et al.*, 2013).

The most common management route for FW disposal is landfilling. Globally, around $1.3 \cdot 10^9$ t of FW are disposed of in landfills, contributing to $3.3 \cdot 10^9$ tonnes-CO_2-eq. year^{-1} of greenhouse gas (GHG) emissions (Salemdeeb *et al.*, 2017; Fisgativa *et al.*, 2017). Unfortunately, in developing countries this is still the most common approach for waste disposal, representing over 90% use rate for FW treatment (Thi *et al.*, 2015).

As well as landfill, FW can be incinerated, recycled or composted. However, each one of them has environmental, social and economic impacts and in most cases does not represent a realistic solution. For example, a total of 24-164 incinerators would be required to meet the needs for the UK waste industry, a costly and environmentally unfriendly option due to the emission of pollutants. On the other hand, the challenges associated with composting of FW are related to structure of food waste with weak porosity, high content of water, low carbon-to-nitrogen relation and fast hydrolysis and accumulation of organic acids during composting, which reduced process efficiency (Wang *et al.*, 2016, Voberková *et al.*,2020).

Due to poor incentives in FW recycling programs and the lack of an official policy to persuade people to participate in recycling activities, recycling faces major challenges in developing countries (Suchada *et al*., 2003). To date, most developing countries have not widely practiced FW recycling and the regulations for FW management are quite incomplete. As a result, most FW is mixed with MSW and landfilled. A large number of countries, including Brazil, Turkey, Malaysia, Mexico, Costa Rica, Romania, South Africa, Belarus, China, Jamaica, Ukraine, Nigeria, and Vietnam, among many others, currently disposes unsorted FW in landfills (Thi *et al*., 2015).

Key to successful FW management in industrialised countries is adequate administrative structures and budget allocations. Only 10% of the total budget is used for waste collection with most of the funds utilized for segregating activities, establishing treatment facilities and programs to enhance communities' awareness of waste recycling and recovery (UNEP, 2011). Nevertheless, experiences from industrialised countries have shown that a country cannot effectively address its FW issue if the government does not establish specific objectives for reducing FW and implement comprehensive legislative regulations to compel sustainable food production (e.g. the closed-loop supply chain model) (Parfitt *et al*., 2010).

Different targets, worldwide, have been set in the waste industry related to the amount of Biodegradable Municipal Waste (BMW) sent to landfill (Price, 2001). (BMW is waste that undergoes anaerobic or aerobic digestion, producing methane emissions). In Europe the disposal of organic waste sent to landfill has been strongly discouraged by legislations such as the EU Directive on Landfills (European Union, 1999) and the Waste Framework Directive (European Parliament and Council, 2008) (Holm-Nielsen *et al*., 2009; Grosso *et al*., 2010).

Table 2.4 presents the compliance for organic waste disposal for members of the European Union by 2020. Therefore, the target beginning in 2006 and ending in 2010 should reduce landfilling of BMW to 75% of the 1995 levels. The percentage of BMW sent to landfill over the next years, are diminished in relation to the 2006 target, possibly due to the fact that the initial higher need for diverting this type of waste in order to reduce environmental impacts would have been successfully met. Therefore, the 2008 target (2013 deadline) represents 33.33% less than the 1995 level and the 2016 target (deadline 2020), 53.3% less.

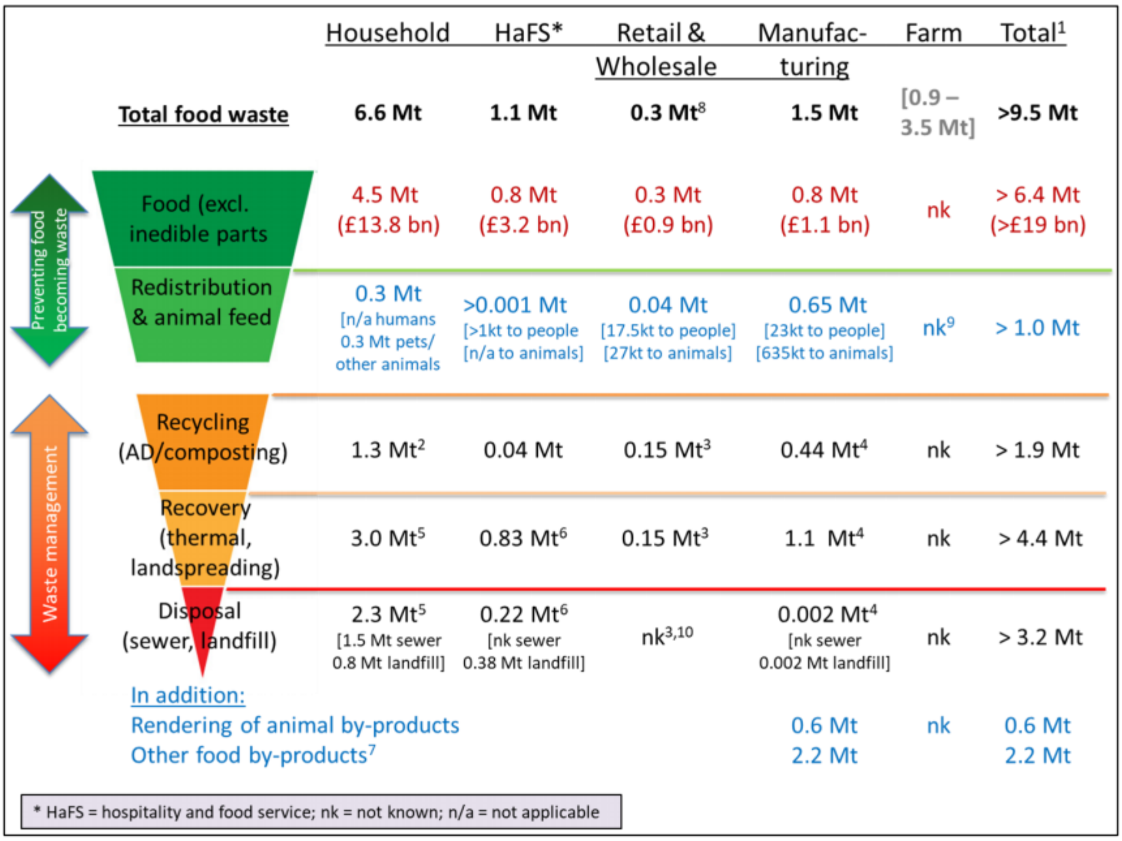
Table 2.4 Landfill directive targets for biodegradable municipal waste. Price (2001).

Target	Deadline[a]
Reduce landfilling of BMW waste to 75% of 1995 level	2006 (2010)
Reduce landfilling of BMW waste to 50% of 1995 level	2008 (2013)
Reduce landfilling of BMW waste to 35% of 1995 level	2016 (2020)

[a] The date in brackets indicates the extended deadline available to countries for whom landfill represents a predominant disposal route (member states landfilling over 80% of municipal solid waste).

In order to achieved such targets, the UK formulated a series of strategies, such as the increase in the costs for disposing waste in landfill from £40–74 to £68–111 of gate fees per tonne between

2009 and 2011 (Lin *et al*., 2013). Additionally, the UK government implemented the Landfill Allowance Trading Scheme (LATS), a scheme designed to benefit local authorities that reduce their disposal of BMW to landfill to a level below their allowance. By doing so, they can trade their excess allowance to lower-performance authorities (DEFRA, 2007).



	Household	HaFS*	Retail & Wholesale	Manufac-turing	Farm	Total[1]
Total food waste	**6.6 Mt**	**1.1 Mt**	**0.3 Mt[8]**	**1.5 Mt**	[0.9 – 3.5 Mt]	**>9.5 Mt**
Food (excl. inedible parts	4.5 Mt (£13.8 bn)	0.8 Mt (£3.2 bn)	0.3 Mt (£0.9 bn)	0.8 Mt (£1.1 bn)	nk	> 6.4 Mt (>£19 bn)
Redistribution & animal feed	0.3 Mt [n/a humans 0.3 Mt pets/ other animals	>0.001 Mt [>1kt to people [n/a to animals]	0.04 Mt [17.5kt to people] [27kt to animals]	0.65 Mt [23kt to people] [635kt to animals]	nk[9]	> 1.0 Mt
Recycling (AD/composting)	1.3 Mt[2]	0.04 Mt	0.15 Mt[3]	0.44 Mt[4]	nk	> 1.9 Mt
Recovery (thermal, landspreading)	3.0 Mt[5]	0.83 Mt[6]	0.15 Mt[3]	1.1 Mt[4]	nk	> 4.4 Mt
Disposal (sewer, landfill)	2.3 Mt[5] [1.5 Mt sewer 0.8 Mt landfill]	0.22 Mt[6] [nk sewer 0.38 Mt landfill]	nk[3,10]	0.002 Mt[4] [nk sewer 0.002 Mt landfill]	nk	> 3.2 Mt
In addition:						
Rendering of animal by-products				0.6 Mt	nk	0.6 Mt
Other food by-products[7]				2.2 Mt		2.2 Mt

* HaFS = hospitality and food service; nk = not known; n/a = not applicable

Figure 2.5 Summary of food surplus, waste and related material arisings in the UK, and their respective treatment and disposal routes (WRAP, 2020).

Nevertheless, according to WRAP, in 2019, of the 9.5 million tonnes of FW generated by the households, hospitality & food service (HaFS), food manufacture, retail and wholesale sectors, 33.68% was diverted to landfill (Figure 2.5). Despite efforts to reduce the biodegradable waste to landfills in accordance with the directives discussed above, this is still the second most utilized form of waste management in the UK, after thermal process or land spreading (46.31%) (WRAP, 2020).

As well as the reduction on the amount of biodegradable waste going to landfill, the UK government committed to achieving the 60% of reductions of GHG emission by 2050 (BERR & DEFRA, 2003). According to the International Energy Agency (IEA), fossil fuels accounted for up to 81% of the world's primary energy supply in 2007 whereas renewable energy sources only contributed a mere 13% (IEA, 2009). Despite a consensus that GHG emissions need to be reduced, it has been estimated that fossil fuels will remain the single greatest energy source worldwide (at 77% for the period 2007–2030) (IEA, 2009). Nevertheless, it is expected that a three-folds increase in energy consumption will happen by 2100, whilst energy generation from biomass is intended to reach 50,000 TWh in 2050, 75,000 TWh in 2075 and 89,000 TWh in 2100 (IEA, 2006).

If large amounts of organic waste are going to be diverted from landfill, sustainable strategies should be investigated as means not to just treat it but to recover valuable products including energy. The shift towards sustainable energy systems offers a promising solution to reduce environmental pollution and provide a sustainable fuel supply (Elsayed *et al*., 2019; Ramanathan *et al*., 2016). In this respect, FW management can play an important role (Weiland, 2010).

2.4 Food to energy conversion technologies

Recovering energy from FW is undoubtedly a sustainable option for reducing methane emissions from waste that would otherwise go to landfill. Furthermore, it can help countries reduce their dependency on energy and fuel imports since organic wastes can generate alternatives to fossil fuel, such as ethanol and butanol. This also contributes towards reducing carbon emissions and meeting renewable energy targets (Weiland, 2010).

The various technologies that are available for energy generation from biomass and waste can be subdivided into thermochemical, biochemical and physicochemical conversion processes (Figure 2.6). FW can be incinerated with other combustible municipal wastes to generate heat or energy. FW combustion may lead to the production of dioxins which can, in turn, contribute to air pollution (Katami *et al*., 2004; Ma *et al*., 2011). Alternative technologies like gasification are still at the demonstration phase and only a limited number of full scale installations has been built (Appels, Lauwers, *et al*., 2011).

Combustion is not a very feasible FW to energy technology as it only offers a net positive energy balance if the water content of the biomass or waste is below 60% (FW has a moisture content between 67-80%) and even then, most of the energy stored in the biomass is used for evaporation of the water. Similarly, the energetic efficiency of pyrolysis and gasification is low for substrates with high moisture content, and the presence of water in the resulting bio-oil is undesirable. These considerations discourage application of these technologies for FW (Van de Velden *et al*., 2010).

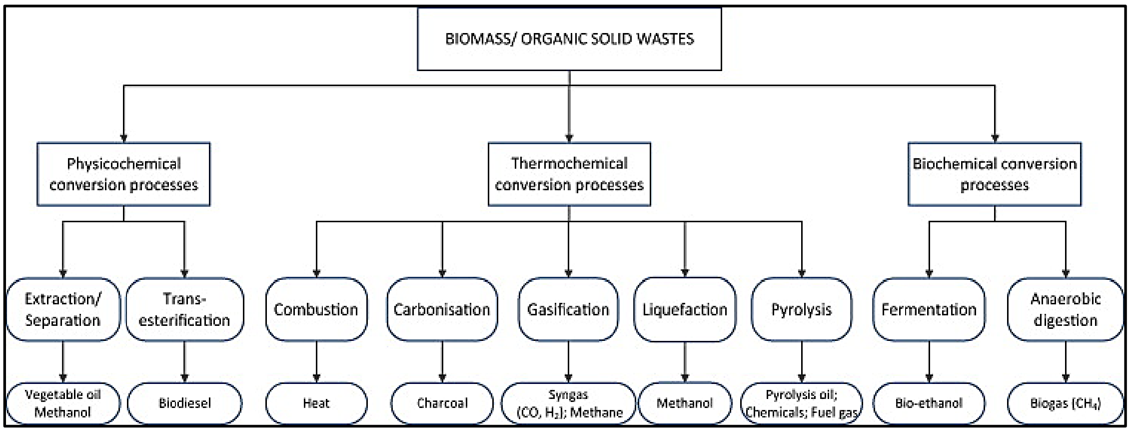


Figure 2.6 Biomass and waste conversion technologies (Appels *et al*., 2011).

In contrast, AD of FW provides both energy (i.e., 0.6 MWh t^{-1}) and biofertilizers (~40% of initial feed) from the same amount of input materials with a lead time and foot print smaller than that of composting (Karthikeyan *et al*., 2018). Therefore, AD is currently the most commonly used technology for FW to energy conversion (Pham *et al*., 2015).

There are several advantages from utilizing FW as a feedstock to generate by-products and energy: **a)** it comprises significant quantities of functionalised molecules (e.g., carbohydrates, proteins, triglycerides, fatty acids and phenolic compounds that can produce materials such as Polyhydroxyalkanoates (PHA's), bio-fuels, syngas, amongst others (Figure 2.7); **b)** it is an abundant and under-utilised resource; **c)** it is available everywhere in different composition and concentrations; **d)** it contains organic matter that is suitable for microbial growth; **e)** it reduces the cost of the waste - as when disposed in landfills (Zhang *et al*., 2012; Lin *et al*., 2013).

According to Sanders *et al*. (2007) fuel applications from FW (US$200–400/ton biomass) usually creates more value than electricity generation (US$60–150/ton biomass) or animal feed (US$70–200/ton biomass). Due to its inherent chemical complexity, FW also can be utilized for production of high-value materials, such as organic acids, biodegradable plastics and enzymes (US$1000/ton biomass). The market demand for biofuels from waste prevails over that for these materials and FW to biofuel technologies therefore need to be developed.

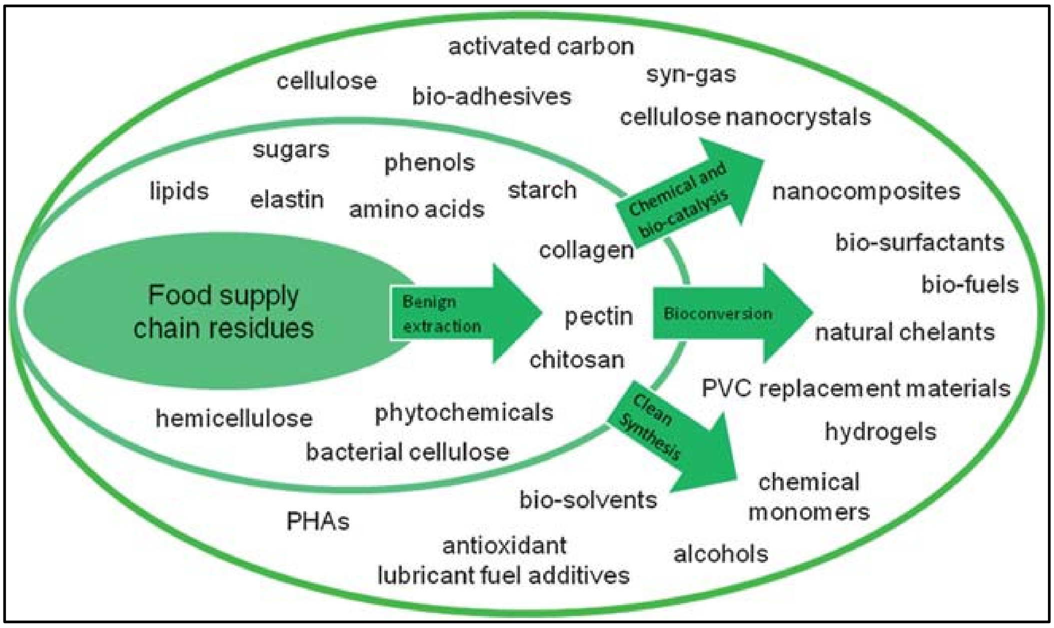


Figure 2.7 Components present in Food Supply Chain and their uses in common consumer applications (Lin *et al*, 2013).

2.4.1 Anaerobic digestion

AD is a process that occurs naturally in the environment, for example in swamps, at the bottom of lakes, in landfills and in the stomachs of animals. When AD occurs, it releases methane and carbon dioxide that escape into the atmosphere and pollute the environment. However, under controlled conditions, the AD process is a versatile technology platform that can serve many purposes in industry and society by generating electricity and/or heat, e.g. in combined heat and power (CHP) systems. Moreover, the methane can be liquefied and used as a transport fuel, or injected directly in to the gas grid. 1 m^3 of biogas from AD at normal conditions (1atm at 20°C) is equivalent to 21 MJ of energy, and it could generate 2.04 kWh of electricity assuming 35% generation efficiency (Murphy *et al*., 2004).

This biological process is complex and comprises several steps. At its simplest, the AD process can be characterized by four sequential processes as depicted in Figure 2.8; namely hydrolysis, acidogenesis, acetogenesis and methanogenesis. The cooperation between key members of the microbial community is essential for the performance of each step as well as the totality of the digestion. The assortment of metabolic and catabolic activities of bacteria determines the transfer of energy content from substrate into biogas/biomethane (Divya *et al*., 2015).

Each of the AD steps is performed by a specific group of microorganisms and enzymes and occurs within a period of time. Hydrolysis is the rate-limiting step for anaerobic digestion of complex organic substrates such as cellulose (Hassan *et al*., 2017). In this case, many days are required for the decomposition of cellulose into monomers, whereas the duration of hydrolysis of soluble carbohydrates takes only a few hours. This difference is due to the compact structure and compositional features of cellulose, hemicellulose and lignin (i.e., lignin content and cellulose crystallinity). Nevertheless, limited hydrolysis reduces the production of intermediate products and, therefore, hinders biogas/biomethane production (Rodriguez *et al*., 2017).

For easily biodegradable wastes such as FW, hydrolysis is not necessarily the rate-limiting step, thus if hydrolysis is increased (due to pre-treatment, for example) it may lead to VFA accumulation, which subsequently inhibits the methanogens (Tembhurkar and Mhaisalkar, 2007). On the other hand, it is expected that under a healthy AD, the methanogenesis step will proceed in seconds to minutes. Therefore, low molecular weight compounds such as volatile fatty acid (VFA) levels should be low in a well-balanced system (Pind *et al*., 2003).

The effectiveness of anaerobic digestion systems is sensitively dependent on feedstock characteristics, operating conditions and digester design. Physical and chemical characteristics of the feedstock such as moisture content, volatile solids, nutrient content, particle size and biodegradability are all important for process stability and biogas production (Uçkun Kiran *et al*., 2014).

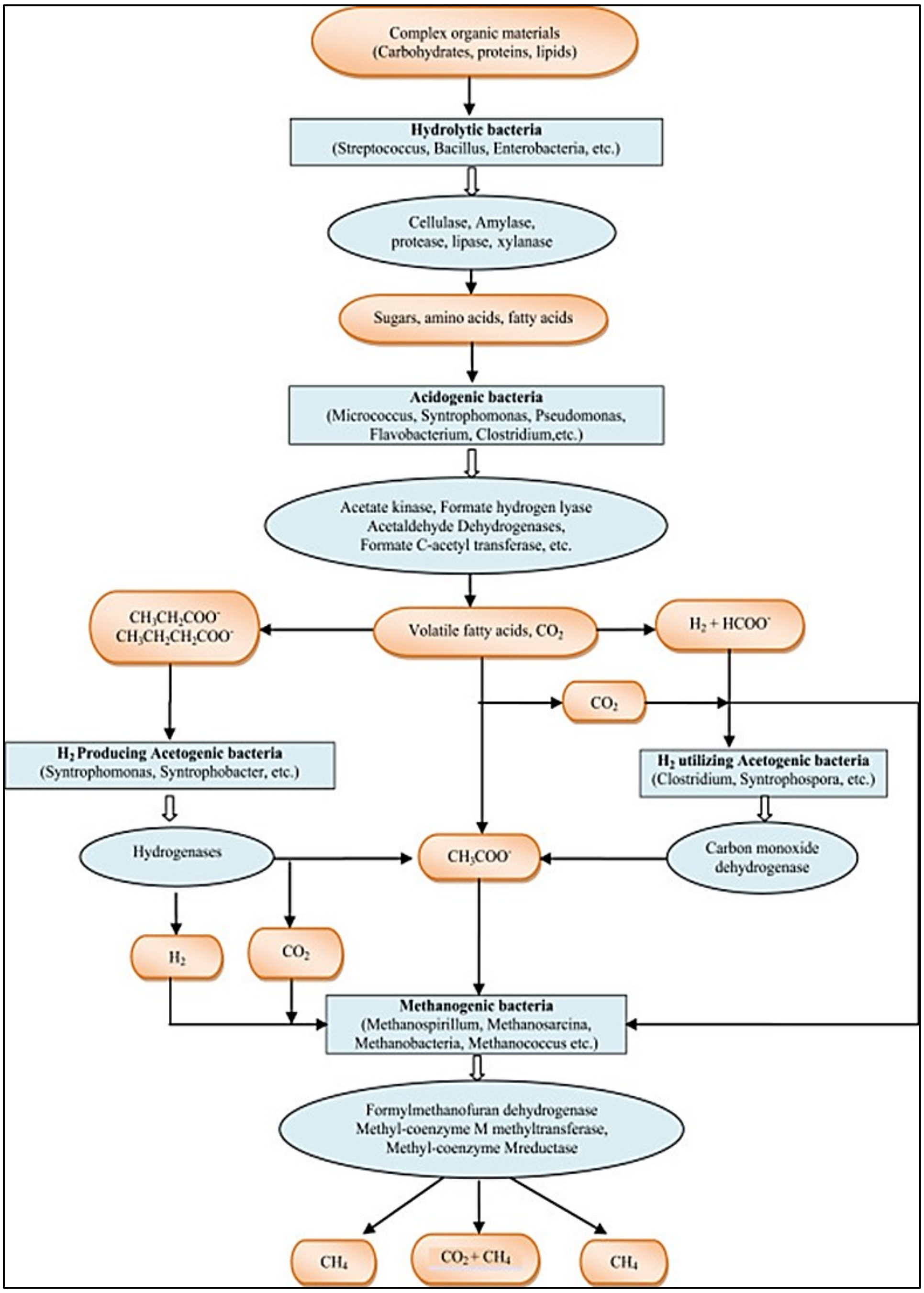


Figure 2.8 An overview of AD process and microbial enzymes responsible for catalysing carbon flow (Divya *et al*., 2015).

The anaerobic digestion of FW is not a simple process. According to Ike *et al*. (2010), a group of microorganisms such as Actinomyces, Thermomonospora, Ralstonia and Shewanella are involved in the degradation of FW into volatile fatty acids, whilst Methanosarcina and Methanobrevibacter/Methanobacterium mainly contribute to methane production. Nevertheless, numerous studies have shown that FW is a good alternative feedstock for AD because of its high degradability and biogas/ methane yield, as shown on Figure 2.9.

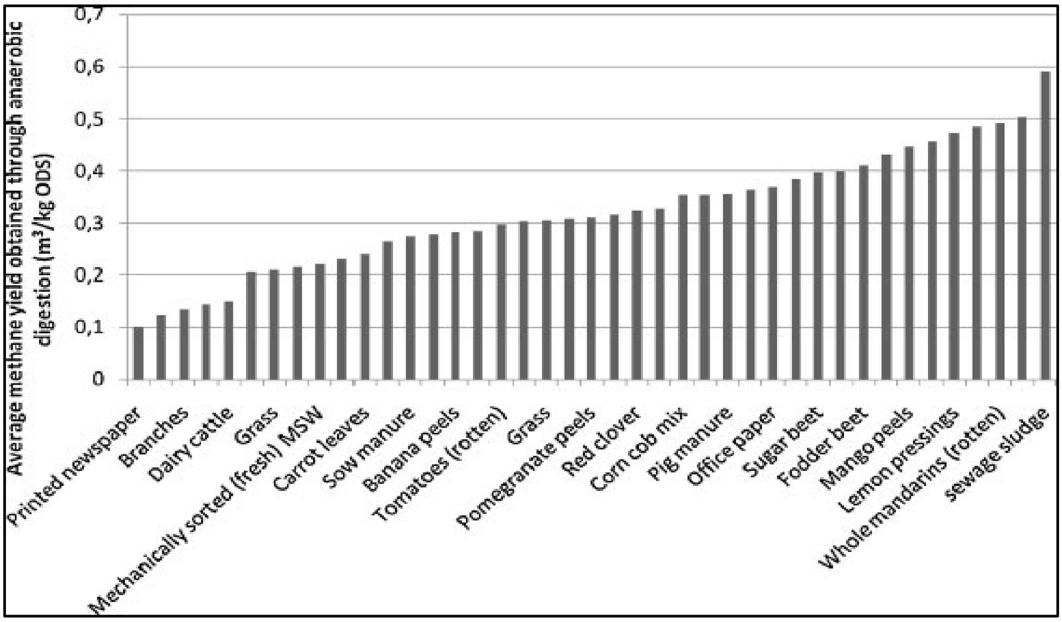


Figure 2.9 Methane yield from different feedstocks including food waste. (Appels *et al*., 2011).

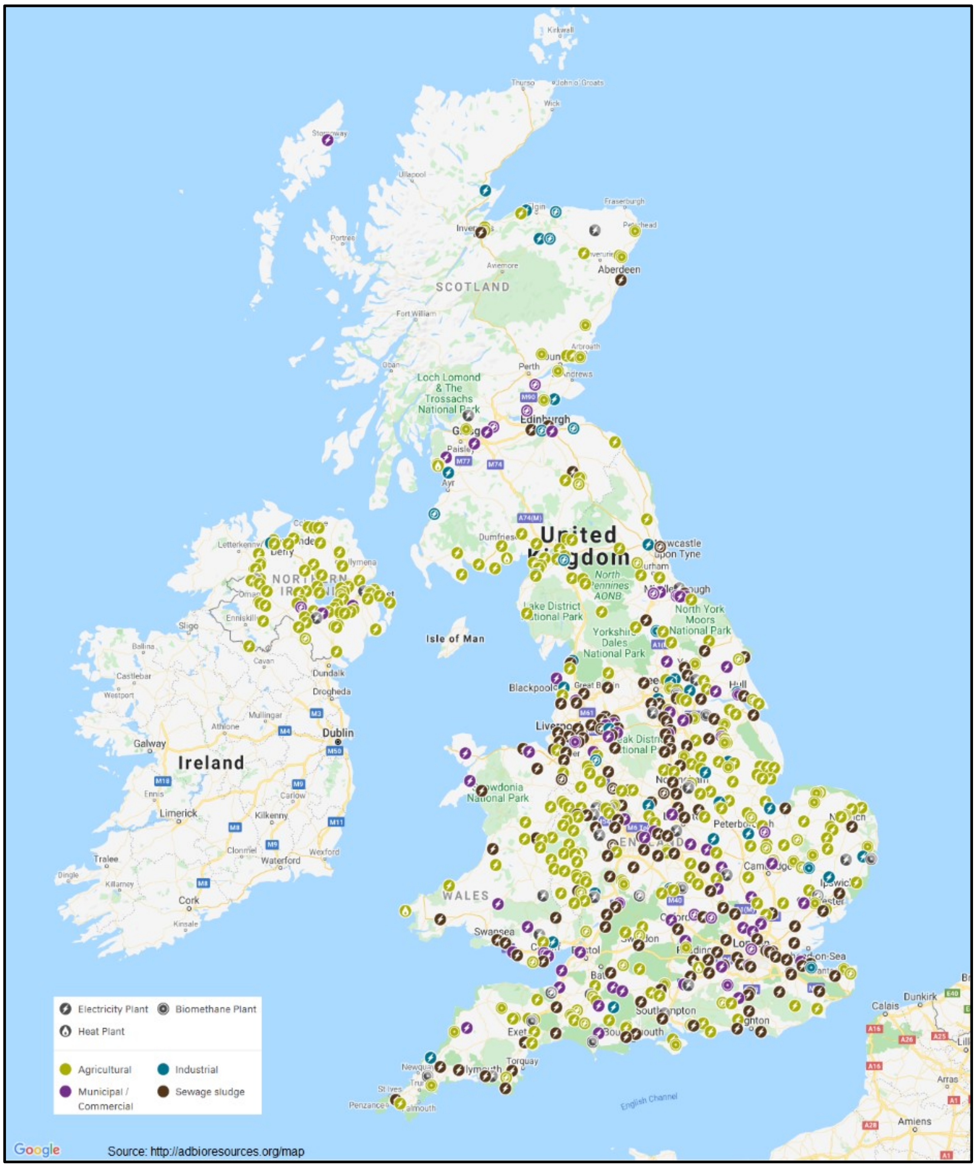
Table 2.5 Reactor type and methane yield from different feedstocks including food waste.

Substrate	**Reactor Type**	**BMY/SMP ($NLCH_4$/kg VS added)**	**Reference**
OFMSW	Full-scale, CSR	360	Bolzonella *et al*. 2006
OFMSW	Lab-scale , CSR	370	Bouallagui *et al*., 2005
OFMSW	Lab-scale ,two-phase	365	Cho *et al*., 1995
OFMSW	Pilot-scale Batch	210	Di Maria *et al*., 2013
FVW	Pilot-scale CSR	420	Lin *et al*., 2011
FVW	Lab-scale, Batch	335	Di Maria *et al*., 2015
FVW	Two stage, N.R	530	Lee *et al*., 1999
FW	Two stage, N.R	440	Gunaseelan VN, 2004
FVW	Single stage, Batch	180-732	Cho *et al*., 1995
Potato waste	Two stage, N.R	390	Zhang *et al*., 20007
FW	Single stage, Batch	440	Forster-Carneiro *et al*. 2008
FW	Two stage CSTR	464	Kim *et al*., 2010
FW	Single stage, Batch	220	Kim *et al*., 2008
FW	Three stage system, UASB	254	Park *et al*., 2008
FW	Single stage	399	Moon & Song, 2011
FW	Single Stage, CSTR	455	Dai X *et al*., 2013
FW & SS	Single Stage ,N.R	465	Molino A *et al*., 2013
FW	Single Stage ,N.R	410	Zhang C *et al*., 2013
FVW	Sequencing batch reactors	420	Bouallagui *et al*. (2005)
Household waste	low-cost household digesters	350	Ferrer *et al*. (2011)

*OFMSW (organic Fraction of Municipal Solid Waste); FVW (Fruit and Vegetable Waste); SS (Sewage Sludge).

Table 2.5 details the methane obtained by different organic wastes as feedstocks under distinct reactor types. Overall, organic wastes including food waste, fruit and vegetable waste, household waste and the organic fraction of municipal solid waste (OFMSW) heralded satisfactory methane yields, ranging from 210 - 732 $NLCH_4$/kg VS added, with higher methane yields usually related to two-stage systems. This can be attributed to the spatial separation between bacteria communities (fermentative and methanogenic) allowing a better control of environmental conditions for each group (Mota and Zaiat, 2018). Compared to other substrates such as branches, office paper and grass, the FW related ones such as vegetable (sugar beet) and fruits (mango and lemon peels) yielded, on average, 400% more methane (Appels, Lauwers, *et al*., 2011).

FW includes fruit and vegetable waste, as well as the organic fraction of municipal solid waste (OFMSW) and any waste originating in households, with each category, giving rise to different methane yields. Cho *et al*. (1995), for example, has reported the methane production capacities of 54 different fruit and vegetable wastes ranged from 180–732mL/gVSadded, depending on the origin of wastes. Di Maria *et al*. (2013) on the other hand, analysed the methane yield from the organic fraction of municipal solid waste and obtained lower value than fruit and vegetable wastes (210 mL/g VS added)(Table 2.5).



Source: http://adbioresources.org/map

Figure 2.10 Digesters sites at the United Kingdom. From: ADBA (2020). http://adbioresources.org/map

Anaerobic Digestion in the United Kingdom is already well established. In 2012, the UK's aggregate AD plant operating capacity was over 5.4 million tonnes of material, and the electrical generating capacity at these plants was 216MW, meaning that more than 1 TWh of energy is recovered by this process (WRAP, 2013). Nevertheless, there is an increasing number of digesters being implemented (Figure 2.10). As well as energy, the higher number of AD plants will generate more of the end product known as digestate.

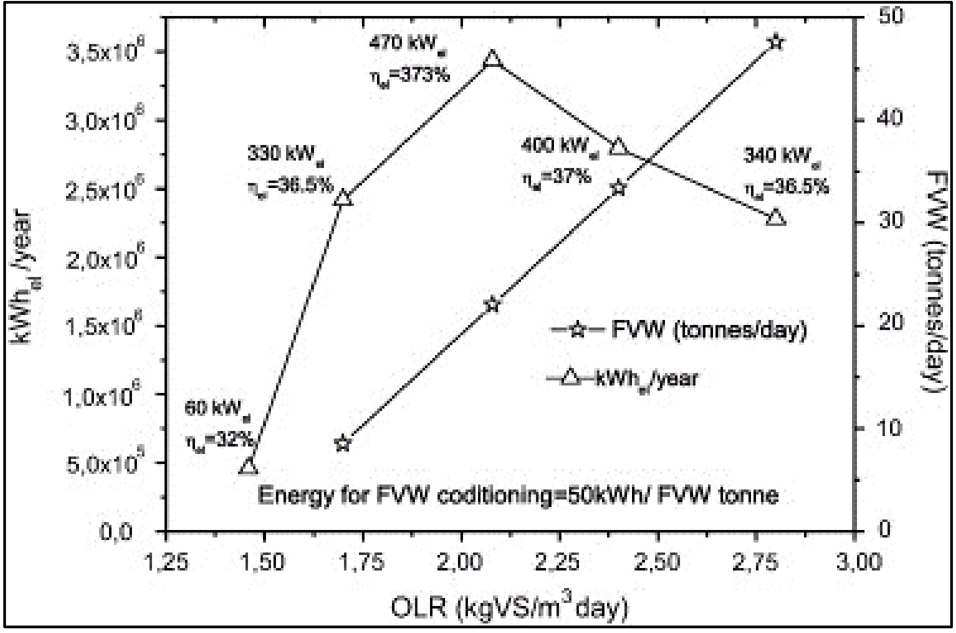


Figure 2.11 Net electrical energy, tonnes of fruit and vegetable waste (FVW) introduced daily in the digester of the full-scale waste water treatment plant (WWTP), co-generator efficiency (ηel) and power output (kWel) (Di Maria *et al*., 2015).

Moreover, just like a Wastewater Treatment Plant - WWTP, AD plants treating FW need to deal with the fact that the influent (substrate) characteristics fluctuates over time and are, at least in part, highly heterogeneous. Therefore, it is not possible to predict future compositions of received feedstock because the dynamics of society and industry constantly results in transients (Madsen *et al*., 2011). Despite the fluctuations in substrate composition and hence the net energetic value produced in a WWTP treating FW, the typical energy net value is usually high (increasing with the Organic Loading Rate - OLR up to 2kgVS $m^{-3}day^{-1}$, with a slight decrease after this value) generally suggesting that this is an adequate and profitable substrate for AD (Figure 2.11).

2.4.2 Food waste pre-treatments for AD

According to European Union Regulation EC1772/2002 (Commission of the European Communities, 2002), substrates such as MSW, FW, and slaughterhouse wastes need to be pasteurized or sterilized before and/or after AD. Considering this regulation, pre-treatment methods could be applied, thus eliminating the extra cost for sterilization (Eggeman and Elander, 2005; Hendriks and Zeeman, 2009).

The main incentive for applying pre-treatments on organic wastes, including FW, is mainly related to improving biomass-to-energy conversion by making the substrate more suitable for digestion. Disintegration methods have recently attracted attention to alter the structure and composition of the biomass thereby addressing the low digestion efficiency often encountered in industrial scale applications of AD (Appels, Lauwers, *et al*., 2011).

The effects that pre-treatment may have on a substrate depend on its type and characteristics. If the solubilised material is inherently easily biodegradable, the effect on biodegradability enhancement may be limited (Lissens *et al*., 2004). The positive effects of pre-treatment are numerous and include: (i) improvement of the surface properties for better microbial interactions; (ii) reduction and/or removal of the toxic compounds that may affect the process; (iii) improvement of the hydrolysis rate kinetics for proteins and lipids and (iv) increase of the accessibility of certain compounds (Carlsson *et al*., 2012; Kudakasseril Kurian *et al*., 2013; Monlau *et al*., 2013; Karthikeyan and Visvanathan, 2013; Fisgativa *et al*., 2017).

Additionally, substrate modifications resulting from pre-treatment have the potential to influence the AD process performance by altering the rate and extent of degradation, both of which are directly correlated to the methane yield, VS reduction and/or productivity. The former occurs by breaking insoluble polymer chains into soluble components, thus making the reaction products more biodegradable as well as more accessible for bacteria to digest, while the latter is increased by the release or exposure of organic material that was originally inaccessible to microorganisms or the transformation of material that was originally not biodegradable (Bougrier *et al*., 2007). The beneficial alterations of the substrate arising from pre-treatment result in acceleration of the process and as a result, reduces digestion time (HRT).

The effort in enhancing AD via pre-treatment methods is not a new strategy. It has been developed over the last 30 years (Haug *et al*., 1978; Stuckey andMcCarty, 1984; Weemaes and Verstraete, 1998; Neyens and Baeyens, 2003; Hendriks and Zeeman, 2009; Pilli *et al*., 2011) with the main objective being to increase operational methane yield in order to arrive as close as is possible to the potential methane yield of the substrate at the highest feasible digestion rate (Carlsson *et al*., 2012). On the other hand, disadvantageous impacts from pre-treatments can also occur such as formation of refractory compounds and/or loss of organic material, hindering methane production (Carrère *et al*., 2010).

Pre-treatment focused AD optimisation has ranged from finding suitable treatments for each substrate (e.g. mechanical, thermal, physico-chemical, chemical, thermochemical and microwave (MW) irradiation) as well as finding economically feasible way of combining them. Each one of these will be briefly discussed below, with a special focus on MW irradiation (Hendriks and Zeeman, 2009).

2.4.2.1 Mechanical

This type of physical pre-treatment is one of the few available for the full-scale biogas process (Lindmark *et al*., 2012). It includes techniques like milling, grinding, sonication, lysis-centrifuge, liquid shear, collision, high-pressure homogenizer, maceration, and liquefaction. It is generally successful in improving AD performance depending on the material properties and the technique used (Lindmark *et al*., 2012). According to Ariunbaatar *et al*. (2014) size reduction using bead mills, electroporation and liquefaction pre-treatments of OFMSW has been studied at lab scale, whereas rotary drum, screw press, disc screen shredder, FW disposer and piston press treatment have been successfully applied at full-scale AD. The principal advantage of this type of pre-treatment is the possibility of disintegration of solid particles of the substrates, which releases cell compounds, increasing the specific surface area (providing better contact between substrate and anaerobic bacteria), thus enhancing the AD process (Skiadas *et al*., 2005; Carrère *et al*., 2010; Elliott and Mahmood, 2012). It doesn't generate odour, is easy to implement, has better dewaterability of the final anaerobic residue and has moderate energy consumption. In contrast to chemical and thermochemical methods, mechanical treatment produces no toxic and/or inhibitory by-products during the disintegration step (Hendriks and Zeeman, 2009).

Major detrimental effects include: a) the additional energy yield may be lower than the energy supplied for disintegration, and b) heating of the substrate which may result in loss of volatile substances with consequent reduction of methane potential (Hendriks and Zeeman, 2009; Kratky and Jirout, 2011; Mönch-Tegeder *et al*., 2013).

The effects of mechanical pre-treatment on the OFMSW reported in the literature are contradictory. Davidsson *et al*. (2007) investigated the biomethane potential of source-sorted OFMSW pre-treated with different mechanical methods including screw press, disc screen shredder, FW disposer and piston press. The authors found small variations in each methane yields per gVS (gram volatile solids) varying from 275 and 410 Nm^3 CH_4/ton VS added, with no statistical difference between them.

Hansen *et al*. (2007) studied the effects of the same pre-treatment technologies on the quantity and quality of source-sorted OFMSW. They showed a positive relation between the use of screw press pre-treatment and a decrease in substrate particle size. Another finding was that a shredder with magnetic separation yielded a higher methane production (5.6–13.8%) compared to the other methods. In contrast, Bernstad *et al*. (2013) reported that although the screw press enhances biogas production in general, there is a loss of biodegradable materials, including important nutrients for bacterial activity via this method, thus suggesting that attention should be paid in order to ensure adequate amount of organics are entering the process.

Mechanical treatments such as Electroporation of OFMSW resulted in 20–40% higher biogas production (Carlsson *et al*., 2008) and liquefaction resulted in 15–26% higher biogas production (Toreci *et al*., 2009) Sonication yielded 16% higher cumulative biogas production than untreated substrates (Cesaro and Belgiorno, 2014).

Palmowski and Muller (2000) investigated the effect on anaerobic biodegradability of comminution of organic material using various machines. The authors demonstrated that size reduction of very fibrous substrates such as maple leaves and hay stems resulted in up to an 18% increase in biogas production. Biogas production from mixtures of apples, carrots, potatoes and meat, which have low fibre contents (very biodegradable), showed no improvement. These two scenarios demonstrate that the effects of particle size reduction depend on substrate characteristics, with highly biodegradable substrates receiving little benefit from this strategy.

A reluctance to adopt mechanical methods originates from a misrepresentation of the pre-treatment effect on the surface area of some substrates, especially for fibrous materials. In this case, there can be substrate damage, which increases the surface area without decreasing particle size (Hartmann *et al*., 2000).

2.4.2.2 Ultrasound pre-treatment

Another category of mechanical pre-treatment is ultrasound. The frequency range for ultrasound (US) is between 20 kHz and 10 MHz. This process is feasible in an aqueous environment. The application of US at frequencies between 20 to 40 kHz results in the formation of small bubbles due to localized pressure drops below the vapor pressure of the aqueous phase. The bubbles oscillate, grow, and collapse in a nonlinear manner and create cavitations, which in turn, cause strong mechanical shear forces and extreme temperature increases inside and around the bubbles (Chu *et al*., 2001).

The advantages of US disintegration are the absence of secondary, or additive, toxic compounds (Khanal *et al*., 2007) The size reduction efficiency depends on the sonication time (Tiehm *et al*., 1997), ultrasonication density (Benabdallah El-Hadj *et al*., 2007; Show *et al*., 2007; Laurent *et al*., 2009), sonication power (Mao and Show, 2006), and sample characteristics (Cao *et al*., 2006).

Köksoy and Sanin, (2010) studied the effect of the F/M ratio under sonicated and unsonicated sludge using batch MAD assays. Sonicated reactors treating sonicated sludge always produced higher cumulative amounts of methane compared to unsonicated reactors with the same F/M ratio. Their results suggest that the extent of waste degradation was increased by US pre-treatment.

2.4.2.3 Physico-chemical pre-treatment

This type of pre-treatment is characterized by the combination of physical (e.g. grinding) and chemical (e.g. acid/alkali) or thermal (e.g. wet type; low temperature) technologies for improving the FW properties prior to AD. Li and Jin (2015), combined physical (grinding to 1–2 mm) and thermal (120 °C, 50 min) pre-treatment on FW, achieving a maximum of 32% higher biogas yield (over control).

Karthikeyan *et al*., (2018) pre-treated FW with acid (alone), but were not successful in improving carbohydrate solubilisation from FW, achieving 45% lower biogas yield. Alkali pre-treatment on the other hand, improved the CH_4 yield by 25% in comparison to the control. However, the most successful strategy was to combine alkali and thermal pre-treatment, which improved the methane yield by approximately 32% over control (Naran *et al*., 2016).

2.4.2.4 Wet air oxidation (WAO)

Wet air oxidation (WAO) is a physico-chemical process applied to solid wastes with the purpose of oxidizing high organic matter content, using high temperatures (150-370 ºC) and high pressures (5-15 MPa). The high temperature is responsible for preparing the system for oxidation, whilst the high pressure oxygen reacts with organic matter to produce inorganic salts and simpler forms of biodegradable and non-biodegradable compounds (Shahriari, 2011).

Lissens *et al*. (2004) tested the effect of WAO on AD enhancement for a variety of solid biowastes including; FW, yard waste and digested biosolids. The pre-treatment conditions were temperatures ranging from 185 to 220 ºC, pressures between 0 and 1.2 MPa and a constant reaction time of 15 minutes They were able to identify SCOD concentrations 4.8 to 5.9 times higher than controls at high pressures, whereas it was only a factor 2.3 to 3 times higher for low pressures. The principal conclusion was that higher oxygen pressures during WAO of digested biowastes (in a full-scale plant) promote an improvement in total methane yield and stabilization kinetics by 35-40%.

2.4.2.5 Chemical pre-treatment

Chemical pre-treatment offers the destruction of organic compounds by means of acids, bases, or extracellular enzymes (Liu *et al*., 2012). Acid pre-treatment is known to be an efficient method to solubilize carbohydrates, while alkali pre-treatment is efficient in solubilisation of proteins and lignin as well as lipid saponification. For this reason, easily biodegradable substrates containing large amounts of carbohydrates such as FW does not benefit from this process, once it accelerates the degradation and subsequent accumulation of VFA, fact known for altering methanogenesis (Wang *et al*., 2011). Other disadvantages associated with the acid pre-treatment include the loss of fermentable sugar due to the degradation of complex substrates, the high cost of acids and the additional cost for neutralizing the acidic conditions prior to the AD process (Taherzadeh and Karimi, 2008; Kumar and Murthy, 2011).

Practical measures need to be adopted when using this method. For example, due to the corrosive nature of chemicals used in acid pre-treatment (e.g. H_2SO_4, HCl, etc.) there is a need for non-corrosive coatings on the equipment. Under acid/alkali pre-treatment, the FW to acid/alkali ratio needs to be optimized based on the total solids contents and strength of the acid/alkali used. Moreover, because of the easy formation of inhibitory compounds such as phenols and furans, the FW characteristics must be evaluated (Monlau *et al*., 2013). Similar to other pre-treatments, the effectiveness depends on the type of method applied and the characteristics of the substrates.

Torres and Lloréns (2008) evaluated the effects of alkaline pre-treatment on the solubilisation degree and AD of OFMSW. The authors found good solubilisation with 11.5% of the COD solubilized under an alkali concentration of 62.0 meq $Ca(OH)_2$/L (equivalent to 2.3 g $Ca(OH)_2$/L) and contact time of 6 hours, considered to be optimal. Furthermore, a VS removal of 94.0% was achieved, responsible for a maximum methane yield 172% higher than the control. Nevertheless, as the concentration of $Ca(OH)_2$ increases, there was a decrease in solubilisation. This was attributed by the authors to the instability caused by the formation of complex, non-soluble, compounds.

2.4.2.6 Thermochemical pre-treatment

A combination of thermochemical and biological pre-treatments were used by Fdez.-Güelfo *et al.* (2011) to enhance solubilisation of OFMSW (TS of 30%). For the biological treatment the authors used mature compost, fungus *Aspergillus awamori* and activated sludge. The thermochemical treatment conditions were NaOH (1-5 g/L), temperature 120-180 °C, oxidizing atmosphere (N_2 and air) and contact time of 30 minutes. The authors found that thermochemical pre-treatment showed higher solubilisation compared to individual biological pre-treatments. Nevertheless, by combining the two methods using optimum pre-treatment conditions of 180 °C with 3 g NaOH/L and 3-bar pressure, it was possible to obtain even better results in terms of solubilisation (2.5 times higher than the control).

2.4.2.7 Thermal pre-treatment

Thermal pre-treatment of organic waste, specifically food/ kitchen waste, has been widely tested with positive results on AD performance and has been successfully applied at industrial scale (Carlsson *et al*., 2011).

The benefits of this treatment are numerous and include: a) pathogen removal, b) enrichment of the spore-forming bacteria in FW (which reduces the accumulation rate of organic acids), c) possible avoidance, or reduction in, toxicity effects of some FW components, d) improvement in dewatering performance of AD end products and reduction in the viscosity of the digestate (with subsequent enhancement of its handling ease (Marin *et al*., 2010; Liu *et al*., 2012; Hao *et al*., 2015).

Similar to mechanical pre-treatment, thermal pre-treatment can also result in loss of volatile organics. Nevertheless, it should be noted that thermal treatment may lead to partial degradation of sugars and other nutritional components, as well as side reactions (e.g. the Maillard reaction) through which the amounts of useful sugars and amino acids are reduced (Mottet *et al*., 2009). It should be chosen as a pre-treatment method carefully, therefore, based on specific waste characteristics.

Ma *et al*. (2011) validated the positive effects of autoclaving kitchen waste at 120 ºC and 1 bar for 30 minutes. The effects on both solubilisation and biogas production were remarkable: a 19% higher COD solubilisation and 48% higher biogas production when compared to the AD of non-treated kitchen waste.

Wang *et al*. (2006) on the other hand, tested different thermal conditions for FW pre-treatment: 70 ºC for 2 hours and 150 ºC for 1 hour, using a hybrid anaerobic solid–liquid system. The authors showed that in general the pre-treatment was beneficial in terms of overall AD process performance since the time needed to produce the same amount of methane as the control (untreated waste) was reduced by up to half.

Schieder *et al*. (2000) pre-treated FW from a restaurant using conventional heating with temperatures between 160 and 200ºC, pressures up to 4 MPa and residence times of up to 60 min.

The authors observed a significantly improvement in the rate of biogas production as well as a shortening of the of AD duration, which were both attributed to an increased rate of hydrolysis.

Different authors have investigated the effects of the final temperature on AD performance. Thermal treatment of FW at low temperatures (<100ºC) did not result in enhancement of the biogas production (Chamchoi *et al*., 2011; Liu *et al*., 2012). Under higher temperatures (>170ºC) this treatment can lead to the formation of inhibitory compounds such as melanoidins.

Liu *et al*. (2012) who tested the effects of thermal pre-treatment on both FW and fruit and vegetable waste at 175°C, reported a 7.9% and 11.7% decrease of the biomethane production, respectively, due to the formation of melanoidins. Bougrier *et al*. (2007) suggested that thermal pre-treatment at high temperatures (>170°C) might lead to the creation of chemical bonds and result in the agglomeration of the particles, affecting methane production.

Minowa *et al*. (1995) changed the phase state of model OFMSW from solid to a liquid slurry by thermal liquefaction using different temperatures (150, 200, and 250ºC). The conclusions were that the change in the form of the waste leads to changed viscosity with a significant decrease in this parameter as the temperature increases and a corresponding improvement in digestibility.

Inoue *et al*. (2002) also used high temperature for liquefaction of OFMSW using thermochemical process with temperatures between 150 ºC and 250 ºC and pressures varying between 3.5 and 7.0 MPa in addition to a mechanical disruption. In this case, a temperature of 175 ºC was considered optimum for the liquidization of the waste as a pre-treatment prior to AD. The authors observed that the liquid slurry had a greater proportion of lower molecular weight organic acids than the mechanically disrupted OFMSW, suggesting that the liquidization of OFMSW could have advantages over particle size reduction when subjecting the treated substrate to AD. 175ºC was also considered optimum for mesophilic methane production resulting in a 27% increase in methane production compared to the control.

Sawayama *et al*. (1997) investigated the effect of mechanical pre-treatment and thermochemical liquidization coupled with sodium carbonate (5% on a dry solid basis) at a final temperature of 175ºC and 4 MPa with one hour holding time as strategies to enhance AD of kitchen waste. The authors found that by anaerobically digesting the liquidized waste, it was possible to obtain twice the biogas yield after 4 days of digestion than that produced from mechanically disrupted garbage for the same period.

The thermal process, with or without mechanical pre-treatment (i.e., grinding), has been proven to achieve a significantly higher CH_4 yield than in the control (Naran *et al*., 2016) and is thus considered by many to be the most reliable pre-treatment option for FW prior to AD.

2.4.2.7.1 Microwave irradiation pre-treatment

In the recent years, microwave irradiation has been investigated as an alternative strategy to pre-treat organic wastes and enhance AD process performance. There are important differences between conventional heating and microwave irradiation. In the former, the temperature increases from the outside to inside the body by means of convection, conduction and radiation. Therefore, in practical terms, the energy is transferred through the vessel and further dissipated throughout the medium causing the hot plates to remain active after the completion of the sample heating, hence increasing the likelihood of heating the sample to dryness (Figure 2.12) (Shahriari *et al.*, 2011).

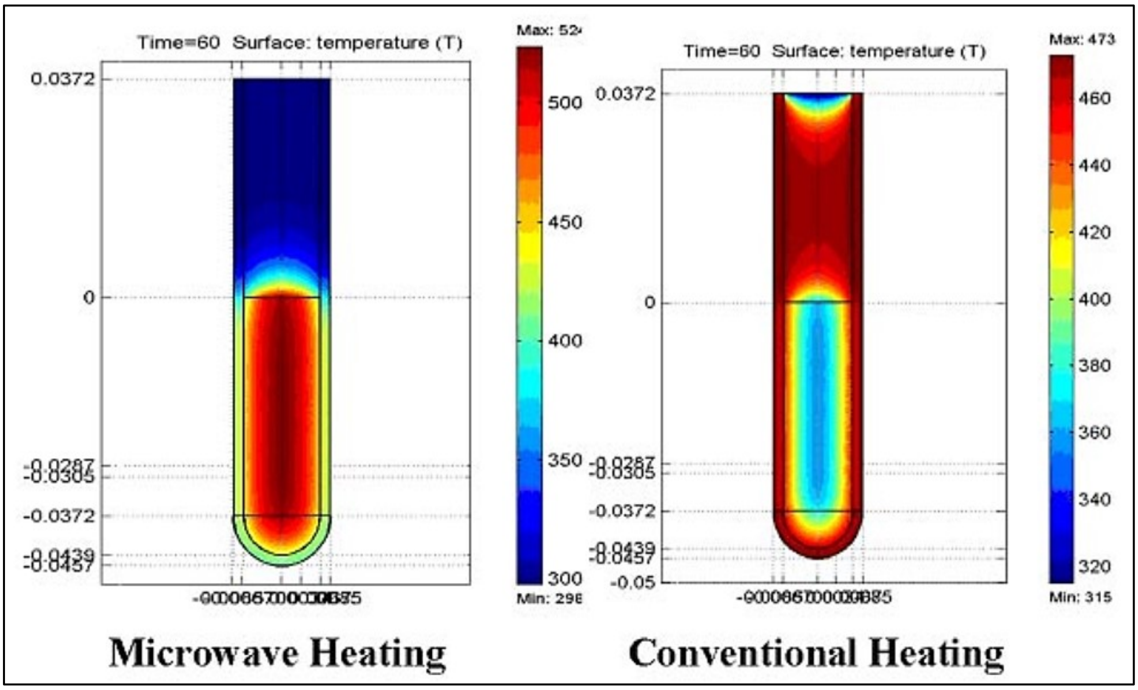


Figure 2.12 Comparison of Temperature Distribution for Conventional and Microwave Heating process (CEM Inc.) Shahriari *et al.* (2011).

In contrast, microwave energy is delivered directly to materials through molecular interaction with the electromagnetic field and therefore MW irradiation promotes a temperature increase throughout (and from within) the subject material. This difference in the way thermal energy is delivered can result in many potential advantages to using microwaves such as reduced processing times and energy savings (Plazl *et al.*, 1995; Thostenson and Chou, 1999).

MW radiation induces molecular and ionic motion (representing mechanical or vibrational energy) in certain materials. Since temperature is a measure of average vibrational (or kinetic) energy the vibrational energy is synonymous with thermal energy. This principle is known as the thermal effect (Vela and Wu, 1979; Fung and Cunningham, 1980). It is especially effective if the subject material contains dipole molecules (i.e. molecules with a positive electrical charge at one end and a negative electrical charge at the other end). Water has dipolar molecules and is thus very susceptible to microwave heating. When a food sample is exposed to MW radiation at a frequency near 2.45GHz (wavelength 12.25 cm), dielectric heating is caused due to absorption of energy by its susceptible components including water, fats and sugars (Shahriari *et al*., 2011). Since the thermal energy is generated in the target material directly, rather than conducted and convected from the outside, the thermal losses of the process are generally lower than for conventional heating (Tang and Chan, 2007).

FW exposed to MW radiation is also subjected to an athermal effect. Differently from the thermal effect, this is caused by possible breakage of hydrogen bonds leading to denaturation and death of bacteria when the polarized parts of macromolecules align with the poles of the electromagnetic field (Loupy, 2002). This effect has the potential to make complex organic molecules unfold and fracture becoming smaller, thus promoting its solubilisation. A consequence is an increase in the hydrolysis rate of anaerobic digestion since the organic matter becomes more readily biodegradable for the microorganisms (Eskicioglu *et al*., 2007; Marin *et al*., 2010)

Several factors influence microwave processing, including the properties (frequency and field strength) of the electromagnetic field, chemical composition of the material being processed, structural changes that occur during processing, size and shape of the material fragments being heated. These are all aspects of the microwave/materials interaction physics (Thostenson and Chou, 1999).

Each material has a specific ability to absorb microwave energy. Materials with a high electrical conductivity such as metals (e.g. aluminium, steel) do not allow electromagnetic fields to penetrate to any significant depth. The electromagnetic radiation is effectively ‘reflected’ from the surface layer and for this reason such materials are not suitable for microwave heating. Materials with low conductivity but low dielectric loss factors (e.g. quartz) have a large penetration depth but little of the penetrating electromagnetic energy is converted to thermal energy. These materials are effectively transparent to MW radiation.

Materials that have low conductivity and high dielectric loss factors (e.g. water) absorb MW energy most effectively. Figure 2.13 compares, qualitatively and schematically, several types of material with respect to their propensity to absorb MW energy. In many studies with FW water is present (as a solvent or as one component in the mixture) and acts as the primary MW energy absorber. The heat energy arising from the dissipation of MW energy in the water is then conducted to the other substances/substrates in the mix. Conventional heating transfers heat most efficiently to materials with high thermal conductivity (Thostenson and Chou, 1999).

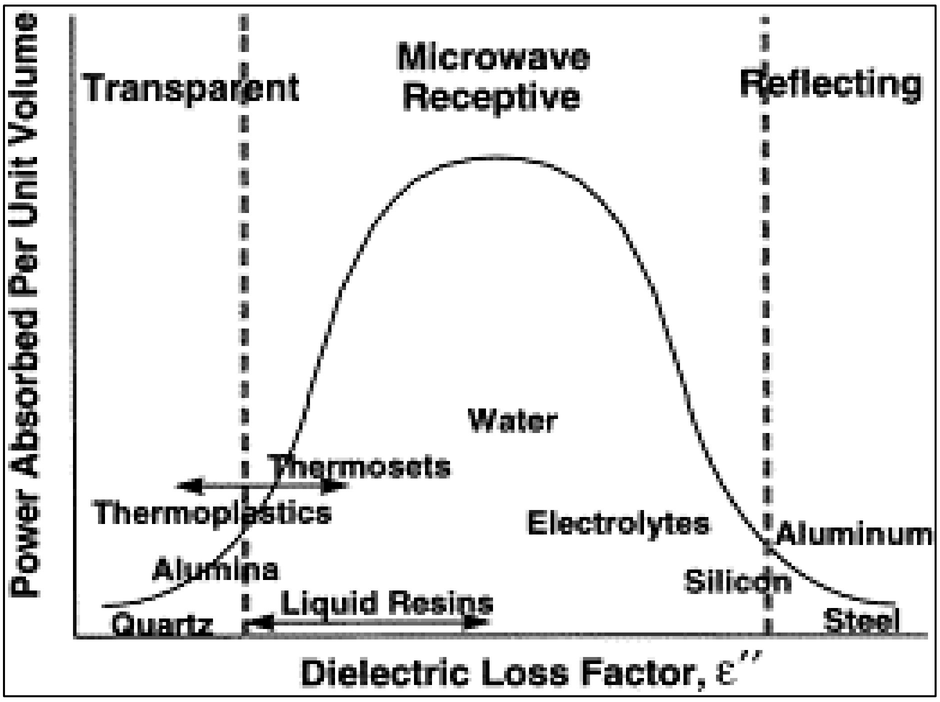


Figure 2.13 Relationship between the dielectric loss factor and ability to absorb microwave power for some common materials (Thostenson and Chou., 1999).

The dielectric loss factor of a substance is generally a function of the frequency of the electromagnetic field. At 2.45 GHz, the dielectric loss, and thus the heating rate, of water is a maximum. The loss factor of water drops rapidly when the frequency reaches 30 GHz (Fini and Breccia, 1999).

In recent years, MW irradiation of sludge has attracted interest as it may simultaneously improve digestion and decrease the pathogen content. Table 2.6 shows some of the work conducted on microwave of sewage sludge in the last two decades, with most of the results/findings being positive, with successful process improvement, including SCOD increase, VS destruction increase and methane or biogas enhancement.

Park *et al*. (2004) investigated the feasibility of applying MW radiation to domestic wastewater sludge for improving AD. The authors reported that when sludge was microwaved to its boiling temperature, there was an increase in solubilisation (SCOD/TCOD increased from 2 to 22%). Moreover, this pre-treatment was able to promote an increase of methane production of 79% in relation to a control. Ultimately, the MW pre-treatment of sludge resulted in a decrease in the HRT of the AD from 15 to 8 days, which would result in a reduction in processing cost.

Hong *et al*. (2006) obtained benefits using MW pre-treatment on primary sludge (PS), WAS and anaerobic digester sludge. When these substrates were irradiated with MWs to a final temperature of 70ºC, there was an increase in solubilization for all of the substrate tested. The increases were 16%, 125% and 45%, respectively. Nevertheless, the biogas production improvement was only significant for PS at 85ºC and 100ºC, which exhibited an increase of 11.9% and 22.7% respectively. MW benefits thus vary depending on substrates.

Zheng and Kennedy (2006) tested the effect of MW irradiation on primary sludge (PS) at different temperatures (65 and 90ºC) and various intensities. They showed a two to three-folds increase of SCOD and a 15% to 30% improvement in the rate of biogas production for 65 and 90ºC respectively. Despite this, the ultimate degradability of PS remained unchanged.

Table 2.6 Microwave pre-treatment of sewage sludge and main effects observed.

Author	**Substrate**	**Temperatures**	**Ramp rate (total time)**	**Hold time**	**Digestion Type**	**Findings**
Coelho *et al.* (2011)	Waste Activated Sludge	96°C	14.4°C/min (6min)	–	Semi-continuous Mesophilic	Improvement: SCOD content, VS removal Biogas production
Hong *et al.* (2006)	Primary (PS) and Waste Activated (AS) Sludge	70 and 100°C	–	–	Semi-continuous Mesophilic	Improvement in SCOD content (18% for A.S); 23 and 15% increase in methane production for P.S and A.S respectively.
Toreci *et al.* (2009)	Waste Activated Sludge	175°C	3.75 and 1.25°C/min (40 and 120min)	1min	Single and dual semi-continuous mesophilic	Increase solubilization at low heating rate but inhibition. Increase in biogas production at fast heating rate;
Zheng *et al.* (2009)	Primary sludge	35, 60 90°C	Fast (80% total Power) Slow (40% total power)	–	Batch tests (BMP)	No sig. difference of methane production and biodegradability between different heating rates.
Eskicioglu *et al.* (2007)	Waste Activated Sludge	50 75 96°C	1.2(36min) 1.3(58min) 1.4°C/min(80min)	–	Batch tests (BMP)	No sig. difference of methane production and biodegradability between different heating rates. 96°C higher biogas production

Qiao *et al.* (2008) investigated the treatment of fresh sludge by MW irradiation combined with alkali pre-treatment (0.2 g NaOH/g-DS). They found that MW heating alone reduced VSS by 40% at 170 ºC within 1 minute. Nevertheless, when the chemical agent was reduced to 0.05 g NaOH/g-DS the VSS dissolution ratio increased to 50% suggesting that a smaller amount of Alkali substances in needed in improving solubilisation with a potential to reduce chemical costs.

Coelho *et al.* (2011) investigated the effects of MW pre-treatment (final temperature of 96°C) on the AD of thickened waste activated sludge (TWAS) on one and two stage reactors under thermophilic and mesophilic conditions at four different HRTs (5, 10, 15, 20 days). For the two-phase reactors, both steps operating under thermophilic conditions with an SRT of 5 days, a 106% enhancement in biogas production was observed in relation to the one stage mesophilic reactor.

On the other hand, when MW pre-treated sludge was digested at the one stage reactor, regardless of being under mesophilic or thermophilic, it exhibited an improvement in relation to the control, with the latter having the best biogas improvement (83%) at an HRT of 20 days. Their study confirmed that for single-stage reactors there was a decrease in waste stabilization efficiency resulting from MW pre-treatment at shorter HRTs. For the two-stage reactors operated at the same HRTs waste stabilization was increased with and without MW pre-treatment.

Yu *et al*. (2010) also investigated the effects of MW pre-treatment on WAS. However, in contrast to Coelho *et al*. (2011), they the studied physical and chemical characteristics of WAS after MW pre-treatment to determine the optimum conditions for microwaving to achieve high SS disintegration. Different set of power (500, 750, and 900 W) and contact times (0-140 seconds) were tested. Their results show a direct relationship between the increase of VSS solubilisation and both MW power and contact time. After longer irradiation (140 seconds), VSS solubilisation was 24.7%, 25.7% and 29.6% at 500 W, 750 W and 900 W, respectively. For the same contact time there was also an increase in the SCOD/TCOD from 0.0622 (raw sludge) to 0.1571, 0.1581 and 0.1611 at powers of 500W, 750W and 900W, respectively. The authors reported 900W and 60 seconds as being the optimum condition for sludge digestibility and energy consumption.

Eskicioglu *et al*. (2007) investigated low temperature (50 and 96 ºC) MW treatment of TWAS. Similar to other studies discussed here, the authors showed positive effects on solubilisation with a 3.6 and 3.2-fold increase in SCOD/TCOD ratio for 50 and 96 °C respectively. MW pre-treatment was also beneficial in improving AD by enhancing methane production, with 13% and 17% increases for 50 and 96 °C respectively. All samples showed similar improvement in VS destruction compared to the controls. Similar to Kennedy *et al*. (2007) temperature was found to be the most important factor affecting WAS solubilisation.

As previously stated, the combination of thermal and athermal effects of MW irradiation can disintegrate organic components in FW into smaller particles making them more available for microorganisms in AD and potentially enhancing methane production. Only two studies, however, have been reported on the effects of this pre-treatment, and understanding of the full effect of such treatment on the chemical and physical characteristics of the waste, microorganisms and process performance is still incomplete. These two studies are now discussed.

Marin *et al*. (2010) reported the effects of microwave irradiation on the anaerobic biodegradability of model kitchen waste (a mixture of different components such as cabbage, cooked rice, oats, dog food and eggplant). The pre-treatment conditions were heating rates of 7.8, 3.9 and 1.9°C/min and a final temperature of 175°C with 1 min temperature holding time. Two different fractions of the waste were tested: liquid/supernatant fraction and the solid fraction designated as whole fraction.

The authors reported that MW irradiation was successful in solubilising not just COD but also proteins and sugars in the supernatant phase, as well as in the whole fraction of pre-treated KW compared to controls. The solubilisation of COD was more evident in the supernatant phase, reaching values of 82, 68 and 99% at heating rates of 7.8, 3.9 and 1.9°C/min, respectively. In the whole fraction, solubilisation increased by 24, 14 and 20% for the same HRs compared to controls. In terms of biodegradability improvement, the authors observed that for the soluble fraction there was an increment of between 5% and 16% relative to controls. On the other hand, for the whole fraction, there was only an improvement for the most rapid heating rate (7.8°C/min) by 9% suggesting that solubilisation rates are not always linked to biodegradability improvement.

As expected, the increased solubilisation and biodegradability rates for all heating rates of the soluble fraction were followed by an increase in biogas production, with an average of 16% more biogas at 3.9 and 1.9 °C/min, compared to 5% at 7.8°C/min. For the whole fraction, the improvement in biogas production (of 11.76%) was only observed for the most rapid heating (7.8°C/min). Overall, the authors concluded that MW irradiation is effective in solubilising organic matter, improving biodegradability and biogas production from kW, especially the soluble fraction. The disposal of the whole fraction and all its environmental and economic impacts needs to be considered, however.

Shahriari *et al*. (2012) investigated the effects of pre-treating OFMSW with a combination of MW irradiation and hydrogen peroxide. The main variables affecting the characteristics of the OFMSW were temperature (115-175°C) and supplemental water additions of 20% and 30% (SWA20 and SWA30). Both fractions (liquid and whole) were evaluated. According to them, the combination of high temperatures (>145°C) and chemical pre-treatment resulted in a larger component of refractory material per gCOD causing a decrease in biogas production. The whole fraction of OFMSW pre-treated at 115 and 145 °C, to the contrary, exhibited a 4–7% improvement in biogas production compared to control. However, at higher pre-treatment temperature of 175°C, there was a decrease in biogas production attributed to the formation of refractory compounds, inhibiting digestion. Regarding the liquid fraction of OFMSW, SWA20 at 145°C exhibited a more pronounced biogas increase of 26% after eight days of digestion relative to untreated samples. The authors concluded that the positive effects of combining MW and H_2O_2 are negligible for the enhanced biogas production.

Carlsson *et al*. (2012) performed a comprehensive literature review of the most frequent types of pre-treatments and their effects on different organic wastes, including household and food industry waste. They state that organic waste from households (Organic Fraction of Municipal Solid Waste – OFMSW) is often subject to mechanical treatments (Figure 2.14). Besides mechanical processing, thermal and chemical pre-treatments have also been frequently studied with OFMSW. Nevertheless, few studies have focused on OFMSW pre-treatment with microwave irradiation, PEF, freeze/thaw and WO pre-treatments.

Regarding OFMSW, the authors reported that particle-size reduction is induced only by mechanical pre-treatments, whereas solubilisation may result from all pre-treatment types. Biodegradability enhancement on the other hand, is an effect only observed for some of the treatments. The addition of acid may cause formation of refractory compound (Hansen *et al*., 2007).

The review made by Carlsson *et al*. (2012) also included slaughterhouse waste (Salminen *et al*., 2003; Luste *et al*., 2009; Hejnfelt and Angelidaki, 2009; Battimelli *et al*., 2009; Cuetos *et al*., 2010) and waste from the dairy industry (Palmowski *et al*., 2006; Beszédes *et al*., 2011). The main pre-treatments applied in this case were thermal and chemical, followed by ultrasonic and microwave pre-treatments (Figure 2.14). Moreover, due to the high initial biodegradability of slaughterhouse waste, some pre-treatments such as thermal (70 and 133°C) and chemical (alkali) may have a negligible effect (Hejnfelt and Angelidaki, 2009; Battimelli *et al*., 2009; Carlsson *et al*., 2012).

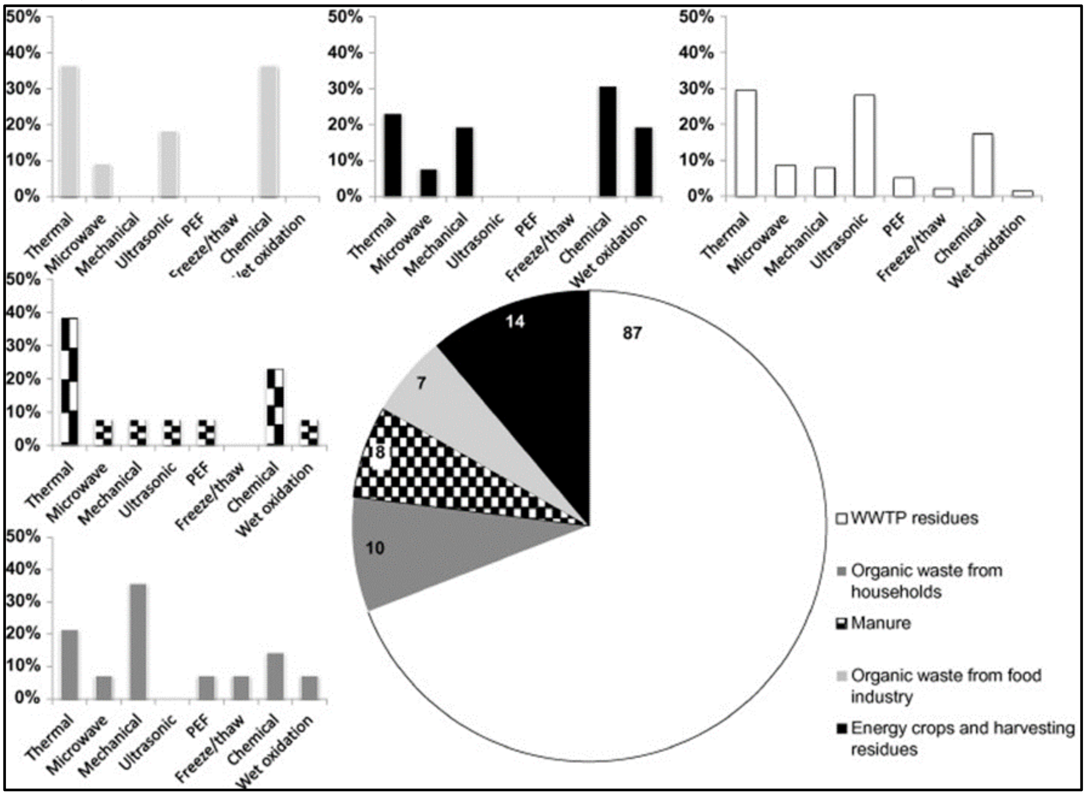


Figure 2.14 Pre-treatments and substrates in the reviewed literature. Substrate pre-treatments applied to different substrate categories in lab-, pilot- and full-scale studies as well as discussed in reviews (112 papers from 1978-2011). The pie-chart illustrates the number of times each substrate-type occurs in combination with a pre-treatment; the total number of occurrences is larger than the number of articles since several articles discuss more than one pre-treatment type. The bar-charts illustrate the distribution among the different pre-treatments for each substrate-type. The literature was selected so as to cover as many different types of substrates, pre-treated with as many processes and/or technologies as possible. (Carlsson *et al*., 2012).

In contrast to OFMSW, particle-size reduction of waste from the food industry is realised by chemical and ultrasonic pre-treatments, while improvement in solubilisation results from all pre-treatment types applied. The effects on biodegradability varies depending on raw substrate characteristics, however it can be said to be present as a result of most treatments cited here.

It can be said with confidence that pre-treatment methods can enhance AD performance. Nevertheless, the high capital cost, high-energy consumption, required chemicals and sophisticated operating conditions (maintenance, odour control etc.) are significant factors hindering their full-scale application (Mata-Alvarez, 2003; Yadvika *et al*., 2004; Carrère *et al*., 2009).

Regarding FW and its variations (e.g. household waste and fruit and vegetable waste), several pre-treatment methods have been applied to enhance methane production. Some of them have already been discussed here, while others are briefly described in Table 2.7. In most cases, the pre-treatment method applied was successful, implying in a higher biogas or methane yield.

It becomes clear from Table 2.7 that pre- hydrolysis is a very common pre-treatment strategy. This is because, by enhancing the hydrolysis step, the total time spent on the AD process can be significantly reduced, hence diminishing process costs. Kim *et al*. (2006) applied thermophilic hydrolysis on food waste and reported a reduction on total AD process time, with an HRT reduced to 10 days, hence suggesting a cost-saving scenario.

The greatest gas (biomethane or biogas) enhancement was obtained when thermochemical process was applied. Ma *et al*. (2011) tested the effect of the addition of HCl until pH reached a final value of 2, final temperature of 120°C and pressure of 1 bar for 30 min. The authors obtained a 24% higher biogas production compared to controls (no pre-treatment). Nevertheless, when FW was pressurized until 10 bar and then depressurized, the increase in biogas was even higher (48% in relation to control). The maximum increase was obtained when the authors froze the FW at 80°C for 6 h, and thawed for 30 min. This resulted in a 56% biogas production increment in relation to control.

Carlsson and Anox (2008) applied 400 pulses with electroporation to FW. The pre-treatment method was efficient in enhancing biogas increment, which varied from 20–40%. This was attributed to substrate cell breakage.

Zou *et al* (2016) used a high voltage pulse discharge (HVPD) as a pre-treatment method for FW. The authors showed that the chosen treatment was effective in enhancing process performance, with a 54% VS destruction and a total cumulative methane production equivalent to 134% higher than the control samples (no pre-treatment applied).

Nevertheless, only a few pre-treatment methods, such as Cambi™, AD with a pre-hydrolysis stage (two-stage AD) and mechanical treatments have been applied at full scale. It is also clear, from the review conducted in this chapter and the high methane yield in Table 2.7, that thermal pre-treatment is one of most feasible technologies for integration providing the temperature and pre-treatment time are optimized.

Table 2.7 Comparison of pre-treatment methods to enhance AD of FW.

Substrate	Pre-treatment	Type of AD System	Results	Reference
Food waste	4 days microaeration with 37.5 mlO2/Ld	Mesophilic wet batch	21% Higher methane yield for inoculated substrate, and 10% higher methane yield for non-inoculated substrate	Lim and Wang (2013)
Household waste	70 °C for 60 min, KOH until pH = 10	Thermophilic batch	Methane yield of 500 mlCH_4/gVS, no enhancement due to Pre-treatment	Chamchoi *et al*. (2011)
Household waste	160–200° C, 40 bars for 60 min	Mesophilic continuous	55–70% COD solubilization, and 3% higher biogas production	Schieder *et al*. (2000)
Household waste	Mesophilic pre-hydrolysis (hydrogenogenic)	Mesophilic continuous	43 mlH2/gVS from first stage, 500 mlCH_4/gVS from second stage which is 21% higher than single stage system	Liu *et al*. (2006)
Food waste	Microwave with intensity of 7.8 C/min	Mesophilic batch	24% Higher COD solubilization and 6% higher biogas production	Marin *et al*. (2010)
Food waste	Addition of HCl until pH = 2 120° C, 1 bar for 30 min 19 ±	Thermophilic batch	13 ± 7% Higher COD solubilization and 48% higher biogas production. 3% Higher COD solubilization and 24% higher biogas production	Ma *et al*. (2011)
	Pressurized until 10 bar and depressurized		2 ± 7% Higher COD solubilization and 48% higher biogas production	
	Frozen at 80° C for 6 h, and thawed for 30 min		16 ± 4% Higher COD solubilization and 56% higher biogas production	
Food waste with polylactide	Hyper-thermophilic/thermophilic prehydrolysis	Thermophilic (TPAD)	15–18% Higher methane conversion ratios than conventional thermophilic digester	Wang *et al*. (2011)
Food waste	Semi-aerobic and anaerobic pre-hydrolysis	Mesophilic continuous	95% COD destruction which resulted in methane yield of 500 ml/gVS	Kim *et al*. (2000)
Food waste	Thermophilic pre-hydrolysis	Thermophilic	HRT can be reduced to 10 days	Kim *et al*. (2006)
Food waste	Thermophilic pre-hydrolysis	Mesophilic	61.3% VS destruction, methane yield of 280 ml/gVS	Kim *et al*. (2004)
Food waste	Mesophilic pre-hydrolysis	Mesophilic continuous (2 stage system)	9% and 13% Higher biogas production than mesophilic and thermophilic AD, respectively	Verrier *et al* (1997)
Food waste	Mesophilic pre-hydrolysis	Mesophilic	Best results of 520 mlCH_4/gTS was achieved at pH = 7	Zhang *et al*. (2005)
Food waste	Mesophilic pre-hydrolysis	Mesophilic continuous	65 mlH2/gVS and 546 mlCH_4/gVS	Wang and Zhao (2009)
Food waste	Microwave = 100 °C, 600 W	Mesophilic batch	Control: 297 mlCH_4/gVS added Microwaved FW: 316 mlCH_4/gVS added	Zhang *et al*. (2016)
Food waste	Thermophilic pre-hydrolysis	Mesophilic continuous	205 mlH2/gVS and 464 mlCH_4/gVS	Chu *et al*. (2008)
Food waste	400 pulses with electroporation	Mesophilic continuous 2	20–40% Higher biogas production due to substrate cell breakage	Carlsson and Anox (2008)
Food waste	70 °C for 2 h 150° C for 1 h	Mesophilic continuous	2.69% Higher methane production, 11.9% Higher methane production	Wang *et al*. (2006)
Food waste	Frozen/thawed and pre-hydrolysis for 7 days Frozen/thawed and pre-hydrolysis for 12 days	Mesophilic continuous	10% Higher COD solubilization, 23.7% higher biogas production. 4% Higher COD solubilization, 8.5% higher biogas production	Stabnikova *et al*. (2008)
Food waste	70 °C thermal and mesophilic prehydrolysis	Mesophilic continuous	91% of FW was converted to biohythane with 8% hydrogen and 83% methane	Kim *et al*. (2000)
Food waste Fruits and vegetables waste	175° C, 60 min	Mesophilic batch	7.9% Decrease in biogas production. 11.7% Decrease in biogas production	Liu *et al*. (2012)
Food Waste	High Voltage Pulse Discharge (HVPD)	Mesophilic batch	54% VS solids destruction with a total cumulative methane production 134% higher than control	Zou *et al*. (2016)

Food Waste	Alkali =0.4N NaOH, pH=12.7, 1h.	Mesophilic batch	339.2 (+25%) $mlCH_4/gVS$removed.	Naran *et al*. (2016)
	Alkali-thermal = 0.4NNaOH, autoclaved at 120°C for 30min.		360.7 (+33%) $mlCH_4/gVS$removed	
	Thermal= 120°C for 30min.		480.8(+77%) $mlCH_4/gVS$removed	
	Ultrasonic= Energy intensity of 360KJ/L, 30min.		432.6 $mlCH_4/gVS$removed	
	Control		271.7 $mlCH_4/gVS$removed	
Food Waste	Commercial enzymes =10U/g dry FW for glyco-amylases at 60°C. 100rpm,24h.	Mesophilic batch	457.36 $mlCH_4/gVS$ (+131%)	Kiran *et al* (2014)
	Fungal Mash=10U/g FW for glyco-amylases, at 60°C, 100rpm for 24h.		468.2 $mlCH_4/gVS$ (+137%)	
	Control		197.9 $mlCH_4/gVS$	
Food Waste	Aeration in 10L reactor, 40°C, 50L/h, 21%02, 2 days.	Mesophilic batch		Fisgativa *et al*. (2016)
	Aeration in 10L reactor, 40°C, 50L/h, 21%02, 4 days.			
	Control			
Kitchen waste	Physical	Mesophilic batch		Agyeman and Tao (2014)
	2.5mm		510-630 $mlCH_4/gVS$	
	4mm		470-560 $mlCH_4/gVS$	
	8 mm		460-470 $mlCH_4/gVS$	
Kitchen waste	Thermal	Mesophilic batch		Li *et al*. (2016)
	120°C, 10min		112 $mlCH_4/gVS$	
	120°C, 30min		152 $mlCH_4/gVS$	
	120°C, 40min		168 $mlCH_4/gVS$	
	120°C, 50min		161 $mlCH_4/gVS$	
	120°C, 60min		129 $mlCH_4/gVS$	
Kitchen waste	Thermal	Mesophilic batch		Li and Jean (2015)
	55 °C, 70min		939 ml biogas (+4%)	
	70 °C, 70min		1135 ml biogas (+25%)	
	90 °C, 70min		1173 ml biogas (+29%)	
	120°C, 50min		1200 ml biogas (+32%)	
	140°C, 50min		885 ml biogas (-3%)	
	160°C, 50min		909 ml biogas (-1%)	
	Control		911 ml biogas	

Adapted from Ariunbaatar *et al*. (2014) and Appels *et al*. (2011).

2.5 Digestate disposal: current challenges in the UK

With the increase of AD worldwide as an alternative method for energy and by-product generation using a variety of wastes, there has been a significant increase in the amount of digestate produced (Curry and Pillay, 2012). Digestate is a product of anaerobic digestion and consists mainly of water (over 90%), undigested material, microbial biomass, and inorganic compounds (minerals/ash) (Lansing *et al*., 2010; Goberna *et al*., 2011; Garfí *et al*., 2011; Alburquerque *et al*., 2012).

At farm scale, digestate is generally mechanically separated into liquid and solid fractions. The former fraction is rich in nitrogen (N) and potassium (K), whereas the latter retains great amount of phosphorus (P) and organic matter (mainly fibres) (Sambusiti *et al*., 2015). Due to the significant concentration of nutrients, the most common global market application for this material is as a soil conditioner/ fertilizer (NNFCC, 2012).

Considering the nutrient content of this material and, predictions for nutrient depletion (which, accounting for population growth and increase in nutrient demand, are 93 to 291 years for phosphorous and 235 to 510 years for potassium), digestate becomes a potential alternative for conventional inorganic fertilizers (Fixen and Johnston, 2012). Moreover, it has the advantage that it can be cheaply produced through anaerobic digestion anywhere, utilizing a wide range of raw materials including agro, commercial and domestic wastes. Using this material in agriculture could be one component in an integrated (cost effective and renewable) nutrient management strategy (Tamil Nadu Agricultural University, 2008; Grigatti *et al*., 2011).

According to Møller *et al.* (2009), land-application of the digestate results in emissions of biogenic CO_2 and N_2O. Emission coefficients of CO_2–C and N_2O–N for these processes were 0.86–0.96 of the C and 0.013–0.017 of the N in digestate, respectively. N and P as nutrients from land-applied digestate replaces chemical fertilizer, thus avoiding the GHG from the chemical fertilizer production. The net change in GHG emission replacing chemical fertilizer with digestates is –11 CO_2 kg/t (Matsuda *et al*., 2012).

Each type of digestate will have a different application. For example, agricultural wastes and energy crop waste are almost exclusively land-applied, while digestate generated from OFMSW and FW needs to be further managed and treated (assuming Article 6 of the EU Waste Framework Directive EEC, 2000 applies)(European Parliament and Council, 2000). This article states that in some cases certain waste shall ceased to be waste when it undergoes a recovery process (considering product quality, input materials, treatment processes and techniques, provision of information, quality assurance procedures, etc.), meaning that FW digestate can be treated as a ‘product’ (Saveyn and Eder, 2014).

Digestate of unacceptable quality should be regarded as “biowaste”, which cannot be used on agricultural land for food or fodder crop production (Trzcinski and Stuckey, 2011). A possible pathway to use this stabilized biowaste is as a daily, temporary or final landfill cover. Other land applications of this type of digestate include landscape restoration in discarded quarries and mines, anti-noise barriers, road construction, golf courses, ski slopes and football pitches (Amlinger *et al.*, 2004).

In Europe, some countries have their own digestate quality standards, e.g. PAS 110:2010 in UK (BSI, 2010). These digestate quality standards have specifications for hygienic standards, impurities, degree of fermentation, odour, organic matter content, heavy mental content, and parameters for declaration. The parameters for declaration include the essential characteristics and constituents of digestate products that help the end user determine the best practice for digestate land-application (Siebert, 2007).

In the UK most of the digestate produced (99%) is spread to agricultural land as fertiliser, either as whole digestate or as a separated fibre, and should be in compliance with quality standard protocols (PAS 110) to ensure it is free from pathogens, chemicals and physical contaminants (Fuchs and Drosg, 2013; Horan *et al.*, 2015). Sale price of qualified raw digestate, used as biofertilizer, varied (in 2015) from -18 to 5€/t in bulking marketing (Dahlin *et al.*, 2016).

The UK's higher demand for digestate began in 2006 with the increase of inorganic fertilizer prices, which lead to the highest recycling percentage of digestate to land as compared to other members of the EU in the same period (a total of 1,050,526 tonnes of Dry Solids went to land at that time in the UK). This product originated mainly from sewage sludge (Evans, 2012). Nevertheless, the digestate from FW is also good substitute for inorganic fertilizer. A research conducted by WRAP (2011), showed that the nutrients concentrations present in the form of whole digestate from FW, are similar and in some cases even higher than in traditional fertilizers. Total Nitrogen and Potassium for example can be found in higher concentrations (172% and 7.8 %, respectively) in the whole digestate derived from FW than in the general-purpose ones, which can be beneficial in the sense that less amount of this matter is needed to enrich the soil (Table 2.8).

The digestate quality of OFMSW or FW usually depends on the type of AD system (e.g., wet or dry, batch or continuous, single-stage or multi-stage, co-digestion or mono-digestion). The moisture content of the digestate will be influenced by the choice between wet or dry AD, with the latter producing a digestate of nearly 35% solids content, having less moisture and therefore, requiring less energy (costs) for drying (Baere and Mattheeuws, 2010).

Table 2.8 Comparison of food waste whole digestate and conventional fertilizer in terms of nutrients (WRAP, 2011). n.d= not detected.

Product	Whole Digestate Derived From Food Waste	General Purpose Fertilizer
Total Nitrogen (%dm)	15.00 (11.90 – 20.50)	5.50 (4.00 – 7.00)
NO_3-N (%dm)	Trace	n.d
NH_4-N (%dm)	10.50 (5.50 – 16.00)	n.d
Total P (%dm)	0.70 (0.30 – 2.00)	3.30 (2.50 – 6.00)
Soluble P (%dm)	0.10 (0.00 – 0.20)	1.47 (0.34 – 2.60)
Potassium (K) (%dm)	4.70 (1.40 – 9.30)	4.36 (2.50 – 5.81)

Later, in 2009, anaerobic digestion in the UK generated 277,000 tonnes/year of digestate; with 50,000 tonnes/year originating from municipal (kitchen/food) waste, and again its application was as a soil conditioner. However, the recent UK agricultural and crops requirements regarding digestate application to land has become stricter with the digestate originating from mixed waste materials being restricted to use on land restoration projects only (WRAP, 2013).

In areas known as Nitrate Vulnerable Zones (NVZ) there is limited field spreading of nitrogen coming from digestate. In England, NVZ represent 58% of agricultural land. In these areas, often animal manure field spreading competes with digestate, as they both contain significant amounts of nitrogen from organic origin (Arbor, 2015).

The surplus digestate needs to be properly stored otherwise it could result in emission of biogas into the atmosphere and/or odour, contamination of surface and underground water, contamination of soil, loss of energetic efficiency and an increased environmental impact of AD plants (Gioelli *et al*., 2011). Alternatively, it can be transported to regions with nutrients deficits (Rehl and Müller, 2011).

Generally, AD and digestate management are separate units in AD plants even though a portion of the digestate can be recirculated back to anaerobic digesters as inoculum (Michele *et al*., 2015; Wu *et al*., 2017). When solid digestate is qualified as a biofertilizer for soil, the “food to waste to food” concept includes the AD of FW with the resulting digestate used as a biofertilizer for vegetable production in a greenhouse (Stoknes *et al*., 2016). This concept could be used for small-scale anaerobic digesters, as small amounts of digestate can easily be utilized on nearby land without costly, long-distance, transportation (Mouat *et al*., 2010).

Alternatives to reducing these costs as well as shifting the concept of digestate from a cost to a revenue centre should be investigated. Many attempts to address these problems have been made over the past decade, including various innovative approaches to digestate valorisation. Most of them involve the dewatering process of the whole digestate into fibre and liquor. According to Reza *et al*. (2014), drying of the digestate is cumbersome and energy intensive. For example, 2kg of dry digestate requires 20.7 MJ of heat to dewater it. Moreover, the potential for ammonia emissions is a drawback to the drying process, with the need for maintaining the temperature constant to avoid ammonia loss through volatilization (Pantelopoulos *et al*., 2016).

Once the digestate is separated into liquor and fibre, the most common techniques for enhancing the fibre are enzymatic hydrolysis, composting, alkaline stabilization land application, all available in the United Kingdom (Figure 2.15). On the other hand, most of these processes are used for converting the cake into a soil improver or act as a pathogen killing mechanism, with none of them resulting in actual energy recovery or elimination of the digestate except the enzymatic hydrolysis process, which reduces the volume of solid digestate by converting it into a bio-liquid that can be further used to produce bio-fuels (WRAP, 2013).

Recovery of energy from the fibre is possible, through process like Incineration, Gasification and pyrolysis (Figure 2.15). However, another drying step would have to be added to the system, implying more investment, and the cost-benefit analysis may not always be positive. In the case of food-based digestate, the dewatering process can be even more complex. The surface charge properties of food-based digestates make it challenging to dewater using standard approaches. While dewatering of food-based digestates has proven possible through selection of the correct polymer, polymer dose and dosing conditions still remains economically challenging (Horan *et al.*, 2015).

Pyrolysis is an alternative strategy for the management of the solid fraction (fibre) of waste. It offers the advantage of converting organic matter into char, bio-oil, and syngas in an oxygen free atmosphere (Neumann *et al.*, 2015). Pyrolysis of the digestate from FW can produce bio-oil with a calorific value as high as 13.5 MJ/kg, syngas consisting of CH_4, H_2, CO_2, and CO, and biochar (Neumann *et al.*, 2015).

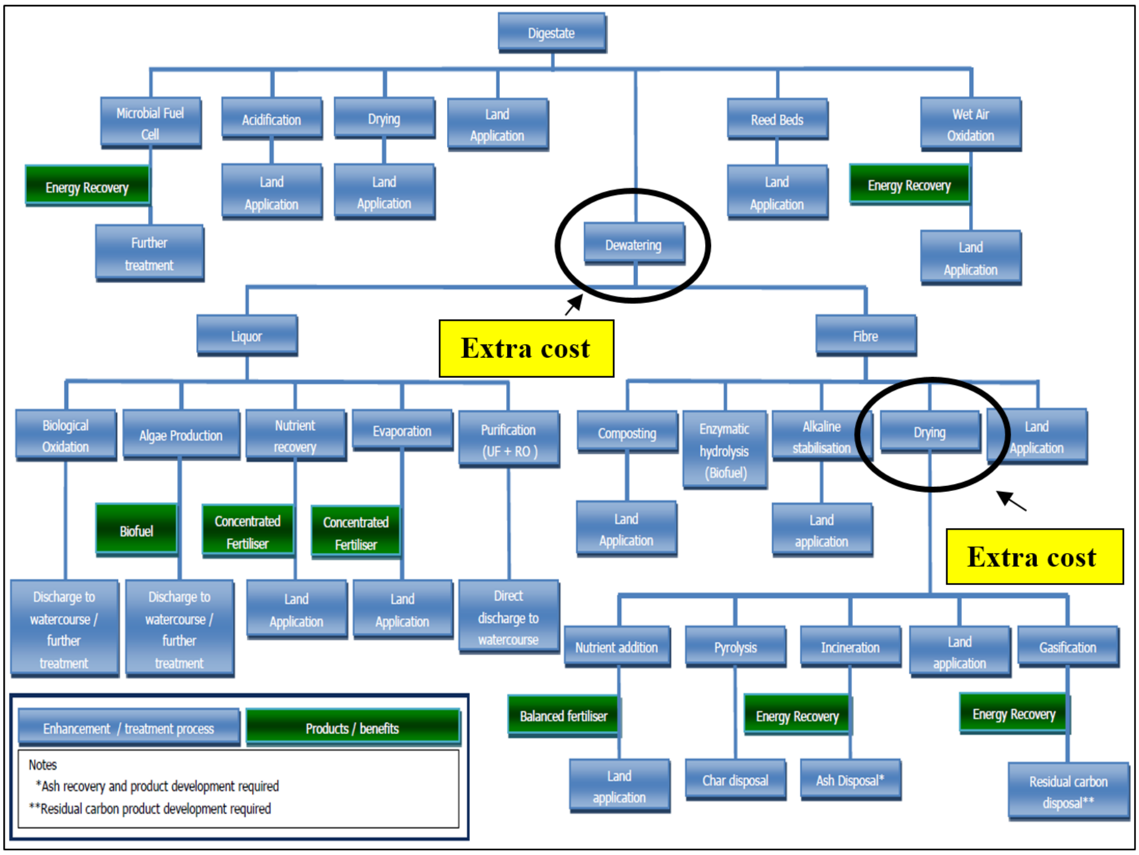


Figure 2.15 Modified Overview of Digestate enhancement and treatment techniques. WRAP (2012).

The biochar obtained from pyrolysis of digestate can also be used to immobilize metals (Cu, Pb, Zn) in industrial soil. Gusiatin *et al.* (2016) showed the efficiency of this by-product on soil remediation. The metal immobilization efficiency of biochar from digestate can be attributed to its high pH (8.83–10.00) and high cation exchange capacity. Moreover, this type of char has a high fixed carbon content, 56.3–83.2%, hence offering resistance to decomposition in soil. This makes it suitable for carbon sequestration (Crombie *et al.*, 2013).

However, pyrolysis of digestate poses certain disadvantages, due to the high operating temperature, which could lead to cross-linking reactions between hydrocarbons and aromatics, decreasing the bio-oil yield (Liu and Zhang, 2008). Alternatively, digestate incineration can reduce a significant fraction of the volume and organic matter and the fertilizer elements such as phosphorous, potassium and calcium present in the bottom ash can be recycled (Kratzeisen *et al*., 2010).

The average investment cost for an incinerator, which depends on the economic conditions of the country, varies from 257 to 772 € yearly per ton of waste (Hogg *et al*., 2002; World Energy Council, 2016). The total investment of a pyrolysis facility is, on average, 670 €/ton yearly based on 10,000 tons waste/year (Delft, 2016). According to Peng and Pivato, (2019), from a transportation point of view, thermal conversion such as incineration or pyrolysis is more feasible than land use (biofertilizer, soil improver or landfill cover) as long as the thermal conversion occurs inside the AD plant. If that is not the case, then the transportation fee can become very costly, since the combusted or pyrolyzed digestate would have to be transported from a treatment facility outside of the AD plant (Peng and Pivato, 2017).

All of the strategies for digestate management discussed above have both advantages and disadvantages, with the latter being mainly attributed to the cost associated with drying the material. Therefore, it is necessary to study alternative approaches for digestate management and utilization options.

A recent alternative view on this matter, suggests the adoption of the Back to Earth (BEA) concept, whose aim is to bring appropriately treated residues back to their non-mobile state. Digestates with appropriate treatment are brought back into the environment either as a soil amendment or as functional component for a landfill (e.g., cover material) (Dahlin *et al*., 2016; Riding *et al*., 2015). Nonetheless, circular economy cannot achieve ambitious goals such as closing the materials loop without addressing the issue of management of residues from production and recycling processes, suggesting that digestate management is not yet sufficiently advanced to implement this strategy (Cossu, 2016).

2.5.1 Digestate pre-treatment and re-circulation on AD

This strategy allows recovery of methane and other by-products and allows nutrient recycling. Although the most common practice is to utilize the liquid fraction of the digestate, the solid fraction has also been used as discussed below.

Balsari *et al*. (2009) were the first ones to propose a recirculation of digestate in the digester. According to them, this option could reduce GHG emissions and reduce the land area required for its storage, while improving the energetic and environmental exploitation of the anaerobic digester.

The residual biodegradability of digestate depends on its compositional and structural characteristics, which vary according to the type of substrates fed to the digester, and the AD plant/reactor configuration. The residual methane yields were also found to be closely correlated to other reactor parameters, such as the Hydraulic Retention Time (HRT) and Organic Loading Rate (OLR) (Menardo *et al*., 2011; Ruile *et al*., 2015). The overall effects off the digestate re-circulation are positive varying from enhanced VS removal to methane yield, with a few studies pointing to the unfeasibility of this strategy, as discussed ahead.

It has been shown that, in contrast to the solid fraction of the digestate, the liquid fraction is rich in VFAs, ammonia, enzymes and bacteria, hence offering additional positive effect on the digestion process. The concentration of each of these components varies across the different stages of the process (Siegert and Banks, 2005; Yuan *et al*., 2015; Tsafrakidou *et al*., 2018). Its volume is superior to the solid fraction, being estimated as high as 50–100 kg m^{-3} (Monlau *et al*., 2015).

The use of the liquid fraction of the digestate on the AD process is known as a Green pre-treatment. This type of treatment is attracting increasing attention because it eliminates the use, or generation of, environmentally hazardous chemicals and minimizes energy demand (Elsayed *et al*., 2019).

Elsayed *et al*. (2019) tested the effects of two different liquid fractions of digestate; acetogenesis digestate (Bio-DAcid) and methanogenesis digestate (BioDMeth), as a pre-treatment for lignocellulosic material (rice straw) and their effects on methane production/yield and nutrient recycle. The liquid fraction from the methanogenesis phase (BioDMeth) was obtained by centrifuging the digestate for 10 minutes at 3000 rpm and the acetogenesis biodigestate (Bio-DAcid), rich in VFAs, was prepared by partial digestion of rice straw using alkali-pre-treatment. The pre-treatment of the rice straw was performed using Bio-DMeth and Bio-DAcid for 24 h and 48 h during which the straw was left to absorb the solution, intended to act as a digestive agent. The treated biomass was then anaerobically digested at 55°C.

According to the authors, the AD of all pre-treated rice straw occurred in a shorter time than the controls due to the high anaerobic biodegradability of destroyed straw fibres. Nevertheless, T80 of the Bio-DAcid-treated straw was longer than that of Bio-DMeth which resulted in lower biomethane yield (208.3 and 226.8L kg^{-1} VS, respectively, at 24 h). The over-load of VFAs (specifically acetate) in the hydrolysate of the Bio-DAcid ultimately causes methanogenic inhibition and delay in biomethane production, and hence, augmentation of the technical digestion time by two days compared to Bio-DMeth. The authors also reported an increase in the biodegradability of pre-treated samples up to 55.7% (Bio-DMeth48h) compared to the control.

The authors conclude that by recycling the Bio-DMeth fraction of the digestate most of the biowaste was converted into biomethane, which results in lower CO_2 production in a cost-effective method. This pre-treatment not only reduces the CO_2 emission through enhanced methanation but also captures the residual nutrients in the digestate to form biomethane, with an additional advantage of saving the freshwater required for the pre-treatment process.

Liu *et al*. (2019) tested the effects of liquid digestate re-circulation and pre-treatment on biogas production from anaerobic digestion of wheat straw. This was based as previously discussed, on the replacing the need for chemical agents, hence reducing pre-treatment costs. The liquid fraction of the digestate was obtained from a small-scale mesophilic (38 ± 0.5 °C) biogas plant fed on chicken manure and used in substitute of a chemical agent and chemical pre-treatment step. Solubilisation and methane yield effects for wheat straw were analysed for treatment times of 3, 5 and 7 days, corresponding to Hydraulic retention times (HRT).

The authors observed that cellulose, hemicellulose, and lignin were reduced by 21.64%–30.22%, 8.5%–25.99%, and 1.22%–11.74% after pre-treatment 3, 5 and 7 days respectively. Additionally, the authors showed the relation between the positive effects of the pre-treatment and the early peak on gas production, which had a straight relationship with treatment total time. Therefore 3-, 5- and 7-days treatment reactors instantly reached to a peak gas production of 197 mL, 208 mL, and 212 mL, respectively, on the first day, and then reached the second peak of gas production of 163 mL, 181 mL, and 173 mL, respectively, on the 13th day. Process improvement was attributed to the breakdown of lignocellulose and cellulose into a small, soluble form, serving as a substrate for methane production.

As well as enhanced biomethane production, the authors reported an ameliorated VS removal. The VS destruction rates of wheat straw pre-treated with liquid digestate for 3 days, 5 days, and 7 days were 43.89%, 48.15%, and 49.72%, respectively. They were found to be 9.92%–17.47% higher than that of the untreated wheat straw (32.25%). Thus, it was concluded that liquid digestate pre-treatment improved the degradability of the raw materials and further enhanced methane production from them.

Similarly, Hu *et al*. (2015) investigated the effectiveness of re-circulating the liquid fraction of corn stover digestate (LFD) on the degree of biogasification of corn stover. The rationale behind their work is that LFD from an anaerobic digester with corn stover as substrate carries abundant specific lignocellulose-degradation microbes, and hence could act in substitution of a chemical agent needed for solubilizing the substrate. For this reason, the substrate treated with the liquid fraction of digestate could benefit from the decomposition of lignocellulosic components, thus enhancing biogas production. The LFD in this case was obtained through a 20-mesh sieve and is referred to as a filtrate. Its effects in the biogasification process were evaluated in four pre-treatment times of 1 (LFD1), 3 (LFD3), 5 (LFD5), 7 (LFD7), days of the corn stover imbibed in liquid digestate solution.

The authors reported that the total biogas productions (TBP) reached 33.91 ± 0.65, 35.26 ± 0.74, 33.01 ± 0.72, 32.55 ± 0.63, and 20.69 ± 0.95 LN (LN = litres at STP) for LFD1, LFD3, LFD5, LFD7, and untreated stovers, respectively. The TBPs of the LFD-treated corn stovers increased by 57.3–70.4% in relation to the untreated/control. Nevertheless, an inverse relationship was observed for the TBP. As the pre-treatment time increased from 3 to 7 days, the TPB decreased significantly. The authors attributed this to the partial consumption of the soluble organic substances during pre-treatment which has a detrimental effect on the consumption of soluble substances. This leads to a reduction of the substrate available for anaerobic bacteria (Yuan *et al*., 2012), The pre-treating time should therefore be controlled to minimize the depletion of soluble organic materials, thus making them available for biomethane production. Another benefit from treating the substrate with the LFD was an increase in the VS destruction with values from 60.2–67.0%, representing 22.1–35.9% increment compared to the controls.

Both benefits of the pre-treatment discussed above could be a consequence of cellulose and lignocellulosic material degradation. In conclusion, the authors determined that, in order to guarantee the complete chemical and biological reaction while minimizing consumption of soluble substances during pre-treatment, 3-days should be applied as the optimal LFD pre-treatment time.

Lindner *et al*. (2015) tested the effects of mechanical treatment of digestate on the degree of degradation and methane yield. The digestate originated from two different sources: a full-scale biogas plant belonging to the University Hohenheim and the acidification reactor of a two-stage laboratory biogas plant, fed daily with a mixture of 43.9% liquid manure, 9% solid manure, 19% maize silage, 21.4% grass silage and 6.8% grain. The pre-treatment consisted of gridding the by-product of AD with a ball mill for four different treatment time periods: 0, 2, 5, and 10 min. The experiment showed no losses of volatile fatty acids through warming by mechanical treatment which is a common finding in this type of treatment. In fact, the authors reported an increase in the TVFAs concentration (between 9 and 15%) for most of the treated samples (the exception were the two- and five-minute treatments of the biogas University's Plant digestate).

Mechanical pre-treatment of the digestate also resulted in an increase in methane yield for all treated samples. The greatest increase was from the digestate from the full-scale plant with 10 minutes of treatment giving triple of the methane yield of the untreated digestate. An improvement of the daily methane production and reduction of the lag phase was also observed for all the mechanically treated digestate samples.

Sambusiti *et al*. (2015) conducted a more detailed study on the use of digestate for the production of methane. The authors investigated the residual methane potential of digestate (DIG) and solid separated digestate (SS-DIG) and the feasibility of applying different kinds of pre-treatments (i.e. thermal, thermo-chemical and enzymatic) in order to enhance methane production. DIG and SS-DIG samples were collected from a mesophilic full-scale AD plant fed on a mixture of maize silage (25%), sorghum silage (11%), olive waste (11%), cow manure (8%), pig manure (18%), turkey manure and coconut chips (26%). Thermal treatment was performed at 80 °C for 1 h under stationary conditions. Alkaline post-treatment was conducted by soaking samples in a NaOH solution at a dosage of 1g NaOH/100g TS at 40°C for 24h without stirring. Lastly, enzymatic post-treatment was conducted by using a commercial enzymatic cocktail, especially developed to enhance biogas production of agricultural substrates. The effects of the treatments varied greatly. There was a detrimental effect, after thermal and alkaline treatments, on methane yields (a reduction of 10–20%) compared to those of untreated samples. Conversely, enzymatic treatment resulted in an increase in methane yield of 13% and 51% for SS-DIG and DIG samples, respectively. These findings are in agreement with other work reported in the literature, e.g. Kaparaju *et al*. (2010) in which a decrease of methane potentials of SS-DIG is observed after thermal treatment performed at 80 °C for 3 h. The decrease is attributed to changes in the chemical composition of the solubilized compounds (SCOD, nitrogen). Nevertheless, Sambusiti et. al. (2015) concludes that methane recovery from digestate and solid separated digestate is feasible through enzymatic pre-treatment considering their residual methane yields (70 NmL CH_4/g VS and 90 NmL CH_4/g VS, respectively).

Digestate was also used for the production of bio-oil by microwave assisted liquefaction. Direct liquefaction of biomass has attracted wide research interest since biomass can be successfully converted into multifunctional bio-oil at lower temperatures and in presence of solvents such as phenol, monohydric alcohols and polyhydric alcohols, and acid or basic catalysts (Soares *et al*., 2014; Lu *et al*., 2015). Microwave (MW) assisted liquefaction has been proven to be an interesting alternative to conventional heating because it results in faster and more uniform internal heating which accelerates the kinetic reaction rates and increases energy efficiency (Li *et al*., 2016).

Thermal treatment was investigated by Barbanera *et al*. (2018) who tested the effects on the production of bio-oil from the solid fraction of digestate resulting from a full-scale mesophilic biogas plant fed on a mixture of pig slurry (15m^3/d), olive pomace (19 t/d), maize silage (19.6t/d), sorghum silage (36.4t/d) and onion scraps (1t/d). Glycerol (at a fixed ratio of 4:1) and 3.5% sulfuric acid were used as solvent together with a maximum microwave power of 600 W. The optimum conditions for the microwave liquefaction of solid digestate were 180 °C, a solvent-to-biomass ratio of 4.2 for a total time of 27.5 min. These conditions gave a liquefaction yield of 59.38%, a High Heating Value of 28.48 MJ/kg and a microwave energy consumption of 115.93Wh.

Peng *et al*. (2016) studied the effects of recycling digestate or effluent liquor on the wheat straw-based CSTRs' (Continuous stirred-tank reactor) performance. Different scenarios were compared. These were: a) no recycling of the digestate used as a control (NR), b) recycling of supernatant after centrifugation (RSN- retention of soluble nutrients), c) recycling of the liquid fraction after filtration (through a 0.5 mm mesh) (RNM-retention of nutrients and microorganisms), d) impact of macro-nutrients nitrogen (NH_4HCO_3) and phosphorous $(NH_4)_2HPO_4$ on the co-digestion of wheat straw with sewage (RSNS), e) recirculation of solution nutrients (RSNS) and macronutrient supplemented-wheat straw and, f) recirculation of nutrients/microbes (RNMS) and macronutrient supplemented-wheat straw.

The authors demonstrated that methane production from the recycled liquor (centrifuged and filtered liquid fractions of the digestate) is small compared to the methane potential of the feedstock (wheat straw). Nonetheless, when the feedstock was digested together with the recycled liquor the methane yields achieved were on average 240 ml CH_4/g VS representing a 21% improvement over the processes without recycling. The energy gain presented in terms of methane yield ranged from 207 to 248 ml/g VS added.

Despite the improvement in methane production the authors noticed that, over time, the processes suffered from declining methane yields and poor stability. The system was then supplemented with macronutrients and, as a result, the digestion processes with digestate liquor recycling was stabilized achieving methane yields ranging from 288 to 296ml CH_4/g VS and low VFAs accumulation and prevalence of methanogenic pH. Amongst the digestate re-circulation with nutrient supplementation scenarios, the highest yield (296 ± 16 ml CH_4/g VS) was achieved by co-digestion with sewage sludge plus recycling of digestate liquor after filtration (retention of nutrients and microorganisms), followed by macronutrient supplemented-wheat straw plus recirculation of nutrients/microbes (RNMS).

The authors reported that, from all tested scenarios, the highest methane yields were obtained from the co-digestion of wheat straw and sewage sludge which was attributed to the additional micronutrients present in the latter capable of balancing any lack in the nutrient content, enhancing buffering capacity of the system and diluting inhibitors (Pöschl *et al*., 2010; Molinuevo-Salces *et al*., 2013). Compared to macronutrient addition (RSNI and RNMI), co-digestion (RSNS and RNMS) showed a 17% and 28% improvement in methane yield.

The authors concluded that re-circulation of digestate liquor can lead to stable operation and relatively high methane yield at a short SRT compared to the process without re-circulation. Under these conditions, however, there was a decline in all the main process parameters (methane yield, methane production rate, etc) and the effects were, therefore, not sustainable. Hence, the authors suggested this strategy may be limited to improving AD process performance, and would not recommend as the optimum operational condition. In turn, this could be delivered from the co-digestion of wheat straw and sewage sludge, without digestate re-circulation.

Table 2.9 summarizes the pre-treatment methods applied to digestate in order to increase methane production. Different pre-treatments methods including chemical (alkaline, ammonia), mechanical (maceration, ball milling), thermal (80°C, 120°C), biological (enzymes) and thermochemical (wet explosion) have been used, with this last one resulting in the highest methane yield.

Most of the pre-treatment method for digestate involved the separation of the whole fraction into liquid and fibre. As previously stated, this strategy allows the digestate to be more valuable, since different destinations can be applied to each fraction, including nutrient recovery, composting, and land spreading.

The methane yield obtained from the digestate is not as high as the one from FW, and this is possibly related to the fact that FW is richer in carbon content, as opposed to the digestate which is known for having high nitrogen content. Therefore, the methane yield of digestate varied from 21- 224 NmL CH_4/g VS.

The lowest methane yield value (21 NmL CH_4/g VS) was obtained by Lindner *et al.* (2015) when digestate (from a mixture of Cattle slurry (35%) Cattle manure (24%), Triticale and sorghum silage (35%), Separated solid fraction (6%), liquid manure (43.9%), solid manure (9%)) was separated by a decanter centrifuge and pre-treated with a ball milling during 10min.

The highest methane yield on the other hand was achieved when the digestate from a mixture of manure (90%), agriculture residues (5%) and industrial waste (5%). The substrate was separated by means of a decanter centrifuge and then pre-treated in two different ways: a) with wet explosion at 180°C for 10min and b) under similar conditions, but with the addition of 6 bar pressure. Biswas *et al*. (2012) reported that the latter pre-treatment condition heralded the highest methane yield at 224, as opposed to 209 NmL CH_4/g VS.

Jurado *et al*. (2013) applied the whole form of digestate (without phase separation) from swine manure to soak in aqueous ammonia at 22 °C for 3 days. The AD at mesophilic conditions heralded maximum methane yield of 200 NmL CH_4/g VS.

Sambusiti *et al*. (2015) used the whole fraction of digestate (from a mixture of maize silage (25% VS), sorghum silage (11% VS), olive waste (11% VS), cow manure (8% VS), pig manure (18% VS) and turkey poultry manure on coconut chips (26% VS) to undergo several pre-treatment methods. The first one corresponded to a thermal pre-treatment, where the digestate was heated at 80 °C for 1hour. In the second pre-treatment, different enzymes (cellulases and xylanase) were used to break down the organic matter. A final temperature of 40 °C and a pH of 5 was used in combination to the enzymes for a period of 24 h. Lastly, a NaOH (1% w/w) solution at 40 °C, for 24 h was tested. According to the authors, the most efficient method to enhance methane production and yield is the second pre-treatment, where enzymes broke down the organic matter. In this case, the methane yield was 90.74% and 152% higher than the thermal pre-treatment and the pre-treatment with NaOH solution, respectively.

Most of these pre-treatment methods are not widely adopted due to the requirement for large amounts of energy and special instruments, as well as a large number of unwanted by-products. A chemical-free and environment friendly pre-treatment method is desired, e.g. MW (Liu *et al*., 2019).

Microwave irradiation of digestate has not yet been tested, despite several studies showing that it improves solubilisation, VS removal and biogas production from sewage sludge. Similar to sewage sludge, digestate is as an end product of a biological treatment, having similar properties to the former such as the incomplete mineralization stage (20-60%) at the end of the AD process (Park and Ahn, 2011), organic matter thus still being available (TCOD and VS). Thermal pre-treatment may enhance the remaining organic matter solubilization further improving AD.

When analysing alternative pre-treatment methods for digestate, it is important to consider the whole fraction rather than the liquid or solid components alone. The disadvantages of utilizing only the liquid or solid fraction are associated with the disposal cost, and the environmental implications, of the fraction that is not being utilized. It is therefore necessary to develop a feasible way to utilize the whole fraction when recovering energy and nutrients from this by-product.

Table 2.9 Comparison of BMP data related to untreated and post-treated digestates.

AD plant feed	Digestate sample	Post-treatment conditions	BMP test conditions	Methane yield (NmL CH_4/g VS)	Ref.
Cattle slurry (35%) Cattle manure (24%) Triticale and sorghum silage (35%) Separated solid fraction (6%)	SS-DIG (screw press separator)	– 120 °C, 30 min	40 °C, 56 days	157 ± 7 176 ± 5	Menardo *et al*. (2011)
Cattle slurry (33%) Cattle manure (23%) Chaff rice (7%) Maize silage (33%) Separated solid fraction (4%)	SS-DIG (compression roller separation)	– 120 °C, 30 min		117 ± 11 98 ± 5	
Swine slurry (76%) Grass silage (8%) Maize silage (16%)				71 ± 5 154 ± 21	
Cow manure (100%)	SS-DIG (sieve separation)	– 80 °C, 3 h NaOH (4% w/w), 20 °C, 48 h Freezing (−20 °C; 24 h) Mechanical maceration< 1 mm	35 °C, 30 days	61 ± 5 48 ± 2 61 ± 1 47 ± 1 51±2	Kaparaju and Rintala (2005)
Maize silage (25% VS) Sorghum silage (11% VS) Olive waste (11% VS) Cow manure (8% VS) Pig manure (18% VS) Turkey poultry manure on Coconut chips (26% VS)	SS-DIG (helical screw press)	– 80 °C, 1 h Enzymes (cellulases and xylanase), 40 °C, 24 h, pH 5 NaOH (1% w/w), 40 °C, 24 h	35 °C, 65 days	90 ± 1 79 ± 7 102 ± 6 81 ± 3	Sambusiti *et al*. (2015)
Manure (90%) Agricultural residues (5%) Industrial Wastes (5%)	SS-DIG (decanter centrifuge)	– Wet explosion (180 °C, 10 min) Wet explosion (180 °C, 10 min, 6 bar O2)	38 °C, 50 days	80 209 224	Biswas *et al*. (2012)
Liquid manure (43.9%) Solid manure (9%) Maize silage (19%) Grass silage (21.4%) Grain (6.8%)	SS-DIG (decanter centrifuge)	– Ball milling, 10 min, eight ball of 30 mm diameter	37 °C, 35 days	21 ± 2 58 ± 5	Lindner *et al*. (2015)
Grass silage (30% VS) Cow manure (70% VS) Of a CSTR laboratory scale reactor	DIG	– NaOH (2% w/w) of 40% NaOH solution, 35 °C, 65 h NaOH (3% w/w) of 40% NaOH solution, 35 °C, 65 h NaOH (4% w/w) of 40% NaOH solution, 35 °C, 65 h NaOH (6% w/w) of 40% NaOH solution, 35 °C, 65 h	35 °C, 118 days	100 ± 6 93 ± 7 99 ± 4 96 ± 4 99 ± 10	Jagadabhi *et al*. (2008)
Solid fraction from swine manure	DIG	– Aqueous ammonia soaking 22 °C, 3 days, 32% (w/w) ammonia	37 °C, 35–50 days	111 ± 11 200 ± 7	Jurado *et al*. (2013)
Maize silage (25% VS) Sorghum silage (11% VS) Olive waste (11% VS) Cow manure (8% VS) Pig manure (18% VS) Turkey poultry manure on Coconut chips (26% VS)	DIG	– 80 °C, 1 h Enzymes (cellulases and xylanase), 40 °C, 24 h, pH 5 NaOH (1% w/w), 40 °C, 24 h	35 °C, 65 days	70 ± 2 57 ± 2 106 ± 4 42 ± 12	Sambusiti *et al*. (2015)

Source Sambusiti *et al*., (2015)

2.6 Identified research gaps from published literature - summary

The literature review has identified the following research gaps:

1) 1.3 billion tonnes annually, or one third of the food produced in the world for human consumption, is lost or wasted throughout the supply chain from production to consumption (FAO, 2011). In the UK the annual FW arising from households, hospitality and food services (HaFS), food manufacture, retail and wholesale sectors in 2018 was around 9.5 million tonnes, with over 85% (by weight) of this waste generated in households (WRAP, 2020). The use of FW in Anaerobic Digesters to produce methane is not a new and previous studies have shown that in most cases such digestion produces satisfactory results. Nevertheless, it has also been found that when treating FW samples high in lignocellulosic content, low methane yields (50-60% of the theoretical maximum value) are obtained (Marin *et al*., 2010). This limitation can be circumvented by adopting different feedstock pre-treatments including microwave (MW) irradiation, which breaks weak hydrogen bonds and has the potential to make complex organic molecules unfold and become smaller, thus promoting solubilisation and making them more readily biodegradable. Although this pre-treatment has been previously tested in FW (Marin *et al*., 2010, Shahriari *et al*., 2012, Zhang *et al*., 2014) studies are still scarce and more information is needed to optimize the process. In the work presented here different operational conditions (final temperatures and ramp rates) are tested on both soluble and whole-fraction FWs and the main factors affecting methane production are determined. As a result, further information on microwave operational conditions will be reported and an alternative route for FW to landfill (globally responsible for 3.3 × 109 tonnes-CO2-eq. year-1 of greenhouse gas emissions (GHG)) (Salemdeeb *et al*., 2017; Fisgativa *et al*., 2017) will be recommended.
2) With the increasing adoption of AD around the globe as an alternative method to generate both energy and by-products from different types of waste, there has been a significant increase in the amount of digestate produced (Curry and Pillay, 2012). The digestate originating from AD of FW arising from post-consumer stages (catering and households - the latter being responsible for 8.0 tons of FW generated in the UK for the year of 2018) has principally been used as a soil amendment (WRAP, 2018). This traditional route is now faced with some restrictions due to: a) the spreading window being limited to certain times of the year (i.e. not allowed in winter) and/or the land already being nutrient sufficient, b) agriculture demand for nutrient supply fluctuating during peak crop (e.g. beginning of spring), c) land application of digestate in some areas not being an option due to the risk over saturating fields with phosphorus and nitrates. Furthermore the rising costs associated with its transportation and spreading has created the need for alternative utilizations or destinations of digestate (Wellinger *et al*., 2013, Neumann *et al*., 2016). The recycling of digestate from AD of lignocellulosic material (rice straw and corn

stover) into AD has had positive results (especially regarding recovery of energy in the form of methane) and for this reason is considered to be a promising alternative route (Hu *et al*. 2015, Elsayed *et al*., 2019, Liu *et al*., 2019). The effects of re-circulation of digestate (of different volumes) from FW from the post-consumer phase on AD will be discussed. The use of this material in the AD reactors, precludes the need for water added to fresh FW, hence offering a sustainable option for scaling–up the process.

3) Several pre-treatment methods to enhance methane recovery from digestate have been tested, including mechanical, thermal and chemical treatments (Kaparaju and Rintala, 2005, Balsari *et al*., 2010, Menardo *et al*., 2011, Biswas *et al*., 2012, Lindner *et al*., 2015). However, to the best of our knowledge, microwave irradiation as a pre-treatment for digestate prior to AD has never been tested. The rationale for this strategy is the success of microwave digested sludge and the recovery of methane coupled with the fact that digestate has similar properties to sludge such as the incomplete mineralization stage (20-60%) at the end of the AD process. This offers the potential for bioenergy recovery (Park and Ahn, 2011). The whole fraction of the digestate is pre-treated and then re-circulated in the AD. The effects are investigated of the resulting potentially energetically favorable, closed system. Such a strategy also avoids costs and environmental impacts that the utilization of only one of the fractions, liquid or solid, would incur.
4) The challenge is how to treat and use such large amount of the whole fraction of digestate in an affordable and environmentally friendly way. In this sense, by adopting this novel strategy there would be several advantages, such as: a) reduction of the final volume of digestate, b) energy recovery from two current substrates of environmental concern: FW and digestate, ultimately offering an alternative management option for them. The combination of microwave pre-treatment on digestate and its re-circulation on the AD for the production of energy comprises the novelty of this research.

CHAPTER 3

3. EXPERIMENTAL METHODOLOGY AND ANALYTICAL METHODS

This chapter provides a detailed description of the materials and methods used in the various stages executed as part of this research. The methods are arranged in four parts corresponding to the four research objectives.

- Characterization of Food Waste, evaluation and comparison of biomethane potential under different I/S ratios.
- Thermal treatment (microwave irradiation) of food waste: influence of final temperature and heating rate on the biomethanation of FW under MAD conditions.
- Microwave thermal treatment of digestate: influence of final temperature and substrate concentration on biomethanation of FW under MAD conditions.
- Integrated study of mass and energy balance of the mesophilic anaerobic digestion of pre-treated substrates (food waste and digestate).

3.1 Materials

3.1.1 Seed inoculum

The inoculum used in this study was obtained from a mesophilic anaerobic digester (MAD), treating sewage sludge at Yorkshire Water's Esholt Waste Water Treatment Works (WWTW) in Bradford (West Yorkshire, UK). This is the second largest WWTW in Yorkshire serving approximately 760,000 people in the Leeds and Bradford region (Aragon Briceño, 2018). This inoculum was consistently used to seed anaerobic batch reactors to test the Biological Methane Potential (BMP) of the tested substrates.

Before each BMP test, this inoculum was passed through a 1 mm sieve to remove any large particles or grit. The only exception was when the acclimatised inoculum (seed) from a particular BMP test (e.g., BMP of microwaved FW at a fast-heating rate) was "recycled" into a subsequent test (e.g., BMP of FW at a medium heating rate), as sieving was not required. After sieving, the seed was incubated at 37°C.

Acclimation of the inoculum with raw/untreated FW occurred prior to BMP tests, at a concentration of 0.34 g-VS/L·day. However, on one occasion (BMP of FW microwaved at a slow heating rate) the inoculum was collected soon before a BMP test set-up and no acclimatization with the substrate was conducted.

Since the total set of BMP tests were carried out over a long period of time, the seed was characterized regarding its main physical-chemical properties (according to section 3.2.1) two days before each BMP set-up.

3.1.2 Food waste

Food waste was collected from the University of Leeds Refectory (Figure 3.1a, b) Leeds, United Kingdom. The refectory serves approximately 3,000 customers per day, with an estimated FW generation of 36 kg/day (Personal Communication, June 2017).

The collection of FW occurred over five consecutive days and samples consisted of a mixture of both plate waste (from the eating area) (Figure 3.2a) and kitchen waste (Figure 3.2b), thus designated Composite Food Waste (CFW). FW sample collected from the university's refectory is assumed to reflect the dietary habits of the typical UK household.

Figure 3.1 Leeds University Refectory at Lunch time (a) and at breakfast time (b).

Figure 3.2 Food waste collection: (a) from customer's plates and (b) from the kitchen area.

The samples were collected on the same day they were discarded, as recommended by Zhang *et al*. (2007), thus avoiding putrescible waste and underestimating the Total Solids (TS) and/or Volatile Solids (VS). The collected waste was manually sorted for any unwanted impurities such as glass, paper, cardboard, plastic and bones, thereby segregating inorganics from organics. The inorganics were discarded (Figure 3.3a, Figure 3.3b).

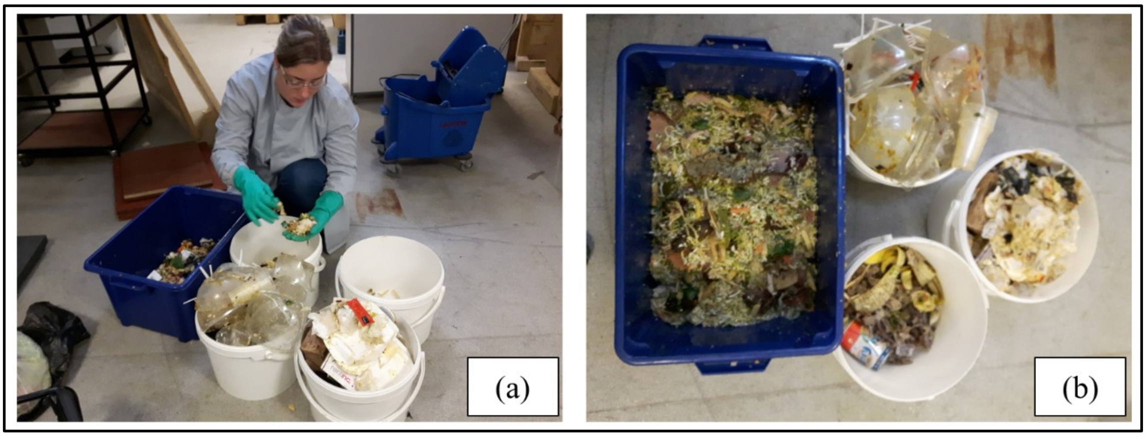


Figure 3.3 (a) sorting of the collected food waste, (b) sorted food waste in organic and inorganic fraction.

Sorted FW substrate was thoroughly mixed (Figure 3.4a), chopped and ground with a mincer. To allow further substrate size reduction and better homogenisation, the sample was blended with a food processer (Figure 3.4b). During this process, no water was added, so that the moisture content would not be affected (Figure 3.4c).

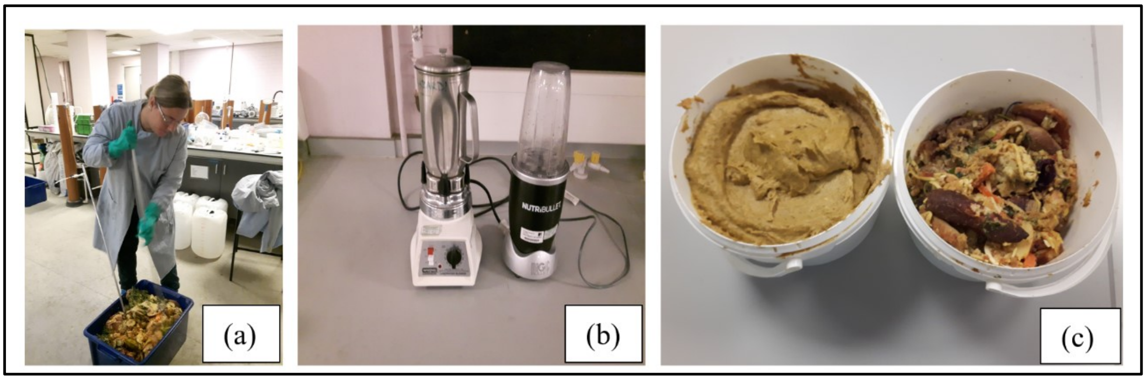


Figure 3.4 (a) mixing step of the food waste, (b) homogenisation of the food waste, (c) original food waste aspect and after processing step.

In order to generate representative samples, the blended waste was mixed and divided into four samples. Subsequently, smaller samples of 500 g were weighed into refrigerator bags, labelled and stored at -20°C until required for the experiments. However, one bag from each sample was stored at 4°C (fridge) in order to carry out a complete characterisation of raw FW. Frozen samples used for the experiments were defrosted at room temperature prior to BMP experiments. This was to ensure that no heat was added in excess of that needed for defrosting, thus avoiding physicochemical changes.

3.1.3 Digestate

In order to ensure satisfactory volume of digestate for the experiments as well as prompt availability of this material, this product was not acquired from a WWTP. Instead, the digestate used in this study was generated along the experimental phase of this research, more specifically during the mesophilic anaerobic digestion of microwaved FW after a period of 28 days, at the Public Health laboratory (School of Civil Engineering, University of Leeds). At the end of each run of BMP tests with microwaved FW as a substrate, the digestate samples were collected and stored at -20°C. Once this experimental step was finalized, the stored samples were defrosted at room temperature and aleatory mixed. The prepared samples were again stored at -20°C until further characterization and BMP analysis Figure 3.5.

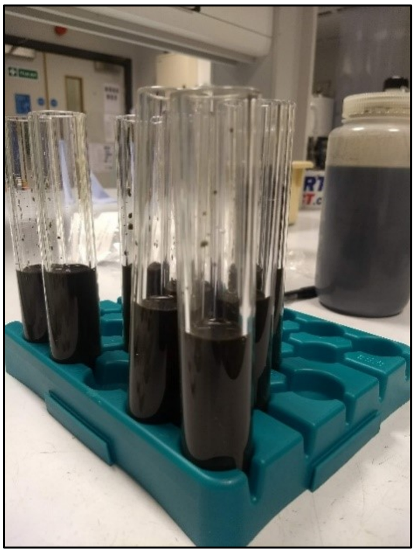


Figure 3.5 Mixed digestate for MW pre-treatment.

3.2 Methods

3.2.1 Food waste, seed and digestate characterization

Total Solids (TS), Volatile Solids (VS), Suspended Solids (SS), Volatile Suspended Solids (VSS) and total phosphorous for the FW, digestate and inoculum were examined according to the standard methods of APHA (2005) and He *et al*. (2013) (Table 3.1).

Table 3.1 Methods for feedstock and seed characterization

Analysis	Method
TS, VS, SS and VSS	2540B, 2540E
Phosphorous	4500P

Elemental analysis (carbon, hydrogen, nitrogen and sulphur) of FW and digestate samples was conducted using a FLASH 2000 Elemental Analyser. FW was also tested for protein, lipid and carbohydrate content. The former analysis was performed by determining nitrogen content using the Kjeldhal method (4500-Norg B), using a Buchi distiller in the distillation step (Figure 3.6), and the lipid content by acid solubilisation and extraction as described in AOAC Method 945.16.

(Figure 3.7). Carbohydrate values were obtained by the differential method; subtracting lipid, protein, ash and moisture content from the total weight of the samples.

Figure 3.6 Kjeldhal method for nitrogen content.

Figure 3.7 Preparation for Lipid method.

Volatile Fatty Acids (acetic; propanoic; i-butyric, butyric, valeric and i-valeric acid) were obtained by centrifuging the sample for 5 min at 153.7 g, and filtering the supernatant with a 0.2 μm filter. The filtrate was then analysed for VFAs using a gas chromatograph (Agilent 7890A) equipped with a flame ionization detector (FID) and NORDION NB-351 column, with a 25 m length, 0.32mm internal diameter and 0.5 μm film thickness. Operating conditions were: injector temperature 150˚C; FID temperature 240˚C; oven temperature program: 95-140˚C (10˚C/min), 140-200˚C (40˚C/min) with helium as a carrier gas. The SUPELCO Volatile Acid Standard Mix was used for calibration (Figure 3.8).

Figure 3.8 Gas chromatograph (Agilent 7890A) at the IPHEE laboratory, Civil Engineering Leeds University.

The pH of all samples was measured using a pH meter (HACH, HQ40dmulti) and alkalinity assessment was done by titration with 50 mM of H_2SO_4 solution, until the pH reached 4.5, using a Mettler Toledo TSO.

The concentration of the various trace elements and metals on the FW and inoculum was established by microwave-assisted acid digestion (nitric acid and hydrogen peroxide), using MARSXpress apparatus. Ammonia, Total bound Nitrogen (TNb), and Total and Soluble Chemical Oxygen demand were determined by HACH 39000 AP Robotic Water System with the LCK 302, APC 338, LCK 514 kits respectively (Figure 3.9).

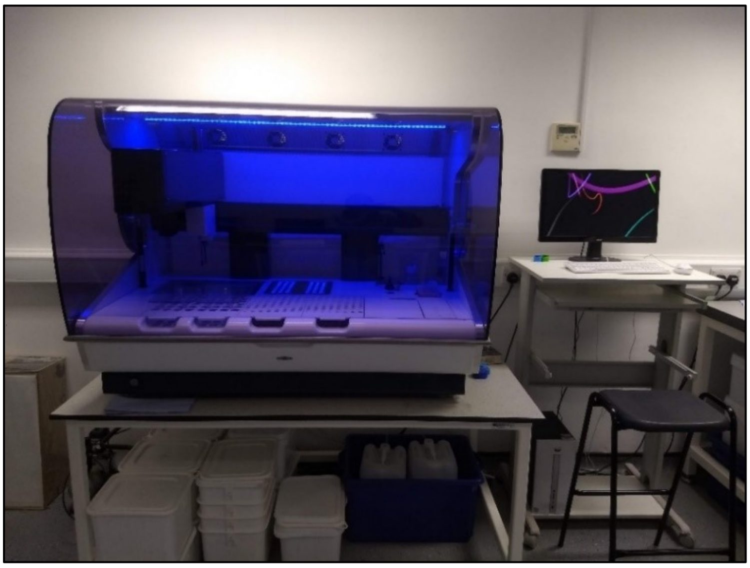


Figure 3.9 HACH 39000 AP Robotic Water System at Civil Engineering Labs, Leeds University.

3.3 Biochemical methane potential tests (BMP tests)

Biochemical methane potential tests (BMPs) are employed to determine the extent of anaerobic biodegradability of a substrate and thus the relative residence time for complete digestion (Labatut *et al*., 2011). In this study BMP tests were made on FW and digestate following thermal treatment (microwave irradiation) and for untreated/raw samples (FW and digestate). These were conducted in batches using 500 ml Duran bottles, with 400 ml working volume, under mesophilic condition (37°C).

The desired temperature was maintained by means of a water bath as part of the Bioprocess Control automatic methane potential test system (AMPTS II), located at the Public Health Lab in the School of Civil Engineering, University of Leeds.

In addition to the water bath for temperature control (Figure 3.10a), the AMPTS II instrument consists of two other main units: a bottle holder with 15 small bottles (80 ml) containing a CO_2 removal solution made from NaOH, ensuring only biomethane was measured (Figure 3.10b), and a flow cell unit responsible for measuring the volume of biomethane produced by each reactor by the displacement of each cell (Figure 3.10c). The volume of biomethane generated during the test is recorded by the equipment software.

The solution in each reactor (Duran bottle) is mixed by a rotating mixing rod (at a rate of one rotation per minute) with an individual electric motor. The mixing step during the BMP test ensures the microbial community is in constant contact with the substrate, hence increasing the probability of substrate degradation (Browne and Murphy, 2013).

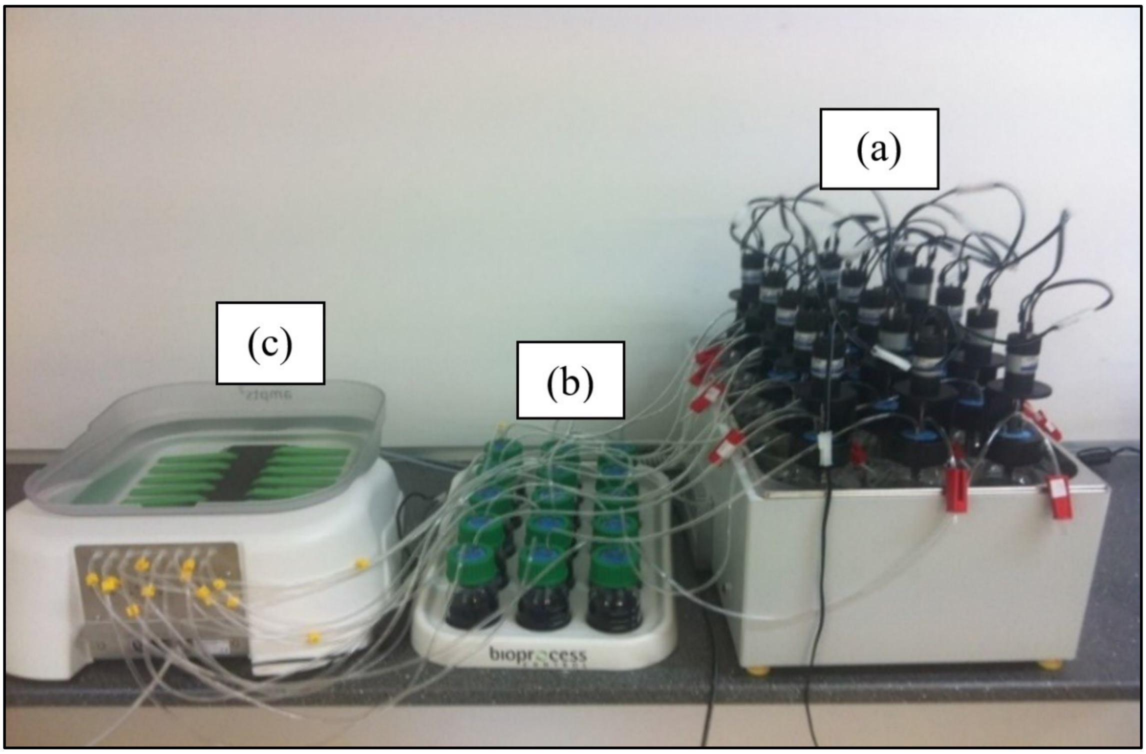


Figure 3.10 AMPTS II equipment used to monitor methane generation during this research. (a) water bath and Duran bottles – reactors, (b) NaOH solution for CO2 scrubber solution, and (c) cell displacement unit. Picture: Browne and Murphy (2013).

3.3.1 BMP test monitoring protocol: sample collection and processing

For each condition tested, triplicates were assembled, as well as control (substrate without thermal/microwave treatment) and blank (inoculum only) samples. Throughout the BMP experimental work, samples were collected from each reactor, including blanks and controls, on day 0, 2, 4 and 7. After this, sampling was carried out once a week, until day 28 (last day of digestion).

Samples from each reactor were analysed including the following: pH, alkalinity, soluble and total chemical oxygen demand, ammonium, total bound nitrogen, total volatile fatty acids, as well as total and volatile solids (Table 3.2). Due to the small working volume of the reactor, the total sacrificed sample volume for the duration of the BMP test was restricted. For this reason, the analysis for day 2 of the test was limited to verification of parameters related to the stability/acidity of the system, i.e. pH, Alkalinity and TVFAs. All analytical monitoring during the BMP process was conducted in triplicate.

Daily methane production by volume from each reactor was automatically measured and reported at normal conditions (Standard Temperature and Pressure (STP) conditions of 0°C and 1 ATM) by the AMPTS II software system. Cumulative methane volumes were corrected by deducting the methane volume originating from blanks reactors containing inoculum/seed but not substrate. Methane yields were normalised against the total amount of substrate added as VS (Equation 1).

$$\mathrm{BMP/CH_4yield} = \frac{\mathrm{VCH_4 - VCH_4blank}}{\mathrm{(mass\ of\ substrate\ fed\ into\ the\ reactor)}} \tag{1}$$

Where BMP = Biochemical Methane Potential (ml CH_4/g VS added), VCH_4 = Volume of methane produced in the reactor (ml), VCH_4blank = Volume of methane produced by the blank reactor, Mass of substrate = Mass of substrate in terms of g VS added in each reactor.

Table 3.2 Gantt chart for monitoring samples from BMP tests.

PARAMETERS	**EXPERIMENTAL SET UP - DAYS**																												
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
VFAS	▨		▨		▨			▨							▨							▨							▨
pH	▨		▨		▨			▨							▨							▨							▨
TCOD	▨				▨			▨							▨							▨							▨
SCOD	▨				▨			▨							▨							▨							▨
VS	▨				▨			▨							▨							▨							▨
TS	▨				▨			▨							▨							▨							▨
TNb	▨				▨			▨							▨							▨							▨
AMMONIUM	▨				▨			▨							▨							▨							▨
ALKALINITY	▨		▨		▨			▨							▨							▨							▨

The total digestion period was 28 days, or the period by which the daily methane production had fallen to less than 1% of the total cumulative methane (produced by the reactor since the beginning of the experiment) (Nielfa *et al*., 2015).

3.4 Data processing and statistical analysis

3.4.1 Solubilisation

The degree of solubilisation of the composite FW and digestate before and after thermal treatment was determined as proposed by López Torres and Espinosa Lloréns (2008). In this case, the soluble organic matter (SCOD) originally present in the samples, as well as the amount produced after the microwave treatment, was taken into account and expressed as a function of the total chemical oxygen demand (TCOD) as defined in Equation (2):

$$\%\ \mathrm{Solubilization} = \frac{\mathrm{SCODf - SCODi}}{\mathrm{TCODi}} \cdot 100 \tag{2}$$

where SCOD, soluble chemical oxygen demand (f-final and i-initial, mg/L) and TCODi, total chemical oxygen demand (i-initial, mg/L).

3.4.2 Theoretical methane potential (TMP)

The theoretical methane potential was estimated according to Buswell and Mueller (1952) and Li *et al*. (2013) as presented in equations (3) and (4). The Theoretical Methane Potential (TMP) calculated in this study was based on the Elemental Composition (C, H, O, N, S) of the feedstock.

According to Nielfa *et al*. (2015), the atomic composition of the waste material together with its ammonia and protein contents offer a fast method for determining the methane production of a given substrate. However, this equation does not differentiate between biodegradable and non-biodegradable matter, and part of the biodegradable organic matter is used by the bacteria to grow, so it does not contribute to the theoretical methane potential value. In practical terms, therefore, the theoretical methane potential method (TMP) will over estimate methane production in most cases.

$$C_cH_hO_oN_nS_s + \left(c - \frac{h}{4} - \frac{o}{2} + \frac{3n}{4} + \frac{s}{2}\right) H_2O \rightarrow \left(\frac{c}{2} - \frac{h}{8} - \frac{o}{4} + \frac{3n}{8} + \frac{s}{4}\right) CH_4 + \left(c - \frac{h}{4} - \frac{o}{2} + \frac{3n}{4} + \frac{s}{2}\right) CO_2 + nNH_3 + sH_2S \quad (3)$$

$$BMP_{thAtC}\ (NmLCH_4/gVSFW) \frac{22.4\left({}^{n}/_{2} + {}^{a}/_{8} - {}^{b}/_{4} - {}^{3c}/_{8}\right)}{12n + a + 16b + 14c} \quad (4)$$

where n, a, b, and c, represent the molar fraction of the Carbon, Hydrogen, Oxygen and Nitrogen respectively.

3.4.3 Anaerobic biodegradability

Sample biodegradability was determined using Equation (5):

$$\%\text{biodegradability} = 100 \cdot \left(\frac{V_{CH_4}}{V_{theoretical}}\right) \quad (5)$$

where VCH$_4$, is the net volume of methane gas produced in the reactor; Vtheoretical, is the volume of methane expected, assuming a complete degradation with 0.35L of methane (STP) per gram/COD degraded (Penaud *et al.*, 1999; Speece, 2008).

3.4.4 Statistical analysis

A kinetic analysis of the methane production was also conducted. The modified Gompertz (MGompertz) growth model, Equation (6), was used to fit the methane production curves, following Zwietering *et al.* (1990), in order to estimate the lag phase and maximum specific methane yield for each assay, using the Origin-Pro® 2016 graphical and statistics software.

$$y = A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t) + 1\right]\right\} \quad (6)$$

Where: y = Cumulative methane yield (mLCH$_4$/gVSadded) time dependent;
A = Maximum methane yield (mLCH$_4$/gVSadded) at time t;
µm = specific methane yield per day (mLCH$_4$/(g^{-1} VSadded • Day^{-1}));
λ = Lag phase (Days);
e = exp(1).

Origin-Pro® statistical package 18 was also used to do an ANOVA one-way statistical analysis to evaluate the significance of the different operational conditions on the solubilisation of organic matter and methane yields.

3.5 Objective 1: Characterization of food waste, evaluation and comparison of biomethane potential under different I/S ratios

A flow chart of the experiment design for Objective 1 is show in Figure 3.11.

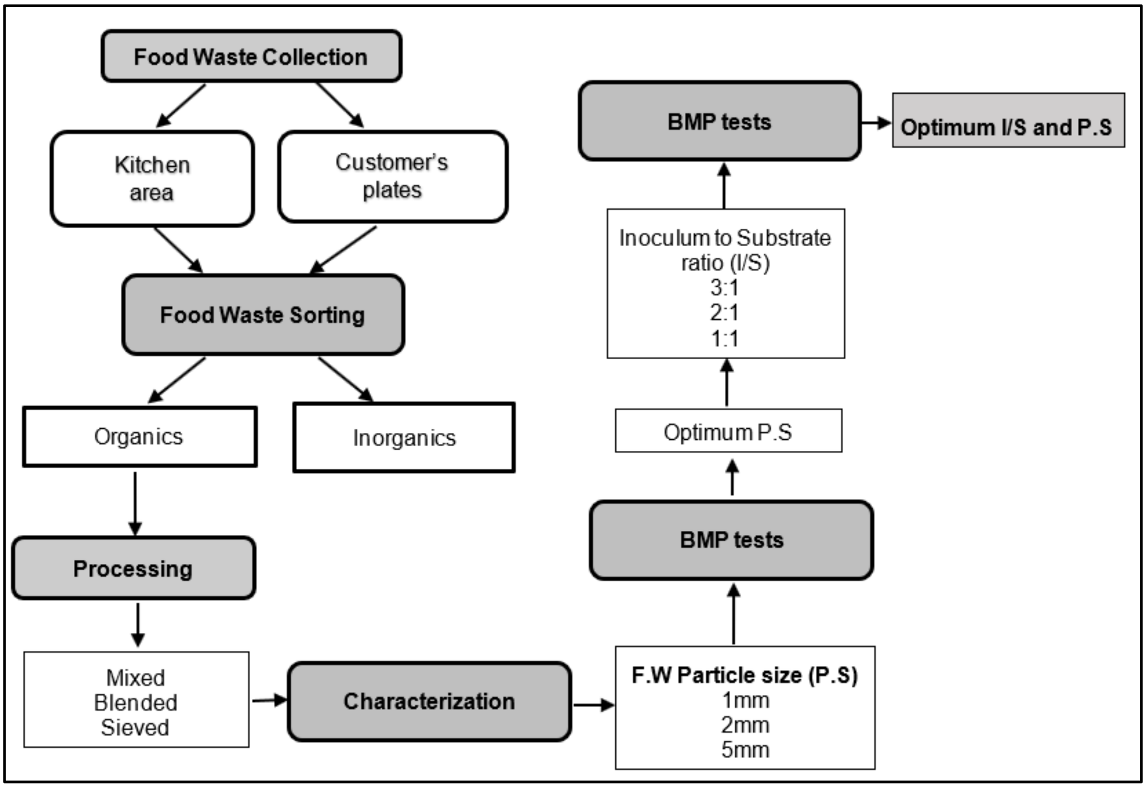


Figure 3.11 Experiment flow chart for Objective 1.

FW was collected and characterized as described in sections 3.1.2 and 3.2. A total of three particle sizes were accessed. The first PS corresponded to the undersize of the processed sample from 1 mm sieve, the second PS was the undersize of the processed sample from a 2 mm sieve and the last was the homogenised sample after processing with PS ≤5 mm; having 95% solids recovery from a 5 mm sieve. The desired PS were obtained by sieving the homogenised food waste sample through the respective sieve sizes with manual application of pressure using a flat metal bar. The choice for these PS was made based on the following literature findings: PS below 1 mm could encourage high VFA concentration, due to enhanced acidogenesis, while at higher PS, (above 5 mm) the biogas yields could be lowered due to poor feedstock degradation (Izumi *et al*., 2010). Subsequently, a first trial on the MAD of FW determined the best particle size for methane generation on BMP tests.

The best Inoculum to Substrate ratio (I/S ratio) for the mesophilic anaerobic digestion of FW was determined by mixing substrate and seed in different proportions based on a Volatile Solids (VS) content (Table 3.3). The I/S ratios tested were selected based on previous works reported in published literature, which suggest that a low I/S ratio value (<1) is insufficient for rapidly-degradable substrates in a batch test (Xu *et al*., 2003; Neves *et al*., 2004; Wu *et al*., 2011). A low I/S ratio (>0.5) increases instability in the anaerobic process, causing high COD content in the effluent and accumulation of volatile fatty acids (VFAs) (Raposo *et al*., 2009). Higher I/S ratios

(> 4), instead, yields poor biogas production, and decreases the effective space in the reactor. The I/S ratios tested were therefore 1, 2 and 3 (Figure 3.11).

The inoculum and substrate were placed and mixed in Duran bottles and distilled water was added to complete a total volume of 500ml in all reactors. 100ml of the mix was then removed for analysis (day 0), so each reactor had an initial working volume of 400ml. The bottles were flushed with 100ml of pure N_2 gas using a syringe to ensure anaerobic conditions and then each reactor was capped tightly with a rubber stopper.

Table 3.3 Total Volatile Solids concentration for each I/S ratio tested.

I/S ratio	PS (mm)	Inoculum concentration (gVS/L)	Substrate Concentration (gVS/L)	Volatile Solids Concentration (gVS/ml reactor)*
3:1	1	15	5	0.05
2:1	1	10	5	0.03
1:1	1	5	5	0.02

*based on a 400ml working volume in each reactor.

The calculation of the required amount of both seed and substrate was determined using Equations (7) and (8), respectively.

$$\text{VS for seed} = \frac{\text{VS of inoculum required (g)} \cdot \text{1000g of inoculum}}{\text{VS in 1000g of inoculum}} \tag{7}$$

$$\text{VS for food waste} = \frac{\text{VS of food waste required (g)} \cdot \text{1000g of food waste}}{\text{VS in 1000g of food waste}} \tag{8}$$

Samples were monitored as described in section 3.3.1. The results regarding the optimum conditions (PS and I/S in terms of methane production) from this set of BMP tests were used as a reference for the further research objectives.

3.6 Objective 2: Thermal treatment (microwave irradiation) of food waste: influence of final temperature and heating rate on the biomethanation under MAD conditions

After the establishment of the optimum FW particle size and I/S ratio for methane production, thermal treatment/microwave irradiation of FW prior to the anaerobic digestion process was investigated as a strategy to enhance biomethane production. Microwave irradiation of the feedstock was chosen based on the existing literature which suggests it is an effective way to circumvent the well-known limitation of the hydrolysis step on AD performance (Figure 3.12).

A flow chart for experiment objective 2 is show in Figure 3.12.

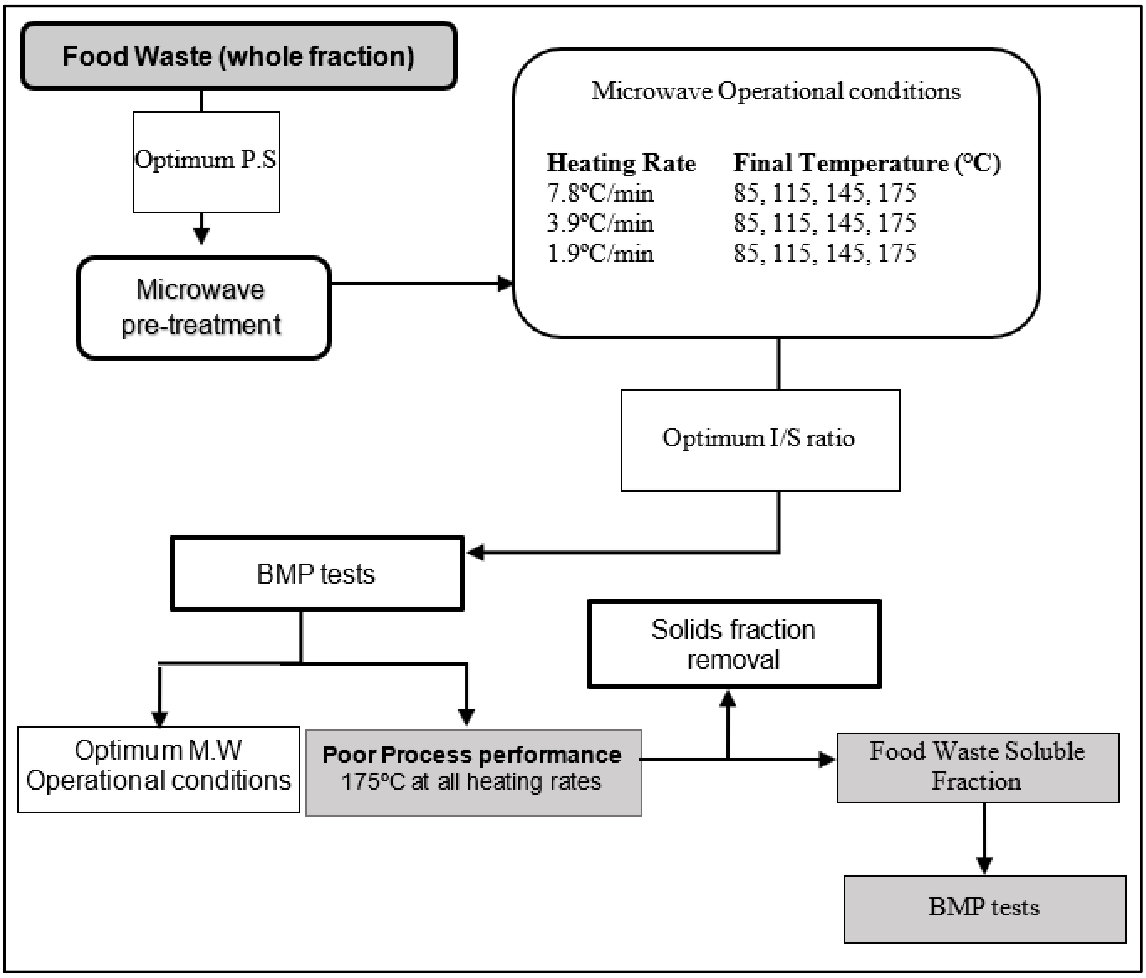


Figure 3.12 Experiment flow chart for objective 2.

3.6.1 Microwave pre-treatment conditions

A laboratory scale STARTSYNTH® microwave oven (Figure 3.13a) was used for the FW thermal pre-treatment step. The equipment has a maximum power output of 2.4 kW, operating at 220 V. The equipment consists of 20 sets of quartz vessels (Figure 3.13b) and a safety shield with springs and caps (Figure 3.13c), providing protection from explosion. It also eliminates volatile losses from the treated samples.

Each quartz vessel was filled with 2g of food waste (composite sample), sieved through a 1mm mesh and 5ml of distilled water. This results in a 10% solids solution (Figure 3.14). The addition of distilled water to the sample before microwave treatment decreased the solid content from approximately 32% to 10%, thus transforming the sample from a thick paste into a less viscous liquid.

The choice of a wet digestion (characterized by a low solids concentration of 0.5-15%) was based on several factors including: a) preventing adherence of solids to the quartz tube walls leading to sample loss, especially for high final temperatures, b) the need for smaller amounts of inoculum compared to the dry digestion (solids concentration ≥15%) and a lower retention time (Li *et al.*, 2011). According to Toreci *et al*. (2010) moisture content of organic wastes such as food waste affects MW pre-treatment efficiency, with higher water content increasing the effectiveness of pre-treatment, hence corroborating the effectiveness of wet digestion.

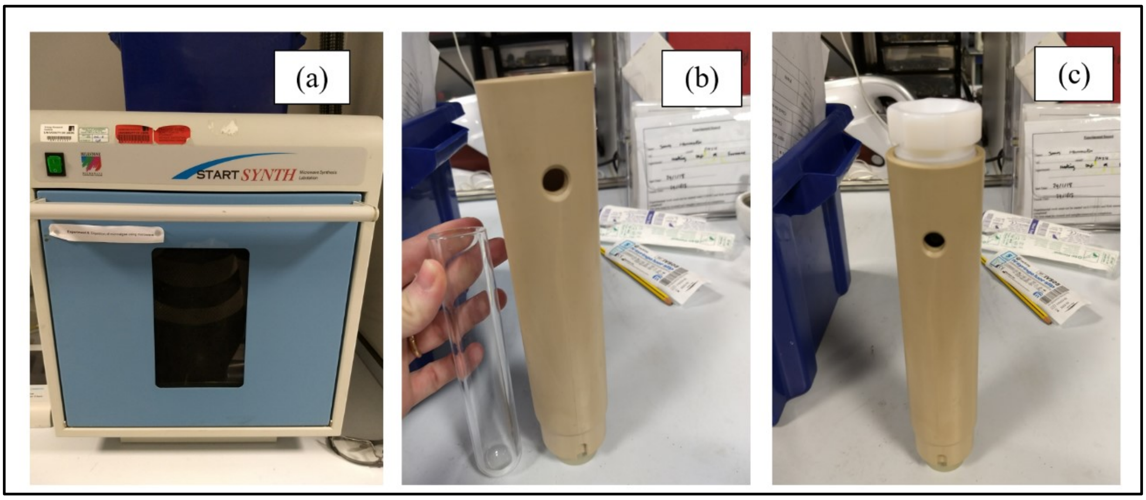


Figure 3.13 (a) Microwave equipment used during this research; (b) quartz vessels and safety shield; (c) safety shields with screw caps.

The vessels were sealed at room temperature at then placed into the STARTSYNTH® equipment.

Figure 3.14 Solids dilution (2 g) prior to microwave irradiation.

Four final temperatures were chosen: 85°C, 115°C, 145°C and 175°C and these were combined with three heating rates of 7.8, 3.9 and 1.9°C/min, which correspond to fast, medium and slow heating rate, respectively. In order to achieve these conditions, a power of 1000 W was applied based on a previous study by Zhang *et al*. (2016). Once the final temperatures were reached, the samples were held at such temperature for 5 minutes, referred to as the hold time. All the existing studies of microwave irradiation of FW have used a hold time of 1 minute. A longer hold time was chosen in this study to investigate its effect on methane production, solubilisation and biodegradability of the FW.

The final temperatures were selected based on the existing literature and the equipment suitability. The STARTSYNTH oven has a temperature limit of 350°C. Marin *et al*. (2010) and Inoue *et al*. (2002) showed that temperatures under 175°C, lead to higher COD solubilisation compared to the controls. In contrast the effectiveness of microwave irradiation on methane/biogas production under temperatures >175°C was proven to decrease. Therefore, the maximum temperature used in this study was 175°C.

Several studies have shown that thermal treatment of solid wastes, including kitchen waste and sludge, at temperatures <70°C are not effective in enhancing biogas production (Chamchoi *et al.*, 2007; Prorot *et al.*, 2011; Gonzalez-Fernandez *et al.* 2012). Furthermore, there is scarce information available regarding the process performance at temperatures <100°C (Zhang *et al.*, 2016). For this reason, 85ºC was selected as the lowest temperature in this study.

Due to the different heating rates, each sample was exposed to different total irradiation times (T_{total}). This is the time required for the equipment and samples to reach the final temperature (t_{ramp}), calculated as shown in equation (9), plus, the hold time (t_{hold}) which is the time the samples are exposed to the final temperature (Table 3.4). It is important to mention that the initial temperature was assumed to be room temperature (20°C), and tramp as well as Ttotal are reported as approximate values.

t_{ramp} was calculated as follows:

$$t_{ramp} = \frac{\text{Final temperature}}{\text{Heating rate}\left(\frac{°C}{\text{min}}\right)} \tag{9}$$

$$\text{based on a 5 min } t_{hold} \tag{10}$$

$$T_{total} = t_{ramp} + t_{hold} \tag{11}$$

Table 3.4 Operational conditions tested on food waste samples.

Microwave Operational Conditions		**Final Temperatures**			
		85°C	**115°C**	**145°C**	**175°C**
7.8 ramp	t_{ramp}	8min	12min	16min	20min
	t_{hold}	5min	5min	5min	5min
	T_{total}	**13min**	**17min**	**21min**	**25min**
3.9 ramp	t_{ramp}	16min	24min	32min	39min
	t_{hold}	5min	5min	5min	5min
	T_{total}	**21min**	**29min**	**37min**	**44min**
1.9 ramp	t_{ramp}	34min	50min	66min	81min
	t_{hold}	5min	5min	5min	5min
	T_{total}	**39min**	**55min**	**71min**	**86min**

After each application of microwave heating there was a cooling time of approximately 10 minutes. During this time, the sample temperature decreases to room temperature by a cooling fan. The cooling of samples to room temperature is a precautionary measure to avoid loss of volatile solids when opening the vessels to remove their content. Nevertheless, sample loss (volume reduction) especially at higher temperatures (145°C and 175°C) occurred, indicating solids loss (Figure 3.15a).

There were only five complete sets of vessels available for this project and all had visible signs of wear, especially the screw caps. This could explain the volatile losses (VS/TS) observed after thermal treatment at high temperatures. After this finding, the microwave FW sample generation method was adjusted, by determining the VS/TS loss for each combination of final temperature and heating rate and then correcting before the characterization and BMP steps. This was to ensure the desired VS loading in each reactor. The processed samples were analysed as described on Section 3.2.

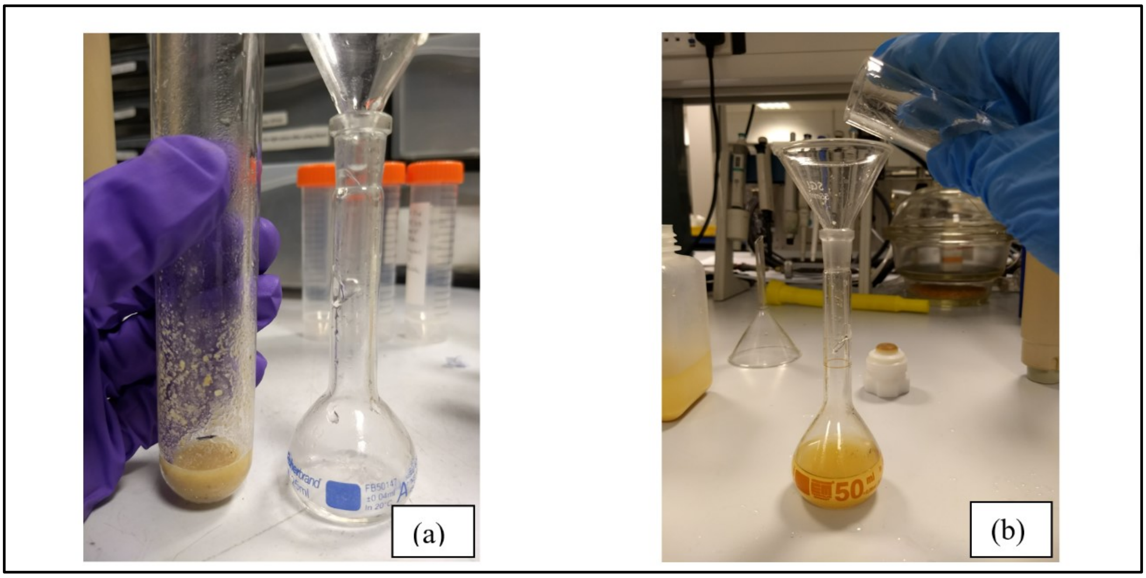


Figure 3.15 (a) Sample loss/volatilization and evaporation at 145°C 1.9°C/min heating rate. (b) Cleaning step of the vessels to ensure complete removal of sample residue.

3.6.2 Experimental factorial design for BMP tests of MW FW

In order to determine the best operational condition (combination of final temperature and heating rate) for methane production from MW FW, a multilevel factorial design was used in the study, with three variables, i.e. final temperature of sample, heating rate and exposure time to MW irradiation (Table 3.5).

Table 3.5 Factorial design for the microwave thermal treatment of food waste.

		Factor 1	Factor 2	Factor 3			
Inoculum to substrate ratio (I/S)	Particle size	Final Temperatures	Heating rate	Exposure time to M.W irradiation (min)			
				85°C	115°C	145°C	175°C
Optimum	Optimum	85°C 115°C 145°C 175°C	7.8°C/min	13	17	21	25
			3.9°C/min	21	29	37	44
			1.9°C/min	39	55	71	86

The BMP tests were assembled according to Section 3.3, following the same calculation for seed and inoculum amount as in Section 3.3.1. Control samples were added to this experimental step. The control samples were not subjected to any microwave irradiation, hence containing untreated FW, as well as inoculum and distilled water.

By virtue of the FW sampling and processing steps, all FW samples were considered representative, homogeneous and consistent in terms of physical-chemical characteristics. A single set of controls (untreated samples) was therefore considered sufficient all the experiments relating to microwave pre-treatment. All the experiments were conducted in triplicate including blanks and control reactors.

3.6.3 Soluble phase of FW assessment

After performing the BMP tests on the microwaved FW, it was noticed that samples treated at 175 °C regardless of the heating rate exhibited the poorest process performance with lowest methane yields. The former could be a consequence of the formation of inhibitory compounds for the methanogenic population such as melanoidins and humic acids (Marin *et al*., 2010). Therefore, a complementary experimental step was added, consisting of analysing the biomethane potential originating from the soluble fraction of microwaved FW at 175°C at all heating rates. This experimental step is important in identifying the origin (whole or soluble fraction) of the inhibition responsible for the low methane yield.

3.6.3.1 BMP tests using soluble fraction of food waste

FW samples were treated at the desired temperature and heating rates (7.8, 3.9 and 1.9°C/min) in the microwave. Control samples were prepared with raw/untreated FW.

An Eppendorf 5810 centrifuge from Sigma Aldrich was used to separate the solids from the solution (Figure 3.16a). This equipment has a maximum G force of 18.0 g. The centrifuge operating conditions for this study were based on previous work on microwave of FW reported by Marin *et al*. (2010), in which a force of 12.0 g was used for 30 minutes. A pilot run of 30 minutes under maximum speed showed that this was insufficient to sediment all the solids. The centrifuging time was therefore increased to 55 min. After the centrifuge step the samples were filtered with a 0.45 μm membrane to remove any colloidal solids and obtain only the supernatant (Figure 3.16b).

The final samples (Table 3.6) were placed on the reactors together with inoculum/ seed (in the proportion of 3:1) and distilled water. The biomethane production was assessed as described in Section 3.3. and the process performance analysed as described in Section 3.3.1.

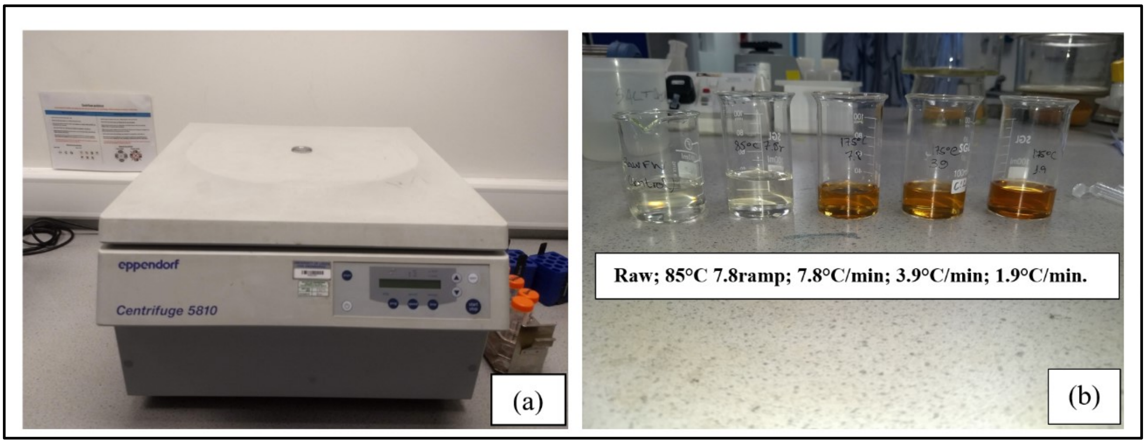


Figure 3.16 (a) Eppendorf 5810 centrifuge used in the phases separation, (b) centrifuged and filtered samples.

Table 3.6 Food waste solution volume before and after solid and liquid phases separation (centrifuging and filtering).

Soluble F.W Sample	Volume I (ml)	Volume II (ml)
175°C 7.8 ramp	52.1	40.5
175°C 3.9 ramp	52.4	40.5
175°C 1.9 ramp	52.2	40.7
85°C 7.8 ramp	52.1	40.6
Control F.W Soluble fraction	52.4	40.9

*Vol I. Before centrifuging and filtering. Vol. II= after centrifuging and filtering.

3.6.4 Soluble FW samples characterization

After phase separation, the samples were analysed, using the methods described in Section 3.2.1, for the parameters described in Section 3.3.1.

3.7 Objective 3: Microwave thermal treatment of digestate: influence of final temperature and substrate concentration on biomethanation of FW under MAD conditions

The MAD of FW is a promising option for renewable energy generation in the form of biomethane. Nevertheless, the end product of the MAD (the digestate) has become a matter of concern due to issues of strict land regulations, land shortages, rising disposal costs (including gate fees) amongst others (Wei *et al*., 2003; Zhang *et al*., 2016). Therefore, alternative management methods are being investigated in order to reduce the volume of digestate produced and thus, reducing costs associated with haulage and disposal.

Similar to sewage sludge, digestate is an end product of biological treatment, having similar properties to the former, such as incomplete mineralization (20 - 60%) at the end of the AD process (Park and Ahn, 2011), and hence retaining residual organic matter (TCOD and VS). Thermal pre-treatment could enhance the remaining organic matter solubilization improving AD. Despite this, in order to enhance the efficiency of the coupled process (microwave irradiation and AD) some corrective measures need to be made. The main measure refers to the correction of the already well-known low C/N ratio (6 - 8) of the digestate. This can be achieved by combining the pre-treated substrate with untreated FW (C/N 10.9).

The objective here aimed to investigate the biomethane potential from the whole fraction of FW when coupled with the re-circulation of microwaved treated digestate under different temperatures and concentrations in order to evaluate the possibility of enhancing energy recovery and biodegradability.

Differently from FW, the final microwave temperatures for treating digestate were 75, 95 and 115°C. The chosen range was based on the literature and previous work that stated that lower temperatures than the ones used to pre-treat FW are sufficient to promote organic matter hydrolysis and methane yield enhancement (Zheng *et al.*2009 and Eskicioglu *et al.* 2007).

The lowest temperature of 75°C was selected after considering previous work on the microwave irradiation on sewage sludge (primary and activated) by Zheng *et al.* (2009) and Eskicioglu *et al.* (2007). These work states that the treatment is inefficient with respect to methane production and biodegradability at temperatures below 60°C.

The highest temperature of 115°C was chosen based on the work of Toreci *et al.* (2009) which reported poor process performance at both single and dual stage semi-continuous mesophilic anaerobic digestion of waste activated sludge pre-treated with microwave irradiation at 175°C. An intermediate final temperature (95°C) was chosen half way between the highest and lowest final temperature. A single heating rate (ramp time) was adopted since previous studies (Zheng *et al.*, 2009; Eskicioglu *et al.*,2007) demonstrated that heating rate was not a significant factor affecting the solubilization and biomethanation of treated sludge.

A heating rate of 1.4°C/min was chosen based on the work from Eskicioglu *et al.* (2007) that used a similar method as the one here (BMP tests in batches). The combination of this ramp time with the final temperatures, results in a similar total exposure time to the ones applied in FW treatment, thus allowing comparisons between them. A hold time of 5 minutes was used for all test conditions, to maintain the same pattern as that for objective 2. Furthermore, in order to allow a better comparison between the microwave pre-treatment of FW and the digestate, the optimum condition for methane generation using FW (85°C, 7.8 ramp) was also applied here (Figure 3.17).

A flow chart for objective 3 is show below.

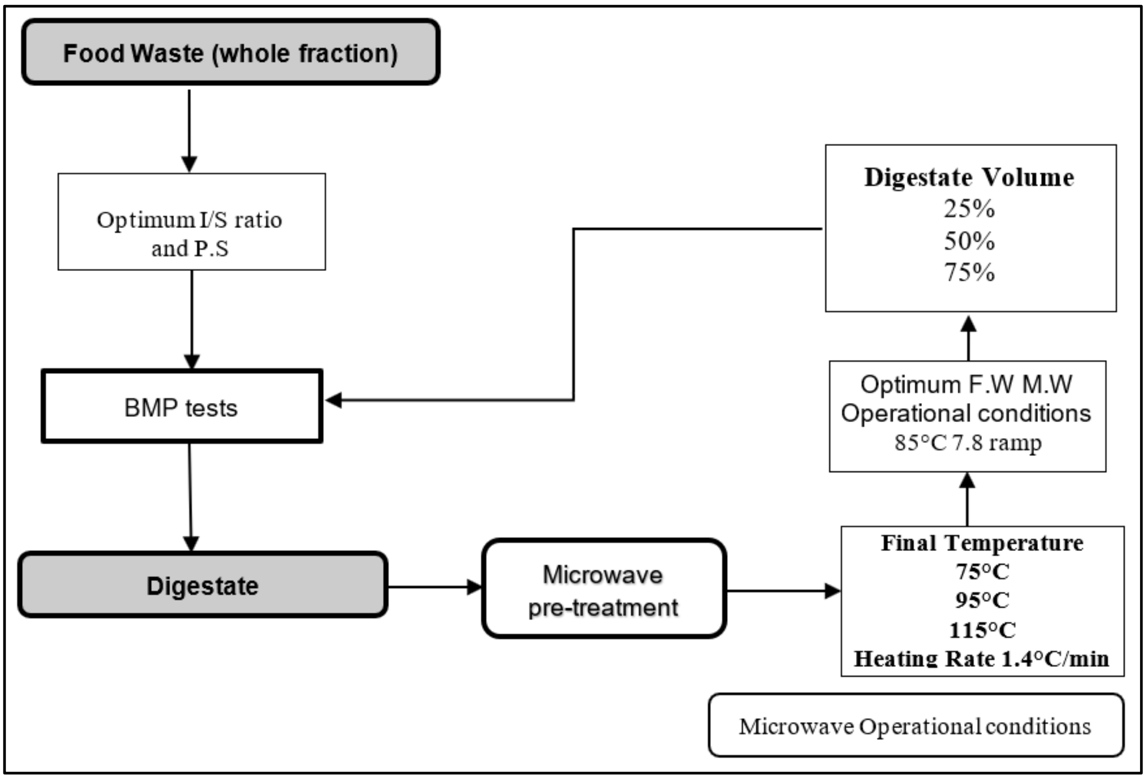


Figure 3.17 Schematic representation of Objective 3.

3.7.1 Microwave pre-treatment conditions for digestate

The digestate used in this experiment phase was obtained as described in Section 3.1.3. A permanent malfunction affected the STARTSYNTH® microwave equipment, which resulted in it only being able to perform part of the planned experimental conditions However, a CEM Mars Xpress Microwave oven became available which was used to complete this step (Figure 3.18a).

The equipment has a maximum power output of 1.2kW. It operates with a power supply voltage of at 240V and has a microwave output frequency of 2444 MHz. The differences between this microwave equipment and the STARTSYNTH® are listed as follows:

(a) the vessels were sealed with a safe membrane as well as new caps (Figure 3.18b, c) which were tightened with a screw driver (Figure 3.18d) reducing the likelihood of volatile losses;

(b) the maximum power used in this case was 1200W, determined after a pilot run with the digestate and the realization that a lower power was not efficient to reach the desired temperatures;

(c) because of the microwave functionality the application of irradiation on digestate had to be done in two distinct phases:

1) the first one corresponded to half of the final power applied (600W) and approximately half of the final temperature;

2) the second phase corresponded to the desired final operational conditions where the equipment reached the final temperature, within a period of time that corresponded to 1.4 °C/min (Figure 3.19). Nevertheless, the total exposure time for microwave irradiation considered here, included phase1.

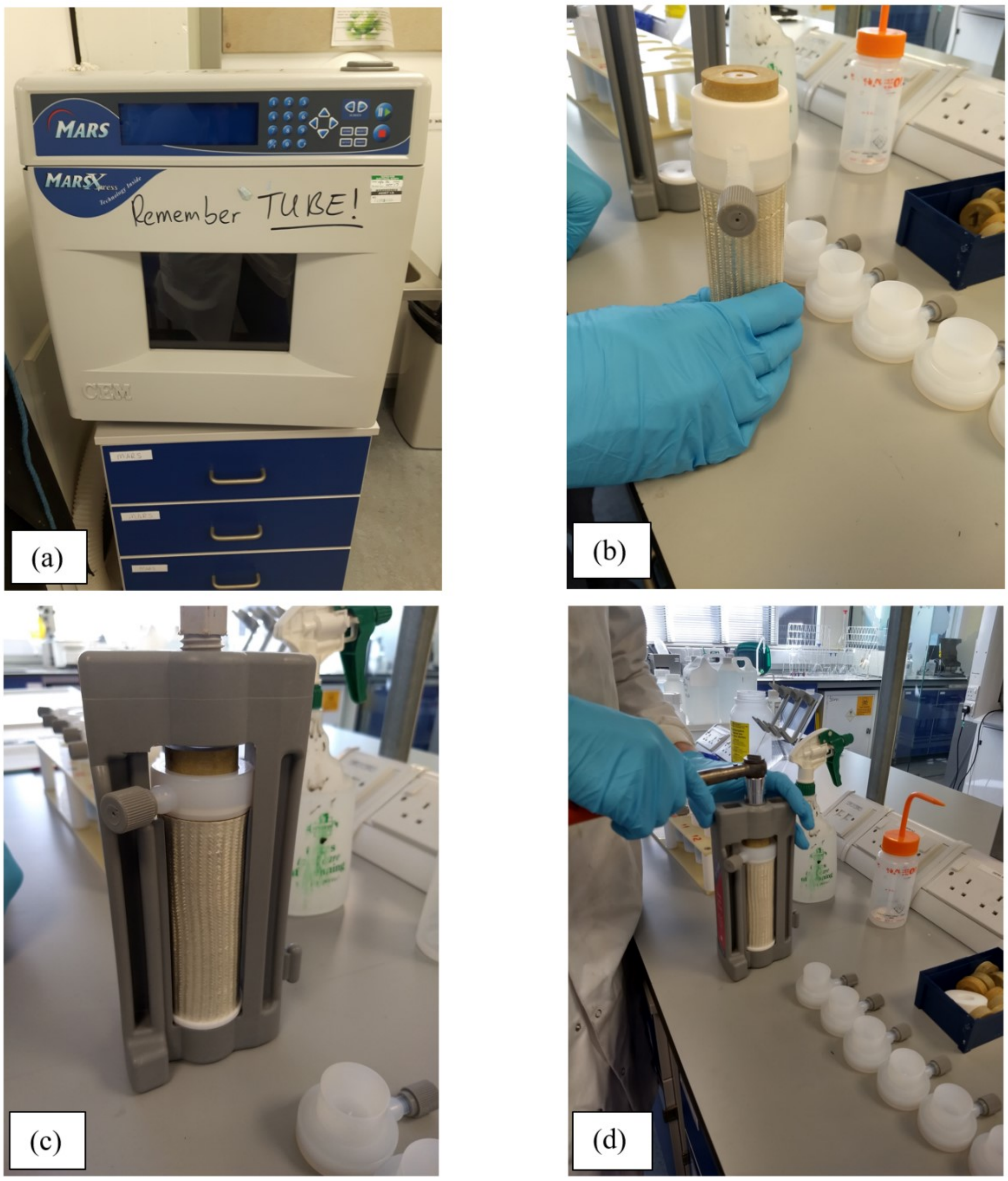
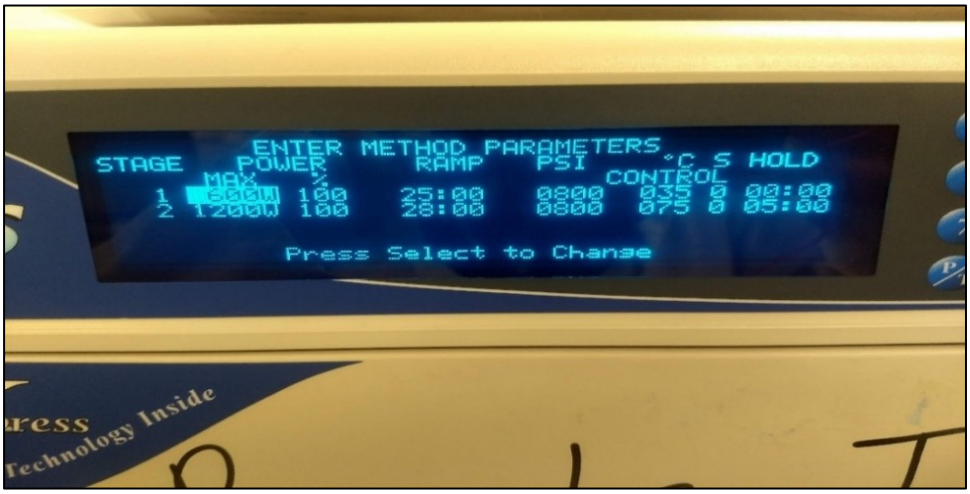


Figure 3.18 (a) Microwave Mars equipment; (b) vessel with safe membrane (c) vessels and caps; (d) tighten mechanisms for vessels.

Despite the differences in configurations and operational methods of the two items of microwave equipment used in the experiment, it is believed that the BMP tests and characteristics of the treated digestate were not significantly affected, hence allowing comparisons between the methane yields and characterization samples.

10 ml digestate were poured into each vessel which was then sealed. The sealed vessels were placed on a carousel inside the microwave oven which rotated the samples so the irradiation and heat were evenly distributed (Figure 3.20a). The temperatures inside the vessels were estimated by a single temperature probe place inside one of the vessels (Figure 3.20b). After each run the samples were cooled to room temperature by an internal fan for an approximately one hour (default time of the equipment).



```
         ENTER METHOD PARAMETERS
STAGE   POWER      RAMP    PSI    °C S HOLD
       MAX  %                 CONTROL
   1  600W 100     25:00   0800   035 0 00:00
   2 1200W 100     28:00   0800   075 0 05:00

         Press Select to Change
```

Figure 3.19 Operational conditions of the MARS Xpress and an example of the microwave irradiation programme used to pre-treat digestate.

The parameters and methods were analysed as described in sections (3.2 -3.4). Subsequently, all the samples were labelled and stored at 4°C until required for characterization and use in the BMP tests.

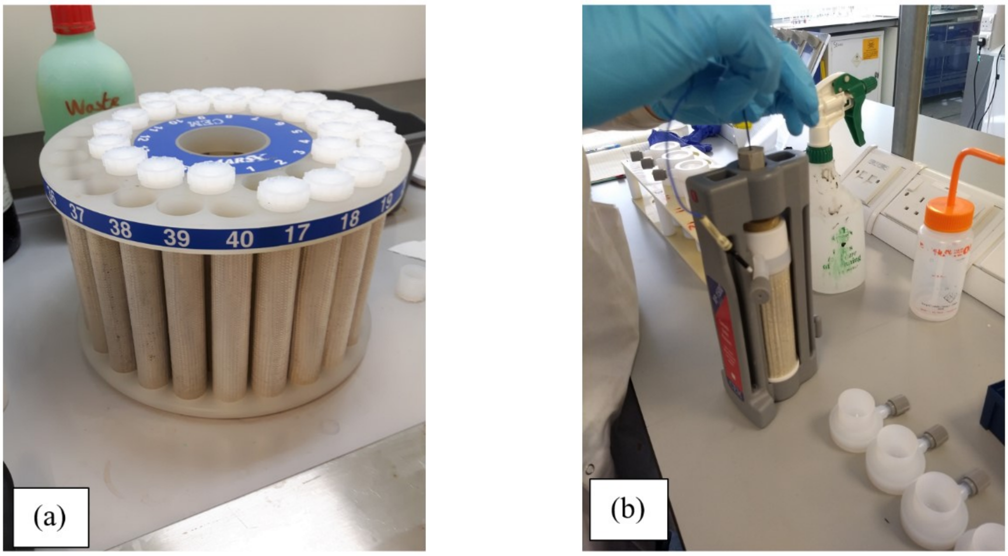


Figure 3.20 (a) Microwave carousel, and (b) temperature probe placement inside microwave vessel.

3.7.2 Experimental factorial design for BMP of MW digestate

In order to determine the best operational condition (combination of final temperature and heating rate) for methane production from MW digestate a multilevel factorial design was used in this experimental phase. Three variables: final temperature, volume of treated digestate and final exposure time to MW irradiation were used, allowing the principal factors affecting process performance (including methane yield) to be assessed. (Table 3.7).

Table 3.7 Factorial design for the microwave thermal treatment of digestate.

Factor 1	Factor 2	Factor 3	Factor 4
Final Temperature (°C)	Heating rate(°C/min)	Concentration (%) of treated digestate	Exposition time to M.W irradiation (min)
75	1.4	25 50 75	53
95			62
115			81
85	7.8		16

3.7.3 BMP tests for the Re-circulation of the Microwaved Digestate

In order to test the impacts of re-circulating the treated digestate on methane production from the whole fraction of raw/untreated FW, similar amount of FW was added to each reactor as in the preceding experiments: 8.0 g (5.0 g V.S) at 1 mm particle size. The inoculum/ seed content of each reactor was also calculated based on a 3:1 ratio, established as the optimum I/S ratio for the MAD of food waste.

Following the method on the BMP tests performed here, the remaining volume of the reactor would be augmented to 500ml with distilled water so that all the reactors have the same working volume (of 400ml after removal of 100ml of sample for analysis). Nevertheless, in this experiment phase, part of the volume of the distilled water was replaced by treated digestate at the final volume proportions of 25, 50 and 75%.

There is a lack of information in the literature on the impact of using digested effluent as a means to reduce fresh/distilled water use in substrate dilution prior to AD (Shariari, 2011), and/or to complete digesters volumes. One of the few relevant studies was published by Sun *et al*. (2010) who evaluated the potential for integrating AD into a water-recycled cassava bioethanol process. The benefits observed from re-circulating an end product in substitution for fresh water has been reported beyond process improvement (i.e. positive impact on yeast growth, enhanced microbial biomass inventory), all attributed to residual enzymes and cofactors that were beneficial to the microbial culture materials), making it a sustainable approach due to its potential to save water and reduce emissions.

3.7.3.1 Monitoring samples of BMP tests with MW digestate

The biomethane production from the re-circulation of digestate into the MAD of FW was assessed as described in section 3.3 and the process performance monitored as described in section 3.3.1.

3.7.3.1.1 Monitoring bacterial concentration via volatile suspended solids during the re-circulation of MW digestate into the MAD of FW

According to Mawioo *et al*. (2017), both effects of MW irradiation, namely thermal and non-thermal action, are involved in the destruction of microorganisms in faecal and sewage sludge. The thermal effect causes rapturing of microbial cells when water is rapidly heated to boiling point (Tang *et al*., 2010; Tyagi and Lo, 2013). The non-thermal effects cause disintegration by breaking hydrogen bonds, which is attributed to the rapidly changing dipole orientation in the polarized side chains of the cell membrane macromolecules (Banik *et al*., 2003; Park *et al*., 2004; Tyagi and Lo, 2013; Serrano *et al*., 2016).

Microwave pre-treatment of the digestate could have an analogous (detrimental) effect on the bacterial population, and for this reason volatile suspended solids (VSS) was used as a monitoring parameter for changes in the bacterial concentration before (control samples), and after microwave pre-treatment, as to evaluate the influence of MW pre-treatment on bacterial density and correlate with process performance. The monitoring of this parameter was also done throughout the digestion process: at day 0, 4, 7, 14, 21 and 28.

3.8 Objective 4: Integrated study of mass and energy balance of the mesophilic anaerobic digestion of pre-treated substrates (food waste and digestate)

Despite the enhancement of AD, the pre-treatment methods available for solid wastes (e.g. food waste, sludge) are not always economically/technically viable, especially when assessing its applicability at an industrial scale (Carballa *et al*., 2011; Ariunbaatar *et al*., 2014). This objective assesses the feasibility of the integrating anaerobic digestion and microwave pre-treatment of both FW and digestate by calculating mass and energy balances based on the experimental results. The energy requirements of the microwave equipment associated with each of the operational conditions (combination of the final temperatures with a ramp rate) used in the pre-treatment phase of both FW and digestate were estimated as described next.

The STARTSYNTH microwaved used for the thermal treatment of FW operated on a discontinuous heating mode, and did not offer a mechanism to record the power fluctuations throughout each run, or a temperature probe to follow the internal sample temperature. In this case, only the maximum power obtained for each operation condition was registered and used in the energy balance, and the final temperature assumed was the one initially chosen for each set of experiments. Equally, the MARS equipment used here to treat the digestate, worked on continuous mode and therefore, the maximum power reached was the one chosen at the programming step (1200W). In any of the cases, the impedance was not design to match the load, and for this reason, some bounds are put on the energy balance of the lab experiment as follows:

a. Upper bound on energy balance–considering the minimum specific energy (energy per gram of substrate) that could have been supplied to the substrate (E_{in}) is given by the product of the substrate specific heat (s) and the temperature through which it is raised (θ), i.e.:

$$\mathrm{E_{in}} = s \, . \, \theta$$

s for the digestate is assumed be that of water (i.e. s = 4.2 kJ/kg/° C). This is a conservative estimate of the E_{in} required because water will have a higher value of s than the non-water component of the substrate. However, the high specific heat of water and the amount of water present in the substrate mean that water is likely to dominate the overall specific heat of the mixture. In this sense E_{in} represents a realistic, estimate of the minimum E_{in} that must be supplied.

A calculation of the energy balance using this figure will thus produce a value close to upper bound on the energy balance.

b. Lower bound –considering the maximum E_{in} that could have been supplied by each microwave oven. For the STARTSYNTH equipment is the maximum power recorded multiplied by the time power was applied divided by the concentration of substrate in each experimental unit (gVS L^{-1}). The maximum E_{in} that could have been supplied by the MARS equipment is the rated power of the oven (chosen at the programming step as 1200W) multiplied by the time this power was applied divided by the concentration of substrate in each experimental unit (gVS L^{-1}). These both represent over estimates of $E_{in.}$ In the case of the first oven it assumes the maximum power observed during the test was delivered to the substrate continuously throughout the test and in the case of the second oven it assumes the maximum possible power capable of being produced by magnetron was delivered the substrate continuously throughout the test. (The degree of over-estimation is greater for the second oven.) It is in this sense that the use of E_{in} so calculated represents a lower bound on the energy balance.

Because microwave of digestate has not yet been tested, there is no reference of its specific heat on the literature. For this reason, its value was assumed to be the same as water, as the moisture content of this material is approximately 95%. In the case of FW, the specific heat has already been shown, and according to Daniel (1998), is equivalent to 1.92KJkg°C (dry FW-solids). Therefore, the final *s* of FW was determined according to both solids (32%) and moisture content (68%) fraction.

The Net Energy (E_{net}) was calculated based on the energy input requirement (E_{in}), evaluated against the volume of biomethane (mlCH$_4$/gVS added) produced (E_{out}) (Figure 3.21).

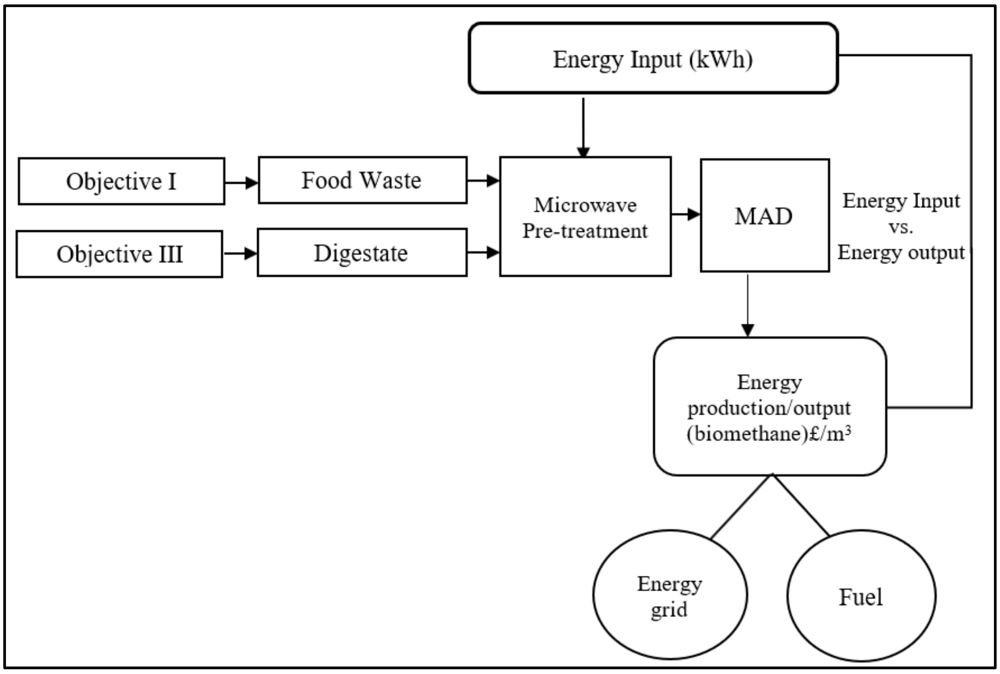


Figure 3.21 Schematic representation of Objective 4.

CHAPTER 4

4. CHARACTERIZATION OF FOOD WASTE AND ITS POTENTIAL USE AS A FEEDSTOCK FOR METHANE (CH_4) PRODUCTION VIA MESOPHILIC ANAEROBIC DIGESTION

4.1 Introduction

FW has been considered an attractive economical source for energy production, with substrate characteristics largely influencing the AD process performance (Zhang *et al*., 2013; Fisgativa *et al*., 2016).

Nevertheless, food waste's composition at household and food service level (restaurants, canteen, etc.) varies significantly from region to region in the world. In Europe, FW is composed by 40% vegetables and fruit, 33% pasta and bread, 17% of dairy products (including eggs) and 9% of meat and fish residues, while in Asia, in particular in Japan, China and South Korea, FW is composed by a 56% of vegetables and fruits, 34% of rice and noodle, and only a small fraction (around 10%) due to fish, meat residues, and dairy products (Braguglia *et al*., 2018). Therefore, the large variation of FW composition/characteristics across the globe can represent a challenge when optimising the supply chain for biogas to renewable energy production (Fisgativa *et al*., 2016).

Despite the development of different reactor designs, such as two stage or multiple-stage reactors, the AD of organic wastes generally relies on single-stage systems, which account for more than 95% of Europe's full-scale plants. Moreover, mesophilic digestion has been preferred over thermophilic one, due to a small energy requirement, and greater stability (Dong *et al*., 2010; Montecchio *et al*., 2016).

This chapter discusses the feasibility of the FW collected from Leeds University refectory as a substrate for methane production via MAD, as well as the use of Inoculum to Substrate ratios (I/S) as a key operational variable to optimize methane yield from MAD of FW in single stage systems (batch reactors).

4.2 Results and discussion

4.2.1 Sample composition

The sample collected at the Leeds University Refectory was analysed for the organic and inorganic fraction. The sample composition varied significantly during the collection period (Figure 4.1, Figure 4.2), with an average daily organic fraction of 69.3% and an average daily inorganic fraction of 30.7%.

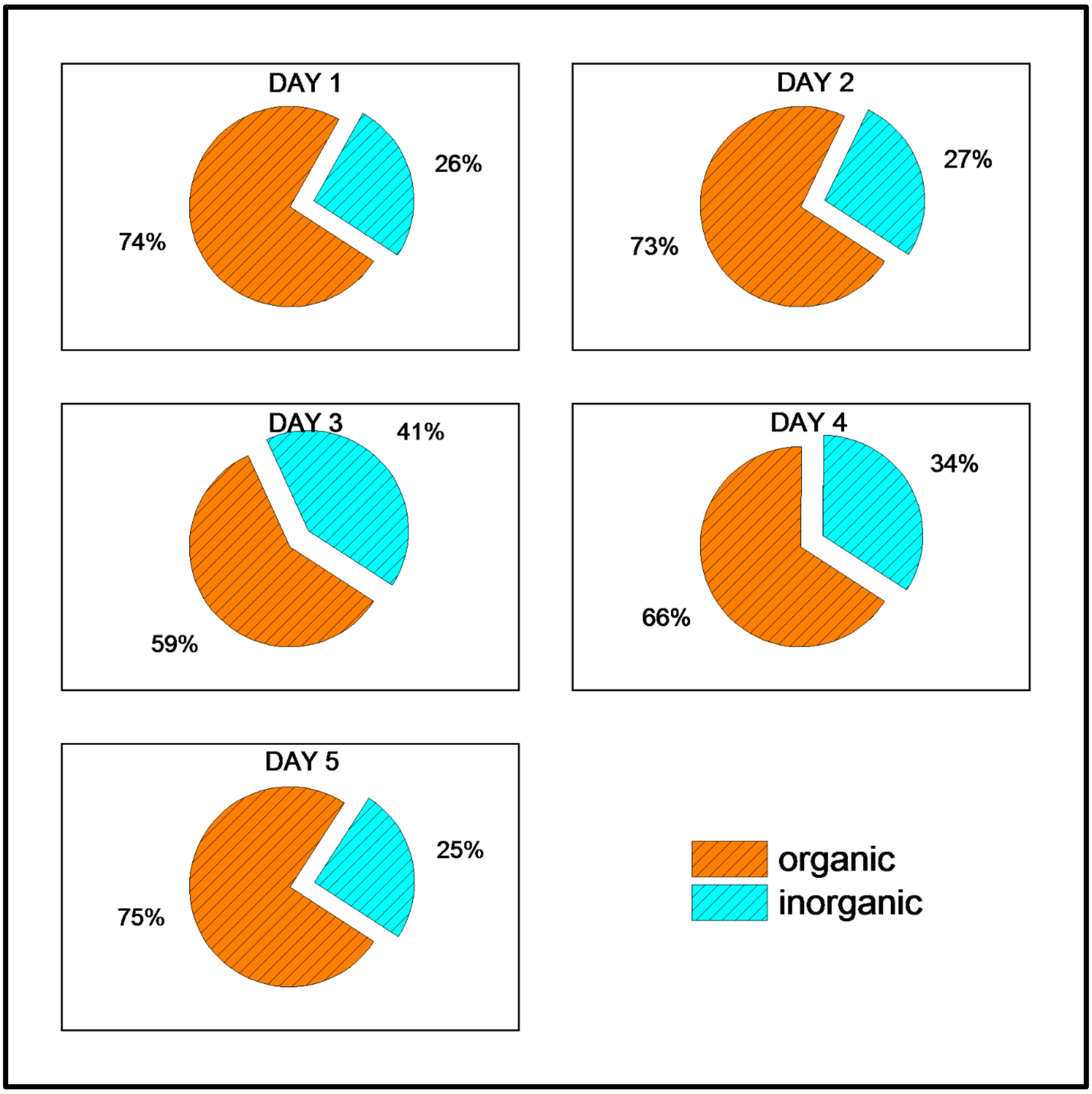
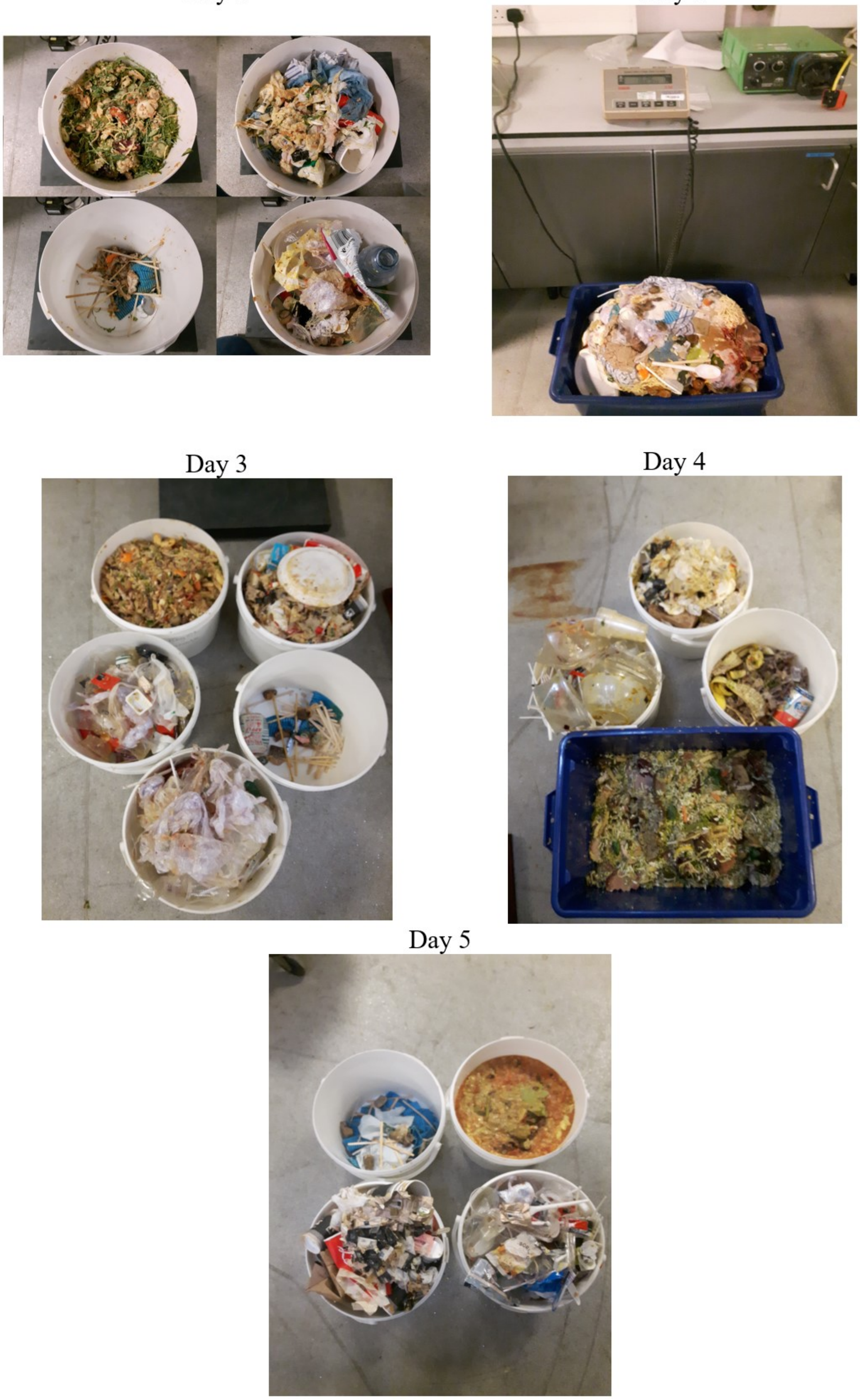


Figure 4.1 Organic and inorganic fraction percentages of sample during the 5 days collection period.

Table 4.1 shows the total weight and composition for each subcategory. A total of 52.95kg of sample was collected, of which 37.40kg corresponded to the organic fraction and 15.55kg to the inorganic fraction (paper, plastic and others).

Table 4.1 Sample composition (organic and inorganic fraction) and weight from Leeds University Refectory.

Fraction of the waste	Organic	Inorganic		
Collection Point	Food Waste Weight (kg)	Paper Weight (kg)	Plastic Weight (kg)	Others Weight (kg)
Day 1	6.15	1.05	0.70	0.40
Day 2	9.30	1.50	0.70	1.20
Day 3	2.05	0.90	0.45	0.10
Day 4	9.65	3.05	1.75	0.25
Day 5	10.25	1.55	0.90	1.05
Total Weight	37.40	8.05	4.50	3.00
Collection Point	Food Waste Composition	Paper Composition	Plastic Composition	Others Composition
Day 1	Tomato, bread, pizza, spaghetti, chickpeas, cucumber, chicken, beef, green peas, mushroom, carrot, fried potatoes, rocket leaves, onions.	Paper towels, napkins, coffee cups, sugar and salt sachets.	Cloth, Ketchup container, wrapping foil, fork, spoons and cups, water bottle, food wrapping boxes.	Chicken bones, metal spoon, wooden stirrer sticks, soft drink can, and chopsticks.
Day 2	Broccoli, carrot, eggs, sausages, bacon, spaghetti, fries, bread, green beans, pizza, chicken and cooked potatoes.	Paper towels, napkins, coffee cups, sugar and salt sachet and merchandising leaflets.	Ketchup container, fork spoons and cups, water bottle, food wrapping boxes.	Soft drink can, Chicken bones and chopsticks.
Day 3	Pizza, minced meat, corn, tomato, spaghetti, fried potato, potato peels, mushrooms, red pepper, carrots, rice, okra, green peas and broccoli.	Paper towels, napkins, Coffee cups, sugar and salt sachets.	Ketchup container, wrapping foil, fork spoons, cups, and food wrapping boxes.	Tea bags, metal knife, chopsticks and beverage can.
Day 4	Pizza, carrots, rice, corn, green peas, corncob, fried and boiled eggs, fried and boiled potatoes, bacon, beef, Yorkshire pudding, mushrooms, tomatoes fish and bread.	Paper towels, napkins, Coffee cups, sugar and salt sachets.	Ketchup container, wrapping foil fork spoons, cups, and food wrapping boxes.	Cloth, Tea bags, metal knife, chopsticks, wooden stirrer and beverage can.
Day 5	Mushrooms, baked beans, sausages, butter, peas, tomatoes, onions, bread, cucumber, cooked eggs, banana and banana peels.	Paper towels, napkins, Coffee cups, sugar and salt sachets.	Ketchup container, wrapping foil fork spoons, cups, and food wrapping boxes.	Cloth, Tea bags, metal knife, chopsticks, wooden stirrer.



Day 1

Day 2

Day 3

Day 4

Day 5

Figure 4.2 Organic and inorganic fraction of sample during the 5 days collection period.

The composition of the organic fraction varied along the collection points, and consisted of mixed cooked and uncooked food such as: cooked white rice, fried potato, bread (white buns), as well as fruit and vegetable peals and pieces (banana, tomato, onion, broccoli, and others). The meat content of the waste included poultry, beef and fish (Table 4.1).

Although the weight and composition of the inorganic fraction was recorded, it was later removed, so there was no interference with fluid dynamics, biodegradation as well as methane yield from the anaerobic digestion of this waste (Steffen *et al*., 1998).

4.3 Composite food waste characterization

The physical and chemical characteristics of the organic waste are important for the design and operation of anaerobic digesters since these affect biogas production and process stability (Zhang *et al*., 2007). The collected FW was therefore characterized and the characteristics analysed in terms of suitability for the anaerobic digestion process.

4.3.1 Solids and moisture content of CFW

The average Moisture Content (MC), VS (Volatile Solids), Total Solids (TS) and VS/TS ratio of the collected FW are shown in Table 4.2. The TS (31.89 mg/kg) and VS (29.60mg/kg) content of the present study are consistent with other works reported in the literature. The TS fraction reported elsewhere ranged from 14.3% to 30.9%, indicating that water accounted for 85.7% to 69.1% of the FW. This is similar to the MC of the FW from the Leeds University Refectory (68.11%). A study, conducted by Kwon and Lee (2004) in a Korean University Cafeteria, showed MC (80%) and VS/TS content (94%), similar to the present study (Table 4.2).

Volatile solids can be used as a measure of the organic content in a sample. The organic content has an influence on the final methane production since it acts as a source nourishment for the bacteria in the reactor (Zhang *et al*., 2014). Tsunatu *et al*. (2014) showed that the percentage of VS content from TS content must be in a range of 80 – 90% to produce the optimum biogas. A large percentage of the TS of the CFW samples was organic (VS/TS 92.91%), hence suggesting the adequacy of the sample to be used as a feedstock for biogas/methane production, without any further adjustments. The VS/TS ratio of the CFW it is also in agreement with previous work reported in the literature (Vavouraki *et al*., 2013, Zhang *et al*., 2013) (Table 4.2).

Table 4.2 Physical and Biochemical Characteristics of food waste samples and comparison with published literature

Parameter/Sample	**Present work**		**References**				
	–	**Average Value (standard deviation)**	**Vavouraki *et al.* (2013)**	**Zhang *et al.* (2007)**	**Zhang *et al.* (2011)**	**Zhang *et al.* (2013)**	**Quiang *et al.* (2013)**
	Number of samples (n)	**CFW**	**Kitchen Waste**	**Food Waste**	**Food Waste**	**Food Waste**	**Food Waste**
Moisture Content %	3	68.11 (0.30)	81.5 (0.66)	-	-	-	-
Total Solids (TS) mg/kg (w.b.)	3	31.89 (3.01)	18.5 (0.71)	30.90 (0.07)	18.1(0.6)	23.1 (0.3)	14.3 (1.75)
Volatile Solids (VS) mg/kg (w.b.)	3	29.60 (4.05)	-	26.35 (0.14)	17.1 (0.6)	21.0 (0.3)	13.1 (1.71)
VS/TS % (d.b.)	3	92.91	94.1 (0.35)	85.30 (0.65)	0.94 (0.01)	90.9 (0.2)	-
C %TS	3	53.06 (0.37)	-	46.78 (1.15)	46.67	56.3 (1.1)	47.4 (0.01)
H %TS	3	7.79 (0.10)	-	-	-	-	6.65 (0.28)
N %TS	3	4.85 (0.07)	-	3.16 (0.22)	3.54	2.3 (0.3)	1.90 (0.09)
O %TS	3	34.18 (0.51)	-	-	-	-	43.7 (0.28)
S %TS	3	0.13 (0.03)	-	-	-	-	0.41 (0.06)
C/N -	3	10.95	-	14.80	13.2	24.5 (1.1)	24.94
Lipid % TS	3	27.62 (1.36)	14.0 (0.51)	-	23.3 (0.45)	-	-
Protein % TS	3	24.31 (1.00)	16.9 (0.69)	-	-	-	-
Carbohydrate % TS	3	42.75 (1.97)	24.0 (1.06)	-	61.9	-	-
Total Chemical Oxygen Demand (TCOD) g/kg	3	327.46 (22.13)	-	-	-	-	-
Soluble Chemical Oxygen Demand (SCOD) g/kg	3	228.30 (21.0)					
Ammonia g/kg	6	13.30 (0.40)					
TKN g/kg	6	16.44 (0.48)					
Total VFAs mg/L	3	785.49 (2.65)	-	-	-	-	-
Phosphorous g/kg	3	0.28 (0.05)					
pH	3	4.20	-	-		-	-

*Data reported as mean values with standard deviation are in brackets, when available.

4.3.2 Elemental composition (C, H, N, S)

The elemental composition of the CFW sample is reported in Table 4.2. There are a number of key factors influencing Anaerobic Digestion. Carbon to Nitrogen ratio (C/N) is an important indicator of potential ammonia toxicity and inhibition. According to Kroeker *et al.* (1979), regarding the AD process, a substance is considered to be inhibitory when it causes an adverse shift in the microbial population or inhibition of bacterial growth. Therefore, inhibition is usually detected when there is a decrease in the steady-rate of methane gas production and accumulation of organic acids.

An optimum C/N is required for bacteria growth and to maintain a stable environment. Generally, a range of 20 - 30 is considered the optimum C/N ratio for AD (Puyuelo *et al.*, 2011), reducing the risk of ammonia inhibition. The CFW elemental analysis showed a similar range of Carbon, Oxygen, Hydrogen, Nitrogen and Sulphur to previous work reported in the literature. However, in this case, with a C/N ratio of 10.95 which is significantly lower than the stabilised threshold (Table 4.2).

Nevertheless, different studies have shown that it is possible to operate anaerobic digesters with lower C/N ratios (15 - 20). This is usually achieved by co-digesting FW with other organic wastes. Kumar *et al.* (2010), for example, co-digested food and green waste showing that organic substrates could be digested at C/N ratio of 19.6. Zhang *et al.* (2013) on the other hand, showed that the optimum C/N ratio when co-digesting FW and cattle manure was 15.8.

It is possible to improve the C/N ratio by adding nitrogen-rich substrates, such as manure, when the nitrogen content is low, or by adding carbon rich substrates, when nitrogen content is high. A similar strategy was used by Musa *et al.* (2014), who tested different C/N ratios when anaerobically digesting FW. The authors originally had a C/N of 17, from a mix of rice and noodles, leafy vegetables, raw chicken and beef and cooked fish. In order to increase this value to 26 and 30, vegetables (corn, lettuce, carrot and broccoli) as well as fruit (pineapple, watermelon, oranges and strawberries) were added to the mix in different proportions. A C/N ratio increase was beneficial for the AD process since it aided the chemical oxygen demand (COD) removal rate (75% to 80% when increasing the C/N ratio to 26 and 30 respectively; compared with 69% when using the original C/N ratio). The ultimate benefit was to improve reactor performance by increasing methane production and yield. The average biogas yield obtained was 0.479L/gVS, 0.620L/gVS and 1.002L/gVS for the C/N ratio of 17, 26 and 30, respectively.

Toxicity and inhibition of the digestion process may occur if sulphur concentration is high (≥0.4%), caused by corrosion effects in digesters, biogas pipes and biogas utilisation devices, thus requiring desulphurisation system(s) (Zhang *et al.*, 2014). Nevertheless, in this study the sulphur content of the CFW was low (0.13% TS), and no corrective measures were implemented, therefore, during the AD process.

4.3.3 Total and soluble chemical oxygen demand of CFW

Total Chemical Oxygen Demand (TCOD) is a measure of organic matter in a particular waste (Gerardi, 2003). Although the TCOD value found on processed samples (327.43g/L) was similar to other studies treating FW (Komemoto *et al*., 2009; Musa *et al*., 2014), they all suggest a high demand of oxygen per litre of sample to stabilise organic content.

The soluble chemical oxygen demand (SCOD) on the other hand, is an indicator of the readily soluble fraction of organic matter that can be used by the microbial community as an available source of energy when anaerobically digesting the FW. In this study, the SCOD/TCOD is 69.71%, representing a higher value than the one reported by Saragih *et al*. (2018), with a SCOD/TCOD of FW equivalent to 43.3%. This latter aspect of the CFW, suggests its feasibility as a substrate for AD, at the same time it demonstrates that there is room for hydrolysis improvement of the FW, which can be obtained through several pre-treatment methods.

4.3.4 Organic fraction composition (lipids, carbohydrates and proteins)

In this study, the CFW was mainly composed of carbohydrates, which added to 42.75 % of the TS. These results reflected the FW composition, where this component was present in its various forms, including: fructose present in fruit peals and pieces, cellulose (non-starch polysaccharides) found in the vegetable peals and pieces, as well as the starch, a polysaccharide found in bread, rice and potatoes (Reilly, 2002).

The organic fraction composition is consistent with previous studies on FW regardless of the differences in (i) waste source (geographic location, type of establishment: restaurants, canteens, households, hotels, etc.), (ii) collection methods (seasonality) and, methods used to characterize the samples, where carbohydrate was the most abundant organic fraction compound of this type of waste (Table 4.2).

Lipids made up 27.62% of the TS content. A low lipid concentration could be beneficial when anaerobically digesting the CFW. According to Bong *et al*. (2018), this is because high lipids concentration can result in a process failure caused by the production of long-chain fatty acids (LCFA) and the formation of oil flock. The latter can be absorbed by the microbial cells inhibiting organic matter degradation thereby suppressing further biogas production.

Although broad in terms of variety, the volume of protein in the sample (24.31% of the total solids content) was lower than the rest of the organic fraction components. The protein content of FW and its effects on anaerobic digestion is controversial in the literature. The nitrogen concentration resulting from microbial digestion of proteins can have either a positive or a negative effect on the process. The positive effect can result from the nitrogen acting as a buffer agent due to the release of NH_3 or NH_4^+, which neutralize the metabolic acids (Volatile Fatty Acids) produced by the microorganisms in system. The negative (inhibitory) effect of high N concentration is by supressing methanogenic activity by several mechanisms such as: a change in the intracellular pH, increase of maintenance energy requirement, and inhibition of a specific enzyme reaction

(Whittmann *et al.*, 1995, Banks *et al.*, 2011). Nonetheless, sufficient N content leads to an increase of 20% in biogas production when compared to digesters which suffers from N deficiency (Garcia-Peña *et al.*, 2011).

Regarding the variation of FW source and its implication on the final characteristics of the waste, Fisgativa *et al.* (2016) conducted a study on the coefficient of variance (CV), based on 102 samples collected in different parts of the world and during distinct seasonality (summer, winter, working period, long holidays, etc.). As expected, the characteristic of FW displayed a high CV. In that study it was observed that FW had an average pH of 5.1 (CV 13.9%), VS of 88.2% (CV 9.3%), BMP of 460.0 NL CH_4/kg VS (CV 19%), 36% of carbohydrates (CV 57.2%), 26% of protein (CV 62.2%), 15% of fats (CV 52.0%), 25% of lingo-cellulose.

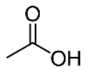
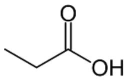
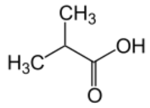
The large variation of FW composition/characteristics can represent a challenge when optimising the supply chain for renewable energy production worldwide. There is a clear incongruity between the variability of feedstock characteristics (with a significant seasonality; and certain FW samples yielding higher biogas production than others) and the constancy of energy demand (such as the fuel required for transportation) which is present all year round (Yue *et al.*, 2014).

4.3.5 pH and volatile fatty acids (VFAs) of CFW

FW is a substrate known for having low pH (acid). The pH (4.20) results found for the Leeds University Refectory waste were consistent with other FW studies, which found a pH range between 4 and 5.2 (Zhang *et al.*, 2007, Elbeshbishy *et al.*, 2012, Browne and Murphy, 2013).

The VFAs content of CFW samples is reported in Table 4.3. Volatile fatty acids (VFAs) which mainly include acetic acid, propanoic acid, butyric acid, and valeric acid, are the main intermediate products during the acidogenesis and acetogenesis phase of organic waste in AD (Pham *et al.*, 2012). It is possible to infer from the results (Table 4.3) that the FW had already started to undergo decomposition (fermentation), reaching the acidogenesis/acetogenesis phase, where intermediate products were formed, this could also justify the low pH range found in the waste.

Table 4.3 Volatile Fatty Acids present in CFW sample.

ACIDOGENESIS/ACETOGENESIS PRODUCTS		
Product	Concentration (mg/L)	Structural Formula
Acetic Acid (mg/L)	757.70	
Propanoic Acid (mg/L)	5.74	
Iso-butyric Acid (mg/L)	22.05	
Total VFAs (mg/L)	**785.49**	———

As previously stated, carbohydrates in their various forms were the main constituent of the food sample studied here. When the polysaccharide (starch, present in the bread and rice-constituents of the sample here studied) are hydrolysed, monosaccharides are released, and their further degradation into the AD process leads to the production of acids such as the acetic acid (Puyuelo *et al*., 2011). Acetic acid was the most abundant intermediate product of the CFW (Table 4.3).

4.4 Biomethane potential tests with CFW

It is well known that substrate particle size (PS) affects significantly the BMP tests. This information is relevant since microorganisms can degrade only the substances present on the organic solid surfaces (Vavlin and Angelidaki, 2005). Smaller particles increase biodegradability by expanding the surface area and consequently, food availability to the microbial community, thus improving methane production (Mshandete *et al*., 2006, Izumi *et al*., 2010).

In this study, preliminary tests on substrate particle size were carried out using FW sample from Leeds University Refectory (results not shown). PS of 1mm resulted in a higher experimental methane yield and biodegradability percentage than other sizes tested (2 mm and 5 mm). 1 mm particle size was therefore chosen as optimum for this study.

Moreover, the bioconversion of organic matter into methane requires sufficient amount of bacteria/ inoculum to be present. Therefore, the Inoculum to Substrate (I/S) ratio has also a significant influence on the performance of BMP tests, and should be previously determined. By applying this step, it is possible to ensure that primary and intermediate products of fermentation can be degraded giving way to bioenergy production at a minimum engineering cost (Hashimoto, 1989; Lu *et al*., 2012).

In order to access the suitability of the CFW sample as a substrate for methane production, a series of Biomethane Potential (BMP) Tests were performed, using 1mm particle size and three different I/S ratios: 3:1, 2:1 and 1:1.

4.4.1 Methane production from CFW

Figure 4.3 shows cumulative methane production over a period of 28 days for three different I/S ratios. The lower I/S ratio (1:1) yielded a higher methane production rate than the higher I/S ratios from day 3 to day 7 with 85.6% of the total methane observed for the duration of the BMP test obtained by day 7 of digestion. After this period, methane production reached a plateau, stabilizing until day 28. On the other hand, the higher I/S ratios methane production increased discretely during the first 5 days of digestion, followed by a significant increase between days 7 and 14 and finally lowering the production until the end of the process.

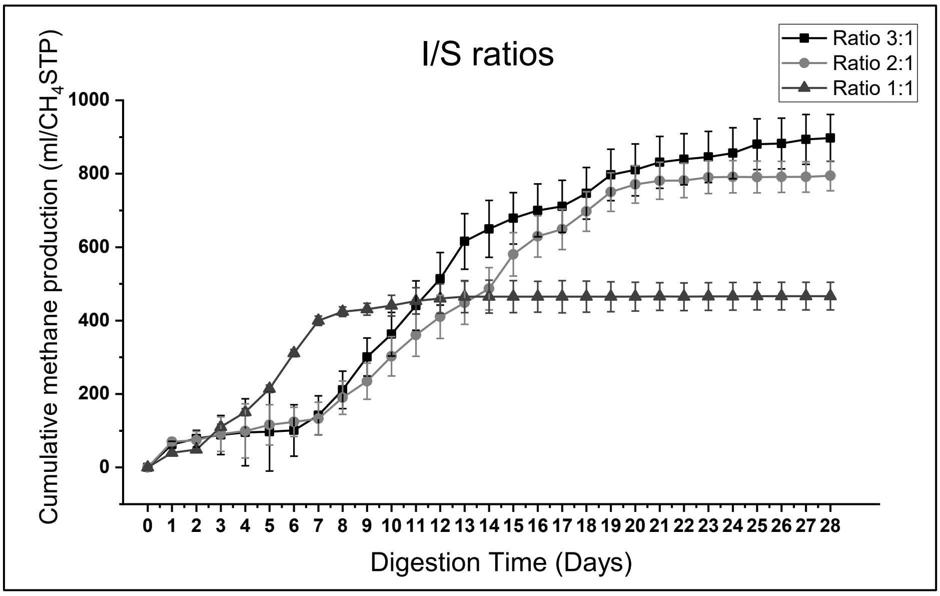


Figure 4.3 Normalized cumulative methane production of food waste samples treated at different I/S ratios. Values are expressed as average values of triplicates.

As shown in chapter 3 Section 3.5, all the reactors contained the same amount of substrate (food waste) varying the inoculum/seed (bacteria) concentration, meaning that latter was the limiting factor on the digestion process. Moreover, the higher I/S ratios, had an elevated concentration of bacteria, which although did not have a positive influence on the methane production rate (especially at the first week of digestion), it definitely help the system to accomplish a higher cumulative methane for the total digestion period (Figure 4.3).

Due to its high biodegradable profile, FW anaerobically digested as a sole substrate in batch systems heralds a high concentration of intermediate products- volatile fatty acids in the start –up phase (first week of digestion) (Angelidaki *et al.,* 2009). The start-up phase is generally considered the most critical step in the operation of anaerobic digesters. Once an anaerobic digester has been started up successfully, it is expected to run without much attention as long as operating conditions are not significantly altered (Hobson and Wheatley, 1993).

According to Kalyuzhnyi *et al.* (2000) when dealing with highly biodegradable substrate as the one used here, higher bacteria amounts is preferred. The reason being that the assimilative methanogenic capacity of the seed reduces as the organic matter or by-products of the organic waste degradation (e.g. VFAs) increases in the system. Therefore, by utilizing higher inoculum concentration, a better process performance can be obtained, since the system can overtake or at least reduce the impacts of the acidification/inhibition phenomena, due to sufficient bacteria concentration, that are capable of metabolizing the excess organic matter and intermediates products into methane, with no system failure (cessing methane production) or significantly lowering its production through the process. (Boulanger *et al*., 2012).

The cumulative methane production during the anaerobic digestion of FW of lower I/S ratio (1:1) confirms this theory. In this case, there was a 52% reduction on the cumulative methane production in relation to the 3:1 and 2:1 ratio. The reduction on the assimilative capacity of the seed on the 1:1 ratio, could have been the result of low bacteria concentration, which were not able to adapt to the typical difficult start-up phase (pH drop, and acidification) of FW. In addition to the reduction on the assimilative capacity of the seed, the slow growth rate of the methanogenic population, could explain the methane plateau observed for the 1:1 ratio after the first week of digestion. The capacity to continue to produce methane in a progressively way after the first week of digestion from the amount of FW added is clearly expressed in the 2:1 and 3:1 ratio performance. Therefore, confirming that the poor process performance (in terms of bioenergy generation) comes from low bacteria activity and number.

Similar to the finding here reported, Boulanger *et al*. (2012) observed a higher cumulative methane yield at higher I/S ratios (2 and 4) compared to other tested (0, 0.015, 0.03, 0.06, 0.12 and 0.25).

On the other hand, the faster methane production rate observed for the lowest I/S ratio during the first week of digestion could reflect a better hydrolysis rate. Considered as a surface process, hydrolysis requires the contact between bioactive agents (either hydrolytic microorganisms or enzymes) and the waste surface for it to be successful (Song and Clarke, 2009).

The improved hypothesized hydrolysis rate of 1:1 ratio could therefore be the result of one or two more factors such as:) a better access to the substrate particles surface which is bioavailable for microbe degradation, or/and b) as a result of easier to digest compounds (protein, lipids and carbohydrates) being more abundant than lignocellulosic compounds which are slowly digested by the microorganism. However, due to the FW sample homogenization before the BMP tests, the last hypothesis is considered very unlikely. Lastly, a higher concentration of hydrolytic bacteria in the inoculum (resulting from a poor mixing method when assembling the reactors) compared to the other I/S could have favoured the better hydrolysis rate.

Boulanger *et al*. (2012) observed a saturation phenomenon regarding the hydrolysis rate when treating MSW under different I/S ratios. According to them, as the I/S ratio increased, the bioavailable surface of waste decreased, due to becoming totally covered by hydrolytic agents which were more abundant than in the lower ratios. Thus, their conclusion was that the increased bacteria concentration responsible for the waste hydrolysis was not necessarily beneficial and could not improve further hydrolytic capacity of the system.

4.4.2 Technical digestion time

The digestion process performance can be accessed by the technical digestion time (T80), which corresponds to the period (time in days) taken by the digestion process to achieve 80% of the maximum yield (Xie *et al*., 2011). Both 3:1 and 2:1 had similar technical digestion times of 17 days, corresponding to a 142.8% longer time than that for the 1:1 ratio (Figure 4.4). The shorter digestion time observed for the 1:1 ratio could be possibly explained by a better hydrolysis rate, as previously discussed.

Zhang *et al*. (2007) digested FW anaerobically in a batch system, under thermophilic temperatures (50°C), and similar to this study, observed that 80% of the total methane produced was obtained after 10 days of digestion.

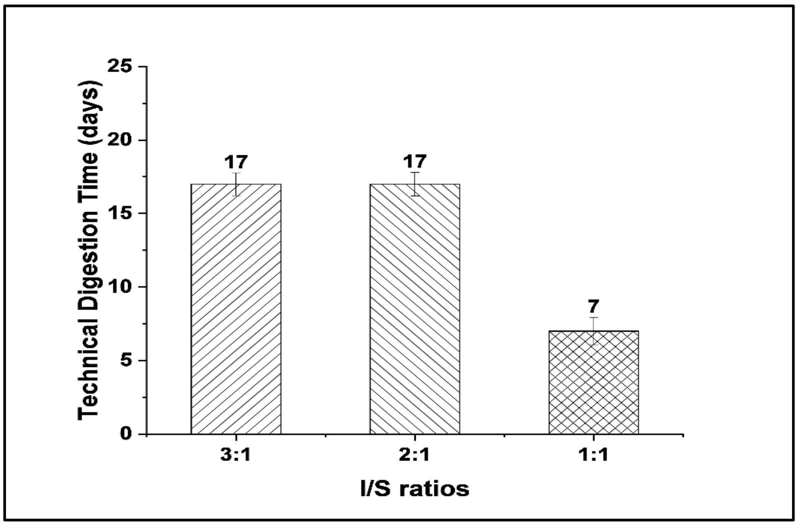


Figure 4.4 Technical digestion time (T80) for the different S/I ratio studied.

4.4.3 Total volatile fatty acids behaviour during the AD of CFW

For all I/S tested, the highest Total Volatile Fatty Acids (TVFAs) production occurred between the second and fourth day of digestion, when it showed an increment of 203.6 % for the reactor 3:1; 225.6% for the reactor 2:1 and, 193% for the reactor 1:1 as compared to day 0, when the experiment was assembled indicating that hydrolytic and fermentative bacteria were active and efficient in degrading the organic waste used (Figure 4.5).

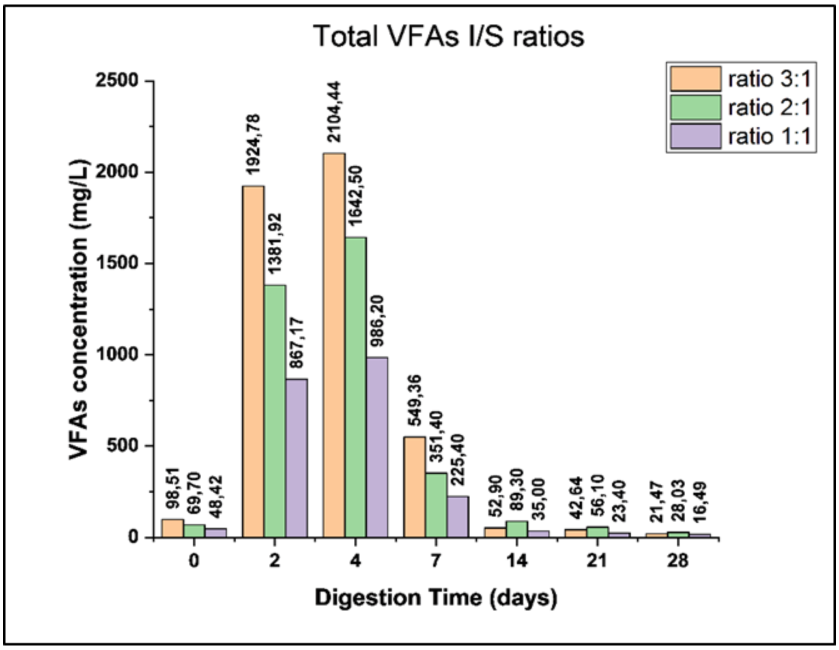


Figure 4.5 Total Volatile Fatty Acid behaviour during the anaerobic digestion of different I/S ratios.

For the higher I/S ratios, the increment of Total VFAs (%) during the Hydrolysis and Acidogenesis phase observed in this study, was similar to the one published by Fernãndez *et al.* (2010), who also treated FW in a mesophilic anaerobic digester using two different TS concentrations: 20 and 30%, obtaining a VFA's increase of 207 and 306%, respectively in relation to when digestion started.

Mineralization and stabilization of solid wastes is one of the desired outcomes of anaerobic digestion process. In order for this to happen, the numerous interactions between the different groups of bacteria (hydrolytic fermentative bacteria, proton reducing acetogenic bacteria, acetoclastic methanogens, and hydrogenotrophic methanogens) present in anaerobic digesters need to be coordinated and fined tuned (Lee *et al*., 2012). Under a stable AD process, these bacteria catalyse organic waste components to volatile fatty acids, hydrogen, and carbon dioxide, which became the main products of fermentation (Chynoweth and Pullammanappallil, 1996). On the other hand, under unstable conditions such as higher substrate loadings; reduced metabolites (e.g., propionic and butyric acids) accumulate (Griffin *et al*., 1998).

In this study TVFAs accumulation occurred for all tested I/S ratios on the 2nd and 4th day of digestion, which is a clear sign of kinetic unbalance, thus suggesting that the metabolic capacity of the methanogens was initially not sufficient to balance the increasing activity of the fermenters on the easily biodegradable FW (Figure 4.5).

Nevertheless, the TVFAs accumulation was greater at higher I/S ratios. This finding is possibly related to the amount of seed in the reactors, since increasing the ratio, increases the number of active bacteria brought into the system, including the ones responsible for VFAs production – acidogenic population. It can be inferred that the higher concentration of bacteria, improved the assimilation of organic matter in the waste, hence implying on a higher concentration of VFAs produced.

On the other hand, the accumulation of metabolites had a detrimental effect on the methane production during this period for 2:1 and 3:1 ratio, which was lower than for the 1:1 ratio. A possible explanation for the lower methane production relates to methanogenic community inhibition, due to a higher pH drop, and system acidification.

The accumulation of intermediate products on the first days of digestion, together with the acidification of the system is a common finding when anaerobically digesting FW as a sole substrate, in a single- stage system, such as batch (Neves *et al*., 2004; Zhang *et al*., 2007; Forster-Carneiro *et al*., 2008; Liu *et al*., 2009). The most common reasons for the acidification of the system are related to the labile organic fraction (LOF) of this type of waste. The LOF is the initial solubilised product obtained as a result of the highly biodegradability rate of F.W under the presence of microorganisms. This fraction is rapidly consumed and transformed into intermediate products (VFAs), which leads to a pH drop and can create an unfavourable environment for methanogenic bacteria, thus affecting the methane production and final methane yield. Nevertheless, the system acidification level is directly affected by the amount of organic matter and LOF of the waste. Thus, the importance of choosing a suitable S/I ratio to maintaining an efficient anaerobic digestion process (Kawai *et al*., 2014).

In anaerobic digestion, there are two main acidification types. In the first one, known as irreversible acidification, the anaerobic digestion process suffers an irrevocable failure, due to a pH drop, which consequently inhibits the intermediate products (VFAs) consumption. In the second type; reversible acidification, the system is capable of recovering itself. In this case, despite the high production rate of VFAs in the initial phase of the process, the produced VFAs are further consumed. Therefore, the declining pH recovers during the digestion period (Eskicioglu and Ghorbani, 2011; Kawai *et al*., 2014).

In this study, the different I/S ratios tested all suffered a reversible acidification, with the high TVFAs concentration at the beginning of the digestion process being latter consumed and transformed into methane.

4.4.4 Propanoic to acetic ratio (p/a ratio)

In the present study, acetic and propanoic acids were the intermediate products to build- up on the first days of digestion. For this reason, the propanoic to acetic ratio (p/a ratio), an indication of process stability, was monitored throughout the experimental period. It was observed that for all the I/S ratios here tested the p/a ratio exceed the recommend threshold of 1.4 mg/L (Hill *et al*., 1987).

Values ranged from 2.95 (2:1 ratio) to 5.4 (1:1 ratio), and occurred at different points of the digestion period. For the 3:1 ratio there was a 3.29-fold increase on the recommend p/a threshold at a latter moment of the digestion (14th day) when compared to the other ratios, on which the main peak occurred at the 7th day of digestion (Figure 4.6).

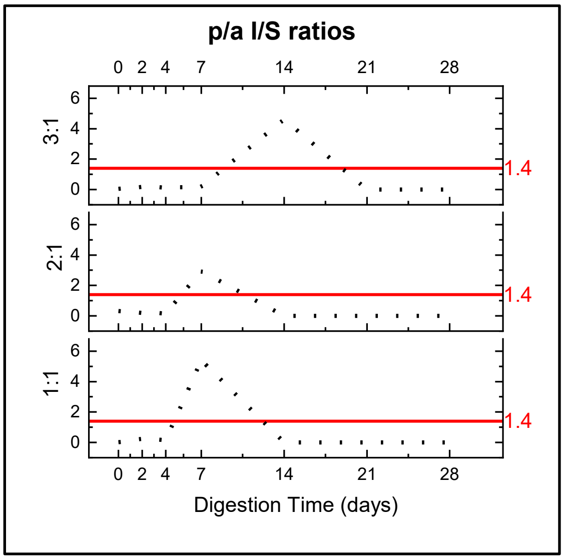


Figure 4.6 Propanoic to Acetic acid ratio concentration during the anaerobic digestion of food waste under different I/S ratios.

The p/a ratio increase can be a result of various factors including: a) a faster degradation of the acetic acid in relation to the propanoic acid by the microorganism causing a higher concentration of the latter in relation to the former, or b) the inhibition of propanoic degrading syntrophs (e.g., *Syntrophobacter wolinii),* leading to an accumulation of propanoic acid (Griffin *et al*., 1998).

According to Pullammanappallil *et al*. (2001), the thermodynamic kinetics of the oxidation of Propanoic acid is exergonic only at low hydrogen pressures. Therefore, under a system perturbation such as: variations in acidogenic and methanogenic biomass concentration and activity (e.g. hydrogenotrophic methanogenesis inhibition, or changes in pH values) there can be accumulation of propanoic acid and, subsequent system failure (Xiao *et al*., 2015).

Xiao *et al*. (2015) demonstrated that a low pH range (4.50 to 6.50) on a two-stage anaerobic digestion had a detrimental effect on this type of acid degradation, once it affects the activity of the main strain of bacteria responsible for its consumption. According to the same authors, it is believed that under single-stage digestion, such as batch, these effects are intensified.

In the present study, all the ratios contained the same amount of substrate (food waste), treated under different concentrations of seed (microorganisms), with the highest ratio containing the highest amounts of microbes. In this context, the propanoic acid degradation on the 3:1 ratio is expected to be faster than in the other tested I/S ratios, and for this reason not prone to accumulation.

However, the 3:1 ratio exhibited a longer period with a lower pH range compared to the other I/S ratios here tested. From the 2nd to the 7th day of the digestion, the pH ranged from 6.50-6.84. The longer exposure to an acidified environment could have negatively affected/inhibited the propanoic acid degrading bacteria activity, which could help explain a posterior peak of the p/a ratio (Figure 4.6).

The 1:1 ratio in turn, exhibited the highest p/a ratio of all tested conditions at day 7 of digestion. However, differently from the 3:1 ratio where the most plausible explanation for the exceeded p/a threshold is an inhibition of the propanoic degrading syntrophs microorganisms, the lower I/S ratio could have benefited from a higher acetoclastic activity (Figure 4.7).

Although the bacterial community structure were not measured in this study, it seems plausible to infer by the VFAs concentration that acetoclastic methanogenic activity would have become the main path for methane formation on the 1:1 ratio, evidenced by the rapid acetate consumption rate (77.14% consumed between day 4 and 7, compared to 61.00% for the 3:1 ratio), thus suggesting either a higher concentration of acetoclastic bacteria within the reactor or a better activity rate of these microorganisms in this reactor.

Figure 4.7 also confirms the hypothesis of inhibition of the propanoic acid degrading bacteria activity postulated for the 3:1 ratio, since the concentration of propanoic acid between 4 and 7 almost doubled (increasing from 282.52 to 407.19mg/L) with an accumulation of this acid within this period. Despite the exceeding p/a ratio threshold for all the I/S ratio, methane production was not significantly affected during the period on which the p/a ratio threshold was exceeded.

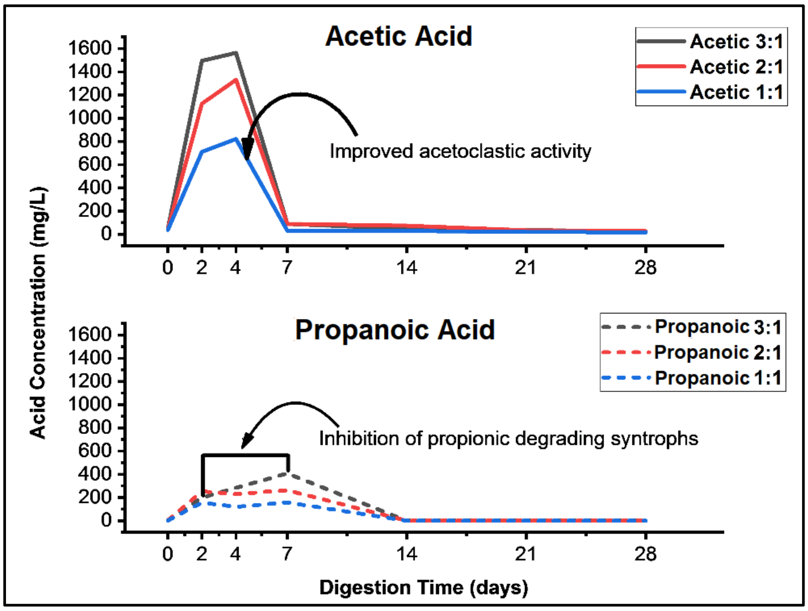


Figure 4.7 Propanoic and acetic acid consumption rate for the different I/S ratios.

These results contrast with the ones found by Boulanger *et al*. (2012), who analysed the influence of different I/S ratios on the degradation Kinetics of MSW. The authors observed that under high I/S ratios (1≤I/S< 4) the cumulated methane production curves exhibit two phases. In the first phase (first days of digestion) hydrogenotrophic metabolism predominated possibly, as a consequence of a high inoculum densities which might have favour spatial proximity between VFA oxidizers and hydrogenotrophic methanogens, thus enhancing syntrophic relationships resulting in earlier methane production by hydrogenotrophic pathway. The second phase in turn (after first week of digestion), corresponded to an increased contribution of the acetoclastic pathway.

The pH drop observed by the aforementioned authors was sharper than then one detected here (Figure 4.8), reaching values of 6. One possible explanation for this difference is the amount of LOF present on the FW used in both studies. As previously mentioned, the LOF is one of the key factors determining TVFAs concentration and, hence the acidification degree of the system. Under their experimental conditions the maximum TVFAs concentration reached values greater than 3g/L for the 0.33 ratio, compared to 2.10g/L for the 3:1 I/S here tested, which in turn suggests a lower LOF of the waste here used.

Nevertheless, previous studies (Moreno-Andrade and Buitrón, 2003; Neves *et al*., 2004; Parawira *et al*., 2004; Kawai *et al*., 2014) have demonstrated that it is possible to rescue a digester from acidification by utilizing a higher concentration of inoculum. In consonant with these previous findings, there was a pH recovery for all the I/S reactors, especially for the 3:1 ratio. In all cases, the pH continued to increase until the end of the digestion period, which coincided with the concomitant, progressive TVFAs removal of the reactors, and further methane production.

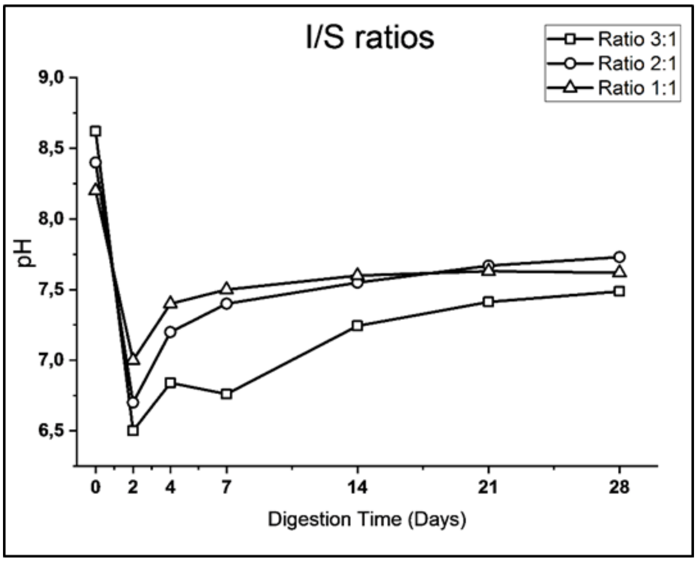


Figure 4.8 pH behaviour during anaerobic digestion of food waste under different ratios.

The recovery of the system after the acidification on the first week of the digestion showed a positive relation with the amount of inoculum in the reactors, with the 3:1 and 2:1 ratio exhibiting a better recovery rate than the 1:1 I/S. This can be evidence by a pH increase from 6.76 to 7.48 observed for the 3:1 ratio, together with a TVFAs removal rate of 58% as opposed to a pH increase from 7.53 to 7.62 followed by an average 56% TVFAs removal rate for the 1: 1 I/S ratio (Figure 4.9).

These results suggest that a combination of higher concentration of bacteria, (with an adequate activity rate), favoured by a balanced anaerobic environment (i.e. with no major inhibitions such as excessively high p/a ratios, or high ammonia concentration-results not shown), leads to a better TVFAs removal rate and further pH increase after the already expected acidification phase, when digesting FW as a sole substrate in a single-stage system (batch).

Similar relation was observed by Kawai *et al*. (2014), who verified that the rates of VFA consumption and pH recovery decreased, as the I/S ratio also decreased, meaning that the recovery rates were negatively affected by the reduced inoculum quantity compared to the amount of the substrate used.

The methane produced after the first week of digestion, under the recovery phase, also showed a straight relation with the different I/S here tested, with the highest methane production observed for the highest I/S ratio. This was already expected, since a TVFAs removal rate was improved under higher inoculum concentrations, and these intermediate products are the precursor for methane formation.

For the 1:1 I/S ratio the methane production occurred mainly in the first week of digestion, therefore in the recovery phase the TVFAs removal was not as efficient as the other I/S ratios. Although the TVFAs removal carried on until the end of the digestion period, the methane production stabilized after 13 days, thus the amount of biomethane produced after this period was negligible and, for this reason not represented in the graph in order to avoid distortions.

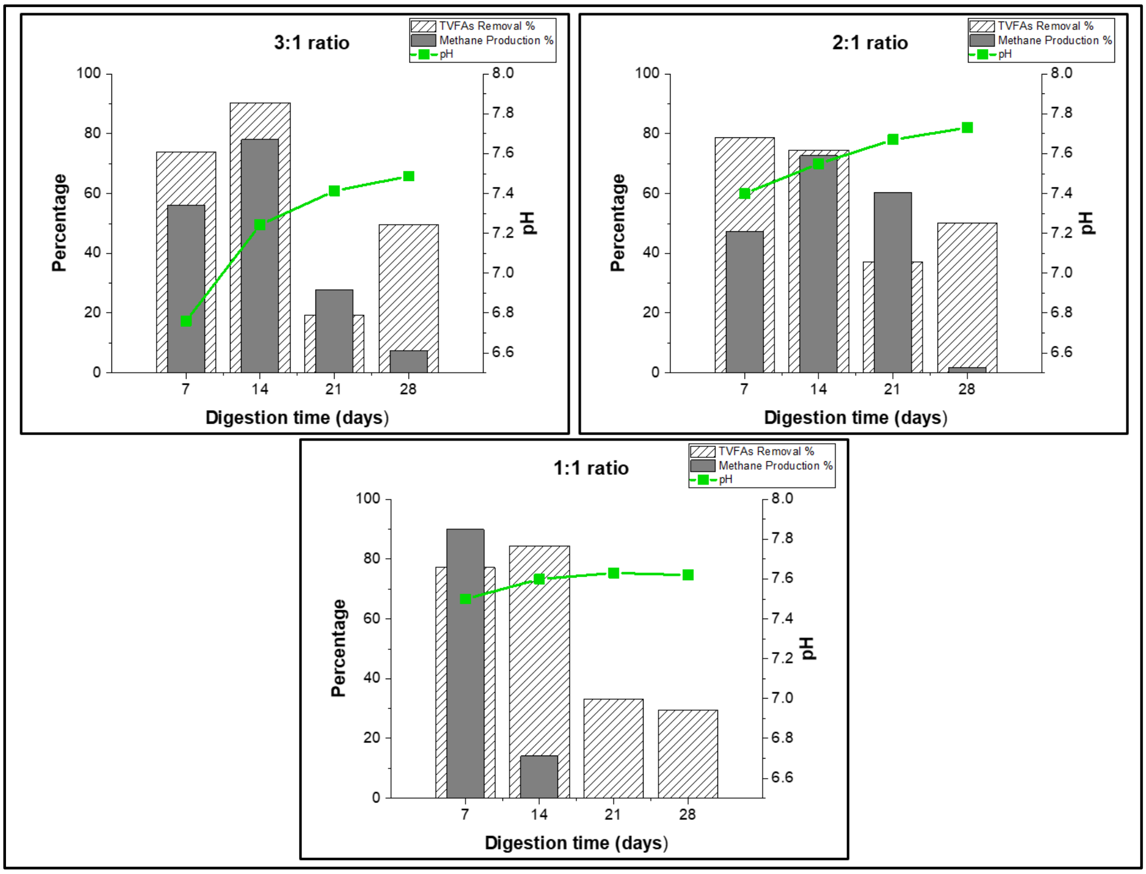


Figure 4.9 Recovery phase profile of different I/S ratios.

4.4.5 Methane yield and kinetic assessment

The methane yields fitted in the MGompertz model obtained for the different I/S ratios are depicted in Figure 4.10. The MGompertz model has been widely adopted for fitting methane yield (Liu and Sung, 2002; Shin *et al*., 2004; Xie *et al*., 2011; Boulanger *et al*., 2012; Pan *et al*., 2013; Wall *et al*., 2013; Mottet *et al*., 2014; Moset *et al*., 2015; Lü *et al*., 2015; Pellera and Gidarakos, 2016; Kong *et al*., 2016). It allows a better understanding of the biomethane production during the experimental work, including the estimation of the duration of the lag phase which is directly associated with hydrolysis rate at the beginning of the anaerobic digestion process.

The maximum predicted methane yield (A), obtained through the Gompertz model for all the I/S ratios here tested, were very similar to the cumulative methane yield obtained experimentally (B) (Table 4.4). For most I/S ratios, B/A was above 90% which, indicates a good fitting; with the greatest fitting observed for the 1:1 ratio (99.38%) and the poorest fitting for 2:1 (90.39%) (Figure 4.10).

The lag phase (ʎ) of the I/S ratios here tested are shown in Table 4.4. The 3:1 and 2:1 ratio exhibited a very similar lag phase 4.47 and 4.48 days respectively, representing twice as much the time needed for this step, as compared to the 1:1 ratio (2.02 days). The lag phase is directly related to the hydrolysis step, and further methane production, and represents the rate on which the organic substrate (bigger molecules such as proteins, carbohydrate and lipids) is solubilised or broken down by the activity of bacterial enzymes into smaller molecules (such as sugars, amino acids and glycerol) (Zhang *et al*., 2011). The smaller molecules then become available for the consumption by the acidogenic bacteria, which will further metabolize them into intermediate products-VFAs, which in the final step will be transformed into biomethane gas.

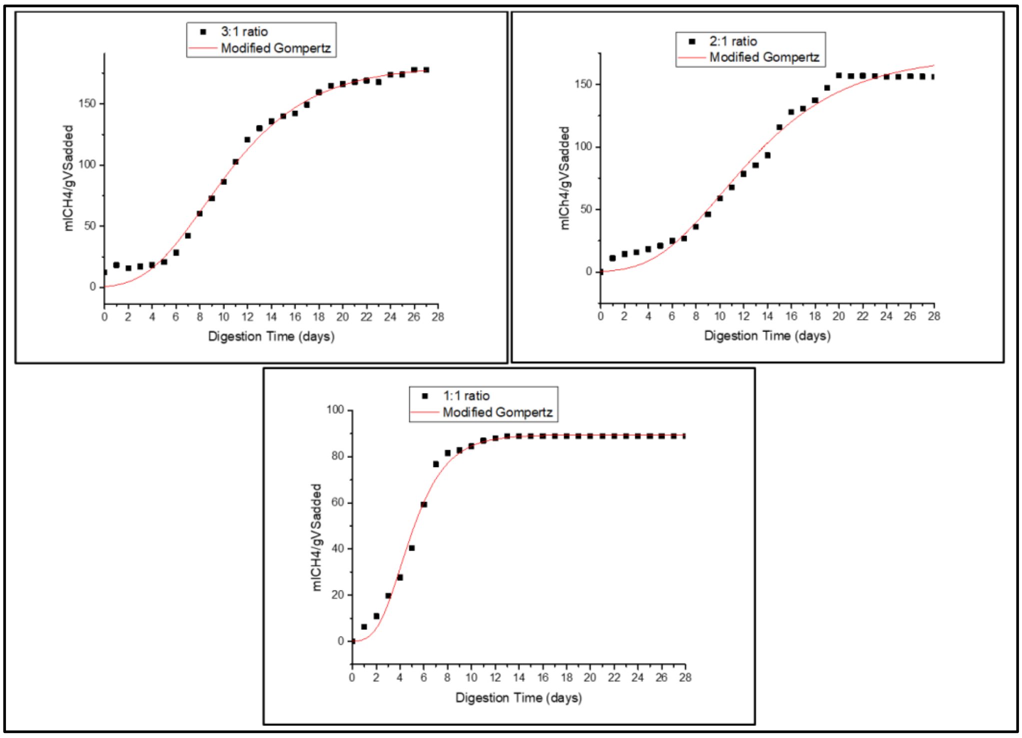


Figure 4.10 Specific methane yield with the MGompertz fitting curves for the different I/S ratios.

In this context, the prolonged lag phase observed for the higher I/S ratios is most likely derived from the greatest TVFAs accumulation (2.10 g 3:1 ratio compared to 0.98g for the 1:1 ratio) during the hydrolysis step. As previously postulated, the higher VFAs concentration for the higher I/S ratios at the beginning of the digestion, could have been the result of a higher bacteria population, which favoured a rapid solubilisation of the FW, followed by an equally fast transformation into VFAs by acidogenic bacteria. On the other hand, the high number of intermediate products caused a greater pH drop, which in turn destabilized the process through the negative effect on the methanogenic population, thus slowing the methane production rate and increasing the lag phase at the first week of digestion, when compared to the 1:1 ratio.

These results strongly suggest that the higher inoculum concentration did not have a positive influence towards reducing the necessary time for the lag phase, but in turn, contribute to a kinetic unbalance between acetogenic and methanogenic activity during the first days of digestion.

Table 4.4 Kinetic assessment of the mesophilic anaerobic digestion of different I/S ratios.

Parameter/ I/S ratios	Predicted methane yield (ml) (A)	Experimental yield (ml) (B)	ʎ (lag phase)
3:1	181.18 (3.29)	177.92 (2.93)	4.77 (0.30)
2:1	172.57 (5.11)	156 (2.51)	4.48 (0.42)
1:1	89.41 (0.67)	88.86 (0.82)	2.02 (0.16)

*Results expressed as means, with standard deviations in brackets.

Despite the improved lag phase, the final methane yield of the 1:1 I/S ratio was 50% lower than the 3:1 ratio, which had the highest methane yield: 177.92mlCH_4/g VS added of all studied ratios.

When analysing different I/S ratios of FW, Kawai *et al.* (2014) also reported the highest methane yield for this ratio, compared to other tested ratios (0.5, 1, 2 and 4). Thus, the present study reinforces that this would be the optimum ratio when treating FW under MAD.

Moreover, the methane yield observed here for the 1:1 ratio was similar to the one reported on the literature when treating FW, however with no irreversible acidification, with typical values bellow 100ml of CH_4/gVSaddded (Neves *et al.*, 2004; Zhou *et al.*, 2011; Lu *et al.*, 2012).

The yields registered for the higher I/S ratios are in agreement with the ones reported elsewhere for the AD of FW under reversible acidification, which ranged from 100mlCH_4/g VS to 300mlCH_4/g VS (Neves *et al.,* 2004; Dearman and Bentham, 2007).

The relatively low methane yields observed in the present study compared to improved values between 400–500ml/ gVS added achieved, for example, by Cho *et al.* (1995); Heo *et al.* (2004); Izumi *et al.*(2010) can be attributed to the acidification process previously mentioned and discussed, that is found in single stage anaerobic digestion. Other authors had previously reported this challenging and disadvantageous aspect of treating FW under anaerobic conditions (Kim *et al.*, 2006; Dearman and Bentham, 2007; Liu *et al.*, 2009), suggesting that this process is better performed when acidogenic and methanogenic populations are spatially separate, in dual stage-systems.

The significantly difference between the final methane yields obtained here and elsewhere can be attributed to various aspects including: a) differences in the chemical composition of FW caused by differences in food cultures and habits, as well as b) methodology used when performing the AD process, such as the operational conditions previously mentioned (batch tests, mesophilic environment, I/S ratio) and amount of volatile solids used.

Methane yield obtained for the different I/S ratio were statistically analysed. The Tukey test showed that there is a significantly difference between the yield obtained for the different ratios (Table 4.5). The Sidak test (post hoc) determines which means differ based on t-statistics, by adjusting the significance level for multiple comparisons. In this case, the Sidak test revealed a greater difference between the 2:1 and 1:1 ratios as well as between the 1:1 and 3:1 ratios, meaning that either 3:1 or 2:1 ratios would be preferred; since the 1:1 ratio had a lower final methane yield (Table 4.6).

However, because 3:1 and 2:1 ratio also exhibited a statistically significant difference between them, and it is already known that 3:1 ratio yielded greater values in terms of methane production and final methane yield, this ratio should be used when optimizing the anaerobic digestion of FW under mesophilic conditions, with particle size 1mm and batch system (Appendix C).

Table 4.5 Tukey test for the different I/S ratios.

I/S	Prob	Alpha	Sig
2:1 vs. 3:1	0,02424	0,05	1
1:1 vs. 3:1	1,15219E-5	0,05	1
1:1 vs. 2:1	5,61449E-5	0,05	1

Table 4.6 Sidak test for the different I/S ratios.

I/S	Prob	Alpha	Sig
2:1 vs. 3:1	0,03089	0,05	1
1:1 vs. 3:1	1,39938E-5	0,05	1
1:1 vs. 2:1	6,82172E-5	0,05	1

4.4.6 Methane yield vs. inoculum/substrate ratio

Several research works have reported the correlation between the methane yield and the amount of substrate versus inoculum used during the anaerobic digestion of FW. In those works, the processes were performed under distinct operational conditions: (batch, single-stages and two-stages), temperatures (mesophilic, thermophilic), as well as particle sizes (≥1mm). The results, including the ones from this work are depicted in Figure 4.11.

In most cases, there was a negative relation between the increase of methane yield and increase of S/I ratios, suggesting that the methane yield from the anaerobic digestion of FW was inversely proportional to the S/I ratio and negatively affected by the decrease in inoculum concentrations (Neves *et al*., 2004; Dearman and Bentham, 2007; Liu *et al*., 2009). Although in this this study I/S ratio was adopted rather than S/I (the values of S/I being equivalent to 0.33 (3:1) 0.5 (2:1) and 1 (1:1)), the general findings regarding the relationship of the final methane yield with the I/S ratios are in consonant with the previous works, where the highest methane yield was obtained when higher inoculum concentration was used or lower S/I was applied (Figure 4.11). Therefore, 0.33 S/I ratio showed improved process performance than ratio 1.

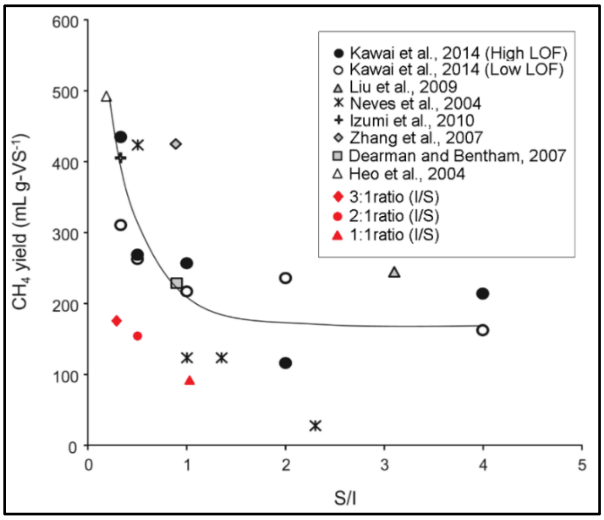


Figure 4.11 Methane yield and S/I ratios relationship from previous and present work. Adapted from Kawai *et al*. (2014).

Nevertheless, similar to the present study, Dearman and Bentham (2007) obtained a low methane yield (lower than 300mL/gVSadded) at a low S/I ratio (less than 1.0) when digesting FW. According to Kawai *et al*. (2014), the low final yield can be an indication of the system not achieving the entire recoverable methane yield, possibly due to intrinsic disturbances affecting the methanogenic population, such as acidification.

Nonetheless, it can be said that FW requires the addition of a large concentration of bacteria/inoculum compared to other substrates, which reinforces the importance of carefully analysing operational conditions which, are not usually taken into consideration, such as particle size and I/S ratios in order to optimize the process.

4.5 Summary

In general, the physicochemical characterization of the composite food waste sample (CFW), collected from the Leeds University Refectory, revealed its suitability as a potential substrate for MAD, with adequate amount of Volatile Solids content, Ammonia, Proteins and Carbohydrates, despite presenting a lower C/N ratio than the recommended threshold.

Biomethane Potential tests (BMP tests) further performed, demonstrated that the CFW is suitable for methane production, although the process stability and efficiency are different under distinct I/S ratios. A lower I/S ratio was responsible for a much faster methane production rate, with 85.6% of the total methane observed for the duration of the BMP test, obtained in the first week of digestion. Therefore, there was a shorter technical digestion time, observed in this case (142% shorter than the higher I/S ratios).

On the other hand, regardless of the I/S ratios, there was a TVFAs build-up on the 2nd and 4th day of digestion, which is a clear sign of kinetic unbalance between the microbial consortia: acetoclastic and methanogenic population, and which affected the process performance, especially at higher I/S ratios. The acidification process, as a result of the TVFAs accumulation was nevertheless reversible, with the system being able to recover itself after the first week of digestion. The recovery capacity of the system decreased as the I/S ratio also decreased, meaning that the amount of inoculum used was a major factor determining the capacity of rescuing the system from a potential failure.

The TVFAs accumulation could have been the main factor responsible for a prolonged lag phase, more than two-folds higher for the 3:1 and 2:1 ratios when compared with the 1:1.

There was a direct relationship between the amount of inoculum used and methane yield, meaning that the higher methane yield from the anaerobic digestion of FW was obtained when higher amount of bacteria was used or lower S/I ratio applied. Therefore, it can be said that FW requires the addition of a large concentration of bacteria/ inoculum compared to other substrates, which reinforces the importance of carefully analysing operational conditions which are not usually taken into consideration, such as particle size and I/S ratios.

Despite the shorter technical digestion time, lower TVFAs accumulation and improved lag phase, the 1:1 ratio exhibited the lowest methane yield, being 50% less than the 3:1 ratio. Statistical analysis revealed a significant difference amongst the yields of the different ratios tested ($p<0.05$), suggesting that the 3:1 ratio should be used when optimizing the anaerobic digestion of FW under mesophilic conditions, with particle size 1mm and batch system.

CHAPTER 5

5. INFLUENCE OF MICROWAVE TEMPERATURE AND POWER ON THE BIOMETHANATION OF FOOD WASTE UNDER MESOPHILIC ANAEROBIC CONDITIONS

5.1 Introduction

FW is an attractive feedstock for AD due to its high biodegradability and moisture content. Due to its complex structure and composition (fat, proteins and in some cases lignocellulosic materials), however, a low methane yield is typically obtained (50 - 60% of the theoretical maximum) (Marin *et al*., 2010). As widely reported in the literature, the poor methane yield can be a result of the limitations in the hydrolysis step when treating complex organic substrates and/or high-suspended solids substrates such as FW (Vavilin *et al*., 2008; Toreci *et al*., 2009; Izumi *et al*., 2010).

The limitation on AD performance caused by the hydrolysis step can be circumvented by adopting feedstock pre-treatments, such as thermal ones. Wang *et al*. (2006), applied thermal treatment to FW, using a hybrid anaerobic solid–liquid system, under the following conditions: 70ºC for 2 hours and at 150ºC for 1 hour. The authors concluded that by pre-treating the waste, the time to produce the same amount of methane as the control (untreated waste) could be shortened by half. Schieder *et al*. (2000) used conventional heating with temperatures between 160 - 200ºC, pressures up to 4MPa and residence time of up to 60 minutes to treat FW from a restaurant. As a result, biogas production rate was improved by 62%, hence shortening the AD duration. Both changes were attributed to faster hydrolysis.

On the other hand, when certain materials are exposed to microwave irradiation, dipole rotation, leads to the breaking of weak hydrogen bonds. This mechanism together with the heating, has the potential to make complex organic molecules unfold and become smaller, thus promoting organic matter solubilisation. The latter increases the AD hydrolysis rate of the organic matter by making it more readily biodegradable for the microorganisms. MW irradiation therefore is a promising pre-treatment for organic solid wastes, including FW (Loupy 2002, Shahriari, 2011).

In this chapter the effects of different microwave operating conditions (namely temperature and power) on FW characteristics and overall MAD process performance (including solubilisation, biodegradability and methane yield) are investigated.

5.2 Results and discussion

The composite FW was microwaved using different operational conditions as described in Section 3.6.1 (Chapter 3) to determine the effects of this thermal pre-treatment on pH, soluble and total chemical oxygen demand (TCOD and SCOD), ammonium, Total Kjeldahl Nitrogen (TKN), TVFAs and solids. The effects of microwave irradiation on composite FW characteristics are shown in Table 5.1, with results expressed as means with the corresponding standard deviation in brackets.

5.2.1 Effects of microwave irradiation on food waste characterization

5.2.1.1 pH

Most of the samples exhibited a drop in pH as a result of microwave pre-treatment. The medium heating rate (3.9°C/min) was the only condition for which all the final temperature samples had a lower pH than the control/untreated sample. The largest pH decrease, and hence the lowest pH value from all tested conditions (final temperatures and ramp rates), occurred for 85°C and 7.8ramp.

A decrease in pH was also observed by Marin *et al*. (2010) when treating the whole fraction of kitchen waste with microwave irradiation. Similar to the present study the authors tested different heating rates (1.9, 3.9 and 7.8°C/min). Their study, however, was limited to a single final temperature of 175°C. The result was a greater decrease in pH for the slow heating rate (1.9 ramp) with respect to the control, suggesting that higher temperatures and slower heating rates (longer exposure to MW irradiation) caused greater acidity. The pH (3.9) for 175°C 3.9°C/min reported by Marin *et al*. (2010) is close to that obtained here.

Shahriari *et al*. (2011) also reported a pH decrease after exposing the organic fraction of municipal solid waste (OFMSW) to microwave irradiation at the following conditions: 20, 40 and 60 min which correspond to fast, medium and slow heating rates, in a similar temperature range to this study (115 - 175°C). Similarly, the highest pH decrease occurred at 175°C for all heating rates with final pH values of: 3.86 (at 20 min) 4.05 (at 40 min) and 3.89 (at 60 min). In contrast to the existing work cited above, the pH decreases in this study had no straight correlation with ramp rate/exposure time to MW irradiation or final temperature.

The decrease in pH values could be a result of the characteristics of the FW sample and different proportions of its components resulting in the formation of distinct soluble compounds such as phenols (which have higher acidities), this especially so at higher temperatures (>145°C).

Marin *et al*. (2010) attributed the observed pH decrease to partial lignin/hemicellulose oxidation and the release of its different components into the liquid fraction. This could explain the pH decrease observed in the current study, since part of composite FW components were vegetables and fruits (tomato, chickpeas, cucumber, green peas, mushroom, carrot, fried and cooked potatoes, potatoes peels, rocket leaves, onions, broccoli, green beans, corn, red pepper and okra). All of these components have lignin as well as hemicellulose as a major constituent of their cell wall.

5.2.1.2 Solubilisation of organic matter

As a result of FW pre-treatment with MW irradiation there was an increase on the Total Chemical Oxygen Demand (TCOD) observed for all the conditions studied. The most significant increase (39.57%) occurred at the higher temperature of 175°C and slower heating rates, suggesting that longer exposures to MW irradiation together with higher temperatures are better for the solubilisation of organic matter (Table 5.1).

The increase in the TCOD (apparent for all test conditions) suggest that the exposure of FW to microwave irradiation causes the solubilisation of recalcitrant compounds in the solution. According to Danso-Boateng *et al*. (2015), thermal treatments are effective in solubilising organic matter from the substrate, and in some cases, recalcitrant compounds.

However, a more plausible explanation for the observed TCOD increase is the change in moisture content of the FW samples after microwave irradiation. Normally, TCOD should not suffer changes during the pre-treatment step, unless there is a loss in the sample´s moisture content (Eskicioglu *et al*., 2007). In this study, most of the parameters were reported in wet basis (w.b%) and supplementary data such as volatile solids, ammonia and TKN demonstrates a general decrease in these values (specially at slow heating rate and higher temperatures) in relation to control ones, hence corroborating this hypothesis (Table 5.1).

The increase of TCOD content after thermal treatment was also reported by Marin *et al*. (2010), for example, reported an increase in TCOD content after treating kitchen waste at 175°C with similar heating rates to this study. A 7.62% increase was reported for the medium heating rate and a 12.42% increase was reported for the slow heating rate.

The soluble chemical oxygen demand (SCOD) also increased with respect to the control for all the conditions studied. Microwave treatment at 85°C, at fast, medium and slow MW intensities, resulted in SCDO increase equivalent to a 3, 16.45 and 18.48%, respectively. These results contrast with the ones presented by Shahriari (2011), which shows no significant increase in SCOD content for 85°C for any of the applied heating rates (20, 40 and 60 min) resulting in a statement that microwave treatment was ineffective in terms of organic matter solubilization for these conditions. The different results between the studies could be explained by the different hold times (5 min, compared to 1 min) which would suggest a dominating influence of this parameter on the solubilisation of organic matter.

For a temperature of 115°C, there was a 11.91, 22.53 and 26.48% increase in SCOD (with respect to the control) for fast, medium and slow heating rates, respectively. Shahriari (2011) in turn, obtained 11.95, 13.04 and 20.65% increases for fast, medium and slow heating rates with 20% moisture content and, 16.90, 22.53 and 28.16% for fast, medium and slow ramps with 30% moisture content.

For FW samples treated at 145°C, there was a 21.27, 53.57 and 59.87% increase in SCOD for fast, medium, and slow heating rates, respectively in relation to the control. Again, these were greater than the ones reported by Shahriari (2011): 14.13, 9.78 and 33.69% for the respective heating rates at 20% moisture content. Nonetheless, by utilizing a higher moisture content of 30%, the author was able to produce better results with 42.25, 54.92 and 54.90%. Lastly, for the 175°C treatment temperature, there was a 37.28% increase for the fast-heating rate, 68.46% for the medium heating rate and a 73.19% at slow heating rate.

Table 5.1 Composite Food Waste characteristics before and after microwave irradiation*

Heating rate (°C/min)	Parameter/ Temperature	Samples (n)	Control (untreated)	85°C	115°C	145°C	175°C
7.8 ramp (Fast)	pH	3	4.20 (0.01)	3.36 (0.01)	4.41 (0.02)	4.75 (0.06)	4.45 (0.09)
	TCOD (g/kg)	3	327.43 (22.13)	402.62 (26.50)	406.16 (22.00)	409.41 (22.14)	413.08 (20.85)
	SCOD (g/kg)	3	228.3 (21.00)	235.20 (13.67)	255.50 (16.40)	276.86 (24.40)	313.43 (29.60)
	Ammonium (g/kg)	6	13.30 (0.40)	13.30 (0.35)	21.52 (0.57)	23.90 (0.67)	24.50 (0.78)
	TKN (g/kg)	3	16.44 (0.48)	16.45 (0.50)	22.57 (0.60)	25.02 (0.87)	25.63 (1.18)
	TVFAs (mg/L)	3	785.49(3.85)	2381.00 (64.48)	2908.63 (67.0)	3018.00 (73.57)	3088.16 (113.31)
	VS (w.b) (g/kg)	3	296.00 (4.05)	239.25 (2.38)	265.12 (2.46)	262.25 (2.95)	249.62 (2.55)
	VS/TS (%)	3	92.91 (0.55)	81.45 (0.35)	91.18 (0.45)	91.50 (0.47)	83.90 (0.38)
3.9 ramp (Medium)	pH	3	4.20 (0.01)	3.86 (0.02)	3.83 (0.04)	3.95 (0.02)	3.98 (0.02)
	TCOD (g/kg)	3	327.43 (22.13)	418.08 (25.50)	427.00 (24.00)	438.00 (29.00)	448.00 (36.25)
	SCOD (g/kg)	3	228.3 (21.00)	265.87 (20.72)	279.75 (20.00)	350. 62 (18.50)	384.60 (25.00)
	Ammonium (g/kg)	6	13.30 (0.94)	0.34 (0.01)	0.47 (0.02)	0.78 (0.01)	1.00 (0.02)
	TKN (g/kg)	3	16.44 (0.48)	12.39 (0.32)	11.65 (0.27)	12.03 (0.30)	11.00 (0.25)
	TVFAs (mg/L)	3	785.49 (3.85)	4887.34 (135.50)	5111.36 (158.10)	3967.50 (114.30)	3987.65 (124.40)
	VS (w.b) (g/kg)	3	296.00 (4.05)	256.25 (2.70)	276.50 (2.92)	285.10 (3.10)	262.50 (2.38)
	VS/TS (%)	3	92.91 (0.55)	91.03 (0.40)	91.94 (0.49)	91.97 (0.51)	91.10 (0.45)
1.9 ramp (Slow)	pH	3	4.20 (0.01)	4. 36 (0.04)	4.01 (0.05)	4.67 (0.05)	4.02 (0.06)
	TCOD (g/kg)	3	327.43 (22.13)	420.42 (16.80)	436.17 (18.92)	445.00 (22.00)	457.00 (25.00)
	SCOD (g/kg)	3	228.3 (21.00)	270.50 (13.40)	288.66 (15.00)	365.00 (16.79)	395.40 (21.00)
	Ammonium (g/kg)	6	13.3 (0.94)	0.15 (0.01)	0.16 (0.01)	0.75 (0.02)	1.69 (0.03)
	TKN (g/kg)	3	16.44 (0.48)	9.14 (0.25)	8.79 (0.17)	9.84 (0.31)	8.71 (0.15)
	TVFAs (mg/L)	3	785.49 (3.85)	2263.25 (64.00)	1265.81 (49.00)	3132.42 (92.00)	3286.49 (95.00)
	VS (w.b) (g/kg)	3	296.0 (4.05)	247.37 (3.79)	238.30 (2.60)	223.40 (2.10)	220.10 (2.05)
	VS/TS (%)	3	92.91 (0.55)	84.50 (0.47)	83.00 (0.42)	82.70 (0.38)	81.00 (0.26)

* Mean values are reported with standard deviation in brackets.

The results from this study are in agreement with other KW studies, which have reported a significant increase in sample solubilization as pre-treatment conditions became more severe (Sawayama *et al.*, 1997; Penaud *et al.*, 1999; Lissens *et al.*, 2004; López Torres and Espinosa Lloréns, 2008). Marin *et al.* (2010) obtained 54.71, 32.70 and 45.91% increases in SCOD content after treating the solid fraction of kitchen waste using a final temperature of 175°C and 7.8, 3.9 and 1.9°C/min heating rates, respectively. Shahriari (2011) also reported 175°C as being the optimum temperature for increasing organic matter content, compared to 115 and 145°C.

Due to an increase in both TCOD and SCOD content for all treated samples, a more accurate degree of organic matter solubilization should be evaluated based on the following: a) SCOD/TCOD content, as well as the (SCOD) produced via microwave thermal treatment expressed as a function of the total chemical oxygen demand (TCOD) - or hydrolysis degree (%) (López Torres and Espinosa Lloréns, 2008). The solubilisation profile of the FW samples treated at different final temperatures and heating rates is shown in Table 5.2**.**

Table 5.2 Solubilisation profile of food waste after microwave pre-treatment under different operational conditions. *

Condition /Parameter	**Microwave Exposure Time** (min)	**Total COD CODt** (g/kg^{-1})	**Soluble COD CODs** (g/kg^{-1})	**SCOD/TCOD**	**Suspended COD (SuspCOD)** (g/kg^{-1})
Control (untreated)	-	327.43 (22.13)	228.30 (21.00)	69.72	99.13
Microwaved FW Whole Fraction					
7.8 ramp (Fast)					
85 °C	16	402.62 (26.50)	235.20 (13.67)	58.00	167.43
115°C	20	406.16 (22.00)	255.50 (16.41)	62.00	150.66
145°C	24	409.41 (22.14)	276.86 (24.45)	67.60	132.55
175°C	27	413.08 (20.85)	313.43 (29.60)	75.80	99.65
3.9 ramp (Medium)					
85 °C	27	418.08 (25.50)	265.87 (20.72)	63.59	152.21
115°C	35	427.00 (24.00)	279.75 (20.00)	65.51	147.25
145°C	43	438.00 (29.00)	350. 62 (18.50)	80.00	87.38
175 °C	50	448.00 (36.25)	384.60 (25.00)	85.80	63.40
1.9 ramp (Slow)					
85 °C	50	420.42 (16.81)	270.50 (13.43)	64.30	149.92
115°C	66	436.17 (18.92)	288.66 (15.00)	66.10	147.51
145°C	82	445.00 (22.00)	365.00 (16.79)	82.00	80.00
175°C	97	457.00 (25.00)	395.40 (21.00)	86.50	61.60

*The results are expressed as means with the standard deviation in brackets.

There were only a few microwave treatment conditions for which an improvement in the amount of soluble organic matter (SCOD/TCOD) in relation to the control was observed. For the fast-heating rate, the only favourable scenario for SCOD/COD improvement was at 175°C, with an 8.26% increase in relation to the control. For the medium heating rate, there was an improvement at both 145 and 175°C, with a 14.74 and 23.06% increase compared to the untreated FW sample. Similarly, for the slow heating rate, 145 and 175°C were the best temperatures to increase SCOD/TCOD with a 17.61 and 24.1% improvement in relation to the control, respectively (Figure 5.1).

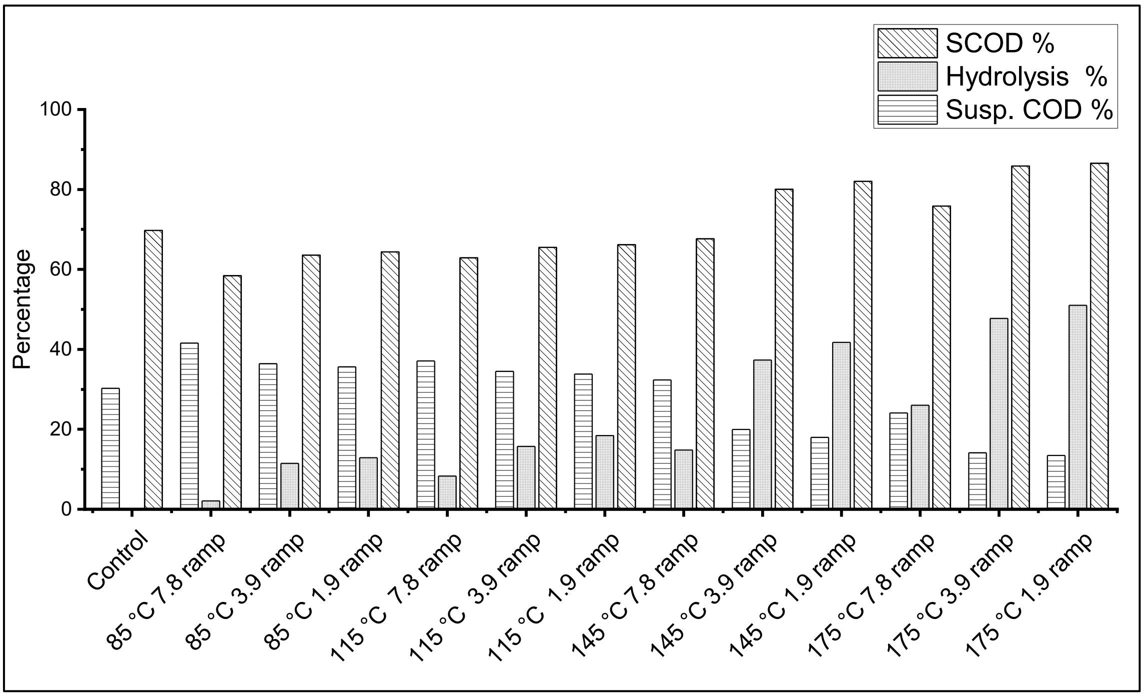


Figure 5.1 SCOD, Suspended COD and hydrolysis percentage as function of final temperature and ramp rate of MW treatment food waste.

The degree of solubilisation or hydrolysis varied significantly for the different temperatures and ramp rates tested here but with a general improvement in relation to the control. Moreover, a direct relationship between the final applied temperature and MW exposure time/ramp rate with the percentage of organic matter solubilization was obtained. For a fast-heating rate and lower temperature (85°C) there was only a slight increase (2%) in the organic matter content of FW. In this case, the suspended organic matter (SuspCOD) was the highest for all treatment conditions, suggesting that in this case, the pre-treatment microwave was not as effective in promoting breakage and solubilization of organic molecules.

Regardless of the ramp applied, 175°C was the optimum final temperature for hydrolysis improvement. At the fast ramp rate (7.8ramp) a 26% increase in the organic content was observed compared to 47.7% and 51.03% for the medium and slow heating rates respectively.

Due to increased hydrolysis for 175°C and all ramp rates, the suspended COD concentration showed a progressive reduction as the exposure time to microwave irradiation increased (3.9 and 1.9 ramps). The greatest suspended COD decrease was for 1.9 ramp, representing a 55% reduction in relation to the control. Additionally, as stated by Eskicioglu *et al.* (2006) and Toreci *et al.* (2010), the degree of pre-treatment can influence the distribution of mass fractions of various sizes for both the soluble and suspended components, ultimately affecting AD performance. As exemplified and validated by both works, an increase in the colloidal component of TWAS in the form of suspended solids after MW pre-treatment, improved the rate of biodegradation, and methanogenesis.

Figure 5.2a summarises the results related to effect of the microwave operating conditions on the SCOD concentrations from FW. Higher temperatures (145 and 175°C) and lower ramp rates of

3.9 and 1.9 (longer exposure to microwave irradiation) increased the amount of soluble organic matter of food waste most.

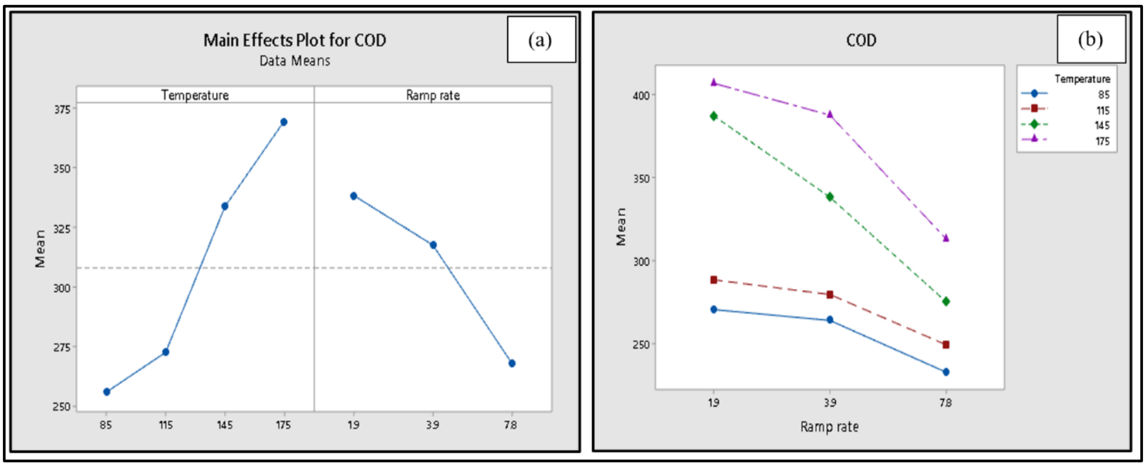


Figure 5.2 Main Effects of MW operational conditions on FW SCOD. (a) main effects (temperature vs. ramp rate) influence on SCOD final concentrations of food waste after MW treatment. (b) Soluble Chemical Oxygen Demand from food waste as an effect of MW operational conditions.

Figure 5.2b shows soluble chemical oxygen demand versus final temperature and heating rate. As the final microwave temperature increased from 85°C to 175°C the SCOD concentration exhibited up to 73.19% increment. Nevertheless, when the heating rate went from fast (7.8°C/min) to slow (1.9°C/min) increasing the time of FW to microwave exposure, the soluble organic matter content increment was up to 26.15%. Since final temperature is deterministically related to the amount of SCOD obtained, it points to this parameter as being the dominant factor influencing SCOD final concentrations.

An analysis of variance (ANOVA) was conducted to evaluate any statistically significant difference in the soluble chemical oxygen demand (SCOD) of the different temperatures and heating rates. When comparing SCOD solubilised between different heating rates and temperatures, most of the differences in results were statistically significant. Only the most relevant combinations, however, will be discussed here.

The Tukey test results suggest that at higher temperature of 175°C and a slow heating rate (1.9ramp) the SCOD obtained is different from when the samples are exposed to the lowest temperature (85°C), regardless of the heating rate. These suggests that in order to obtain greater SCOD content, the higher temperature should be chosen over lower one in combination with a medium heating rate (over a slow one), since there is no statistically significant difference between these two last operational conditions. However, when comparing the economic feasibility of this parameter combination (175°C 1.9 ramp and 85°C 3.9 ramp), there is a 41.69% increase on energy consumption (kW) in relation to the final COD yield (12.70%) suggesting that the extra organic matter solubilized is not compensated by the extra costs (energy requirements) applied to the process (Table 5.3).

When analysing the two extreme conditions, i.e. lower temperature at a slow heating rate and higher temperature at a fast-heating rate (85°C 1.9 °C/min and 175°C 7.8C/min) the difference is statistically significant. In this case, there is an even higher increase in energy consumption, for the SCOD yield, again suggesting the economic unfeasibility of the latter operational condition (Table 5.3).

Table 5.3 Statistical and economic analysis of relevant microwave operating condition combinations regarding SCOD profile.

Ramp Rate vs. Final Temperature	Statistical Value (*p* value)	SCOD increase (%)	Energy consumption increase (%)
85°C 3.9°C/min and 175°C 1.9°C/min	$p = 0.0020$	35.40	119.27%
85°C 1.9°C/min and 175°C 7.8°C/min	$p = 0.0000$	31.41	169.00%
175°C 7.8°C/min and 175°C 1.9°C/min	$p = 0.0024$	20.38	-

There was also a statistically significant difference between the SCOD produced after microwave pre-treatment at 175°C and fast and slow heating rates, with the latter yielding a higher solubilisation. In this case, there was there was also a 27% decrease in energy consumption (due to an intrinsic microwave power fluctuation behaviour), and therefore should be preferred over the former condition. Overall, these results suggest that despite the higher organic matter solubilization of higher temperature, this is not compensated by the extra energy input of the process.

Despite the organic matter increase being used as a metric of treatment efficiency for many authors, Appels, Assche, *et al.* (2011) presented some indications that the increase of SCOD, itself, is not a good indicator of biochemical methane production potential for waste activated sludge. This is because within the solubilized organic matter there is recalcitrant form, that will be inaccessible and resistant for bacteria degradation. However, the solubilisation of organic matter in MW pre-treated FW is evident and constitute a promising process for increasing methane yields when samples are anaerobically digested. The composition of SCOD is therefore, extremely important and should be further investigated.

5.2.1.3 Ammonium and TKN

At a fast-heating rate there was an increase in the ammonium concentration in relation to the untreated samples at most of the final temperatures. At a fast-heating rate (7.8°C/min) and final temperature of 85°C, microwave irradiation was ineffective in terms of protein breakage, with sample concentrations being similar to the control (13.30 g/L and 13.36 g/L respectively).

The exposure of FW to a fast-heating rate (7.8°C/min) and temperatures higher than 85°C caused the ammonium concentration to increase in relation to the control as follows: 61.07, 78.92, and 83.38% for 115°C, 145°C and 175°C respectively. This suggests that for a longer exposure time, microwave irradiation resulted in the breaking of proteins, with further ammonium release to the solution, thus having a hydrolytic effect, showing a straight relation with the final temperature.

Without substrate pre-treatment organic complex material such as carbohydrate, proteins and lipids need to be disintegrated into smaller and soluble compounds for further methane production during anaerobic digestion (Vavilin *et al.*, 2008). The hydrolysis of organic polymers is carried out by several specific hydrolyses (extracellular bacterial enzymes), such as cellulases (acting on carbohydrates), proteinases (acting in proteins) and lipases (acting in lipids). The microorganisms can either release them to the bulk liquid where they will be absorbed by a particle or react with a soluble substrate (Jain *et al.*, 1992), or the microorganisms will produce them when attached to a particle, releasing them in their vicinity, benefitting from the soluble products of the enzymatic reaction (Vavilin *et al.*, 1997). In this sense, microwave irradiation has been shown to accelerate the hydrolysis of nitrogen compounds to ammonium, thus having an analogous effect to the microbial enzymes on AD process.

Higher ammonium concentrations on the system, however, resulting from microwave pre-treatment can have detrimental effects on the final methane yield. Optimum microwave irradiation operating conditions related to nitrogen release by the substrate should, therefore, be determined and evaluated when designing AD processes.

On the other hand, when FW was exposed to a medium heating rate (3.9 ramp), there was a 97.45, 96.45, 94.13, 92.51% reduction on the ammonium concentration compared to the control for 85, 115, 145 and 175°C, respectively. For the slow heating rate (1.9 ramp) at lower temperatures 85 and 115°C the ammonium content decrease was greater, 98.82 and 98.75% respectively. Higher temperatures of 145 and 175°C resulted in decreases of 94.37 and 87.35%, respectively.

These results are in partial agreement with the ones reported by Marin *et al.* (2010). The authors observed ammonium loss when treating kitchen waste with microwave irradiation with a final temperature of 175°C. The most significant reduction occurred for a fast-heating rate (7.8°C/min), with a 28.57% decrease in relation to the untreated samples, followed by medium heating rate (3.9°C/min) with a 14.28% decrease. As opposed to the present study, the authors reported a 21.42% increase in ammonium content for the samples treated at slow heating rate (1.9°C/min).

A possible explanation for the observed ammonium decreases in the work reported here is the volatilization of this compound. Lin *et al.* (2009) reported previous ammonium volatilization under thermal microwave treatment. In their case, however, this was done intentionally and used as a mechanism to remove ammonium from wastewater. The relation between ammonium removal and temperatures was the following: minor ammonium decreases below 80°C, and significant removal when temperatures exceeded this value (80% removal at 100°C after 3 minutes of microwave irradiation and a power of 350 watts), as a result of athermal and thermal effects of the microwave.

The thermal effect is the main mechanism from which ammonium volatilization occurs under MW irradiation. Both H_2O and NH_3 are polar molecules and are connected by intermolecular hydrogen bond: N-H...O and O-H…N, thus having the potential to be polarized in the microwave field. Depending on the power applied, such dipole molecules rotate and line up rapidly (4900 million times/s). The vibration of water and ammonium molecules leads to weakening and eventual rupture of the hydrogen bond resulting in the escape of molecular ammonium from the liquid into the gas phase (Lin *et al*., 2009). It is possible that similar behaviour occurred in this study.

On the other hand, the significant loss of ammonium observed here could have been a result of the poor state of the vessels used during the microwave treatment. The worn screw caps may have prejudiced the vessel's ability to completely contain volatile components. Nevertheless, ammonium volatilization could be beneficial for the anaerobic digestion of FW, especially in cases where the samples are rich in nitrogen compounds. This is because methanogenic activity can be inhibited by high concentrations of ammonium in the system (Zhang *et al*., 2007).

Total Kjeldahl Nitrogen (TKN) measures the sum of NH_3-N and organically bound nitrogen (Abbey *et al*., 2017). A similar effect to that of ammonium concentration was observed after microwave treatment. That is, an increase TKN was observed for a fast-heating rate for all final temperatures except for the 85°C and a sharp decrease for all the remaining microwave conditions tested.

The greatest TKN increase was observed at a fast-heating rate and 175°C. In contrast, the biggest reduction occurred at 175°C a slow heating rate representing a 47.68% in relation to the control.

Marin *et al*. (2010) also reported a decrease in TKN concentration for MW treated FW with respect to the control. The greatest decrease (63.75%) occurred at a slow heating rate (1.9 ramp) compared to the medium and fast heating rate (23.97 and 28.36% decrease respectively) and a final temperature of 175°C. According to the authors, the decrease on TKN concentration after MW treatment was due to solubilisation of organic nitrogen forms from the solid (whole fraction) to the soluble fraction.

5.2.1.4 Total volatile fatty acids

Measurements of TVFAs were based in the type of acids present at the characterization step, and reported as Total VFAs (TVFAs). For the fast-heating rate, the TVFAs concentration increased significantly in relation to the control and the increment was linked to the temperature. The values ranged from 2381.9 to 3088.0mg/L, representing a 203.23 and 260.02% increase compared to the untreated FW for the lowest and highest temperatures, respectively (Table 5.1).

For the medium heating rate, the TVFAs concentration ranged from 3967 to 5111mg/L compared to 785.4mg/L from the untreated samples. However, no trend for the TVFAs concentration versus temperature was observed. The maximum increase in TVFAs occurred at 115°C, where there was a 550.67% increase relative to the raw FW. The lowest TVFAs increase (405.90%) was for 145°C. The greatest TVFAs values caused a lower pH range (3.83 - 3.98) due to the increase in the media acidy.

Contrary to what was expected, the TVFAs concentrations for the slow heating rate were not greater than those for the medium or fast heating rates. In the slow heating rate case, the values ranged from 1266.00 to 3287.00 mg/L and showed no relationship to the final temperature applied. Samples treated at 175°C showed the highest increase in TVFAs with respect to the controls with a318.46% increase. FW treated at 115ºC exhibited the lowest (61.17%) TVFAs improvement. The latter microwave operational condition resulted in the lowest VFAs increase amongst all those tested here.

These results contrast with previous findings by Marin *et al*. (2010), who reported that after microwaving the whole fraction of kitchen waste, at 175°C, there was a decrease in the TVFAs concentrations in relation to the untreated sample for both fast (7.8°C/min) and medium (3.9°C/min) heating rates. The reductions were 47.87% and 61.18% respectively. The exception occurred when the waste was heated at a slow heating rate (1.9°C/min), which resulted in a 19.56% increase in this parameter.

Shahriari (2011), observed a different TVFAs production pattern for FW with 20% and 30% moisture content. In both cases the increase in TVFAs concentration varied according to the final temperature applied. All the samples treated at a final temperature of 115°C, regardless of the heating rate (fast, medium and slow), exhibited a negligible TVFAs increase. When the samples were exposed to the same heating rates and a final temperature of 145°C, however, a significant increase was observed for both 20 and 30% moisture content samples. The reported TVFAs concentrations ranged from 647 - 2028 mg/L compared to 5.00 and 4.00 mg/L for the controls at 20 and 30% moisture content, respectively.

The differences regarding the production of TVFAs found here and elsewhere cannot be easily explained since the mechanisms by which thermal transformation of the organic matter into volatile fatty acids during microwave treatment are still poorly understood. Nonetheless, it can be speculated that certain microwave operational conditions such as high temperature (175°C) and slow heating rate (with longer exposition to MW radiation) favours the production of volatile fatty acids.

The thermal transformation of organic matter into volatile fatty acids by this type of pre-treatment is promising in the context of enhancing the anaerobic digestion process. The reasoning behind this statement is that by producing the “right” type of VFA such as acetic acid, the main biomethane precursor (Feng *et al*., 2013), the AD process might be improved by augmenting the volume of biomethane obtained. No previous study has related the VFA types obtained with the applied microwave operating conditions. For this reason, a more comprehensive relationship between the VFA types and each combination of ramp time and temperature is now discussed.

Figure 5.3 depicts the different VFAs produced after the FW sample was exposed to a fast-heating rate and a range of final temperatures. Regardless of the final temperature, acetic acid was the most abundant intermediate product after microwave irradiation. Its increase with respect to the control was 108.7% at 85°C and 242.5% at 175°C.

Similar to the fast-heating rate, acetic acid was the main acid produced after the microwave irradiation of FW at a medium heating rate. However, this time its final concentration was not temperature related. Lower temperatures exhibited a greater increase with respect to the control than the higher ones. There were increases of 521.30% for 85°C, as opposed to 374.81% for 175°C (Figure 5.3).

For the slow heating rate, the acetic acid once again was dominant amongst the TVFAs, for all the tested temperatures, and also exhibited an increase in relation to the control. The highest temperatures (145 and 175°C) showed the greatest increase of acetic acid with 273.5% and 265.41%. 115°C resulted in the lowest increase in acetic acid (37.02%) and also the lowest increase in TVFAs of all tested conditions. No clear explanation can currently be offered of why this occurred.

The main factors influencing acetic acid concentration were predicted and plotted using Minitab 2018® (Figure 5.4). Ramp rate had a significant influence on the final acetic acid concentrations after FW was exposed to microwave irradiation. Extreme heating rates (7.8 and 1.9°C/min) yielded lower acetic acid concentrations compared the medium heating rate. The difference in this acid concentration was equivalent to 114% comparing medium and fast heating rate and 304% between the medium and slow heating rate.

It can be postulated that acetic acid concentrations would reach a maximum concentration of 4500mg/L at a ramp rate of approximately 5 units. Minitab prediction of acetic acid under heating rates varying from 2 to 8 formed a perfect curve. This is because, the medium heating rate exhibited maximum TVFAs values, significantly decreasing under extreme heating rates (under 2 and over 7.8).

Compared to the ramp rate, final temperatures had a lower influence on the acetic acid final concentrations. The difference between the highest acetic acid concentration obtained at the lowest temperature (85°C) and the highest one (175°) is 23.6%. Ramp rate was therefore the dominant factor influencing acetic acid production.

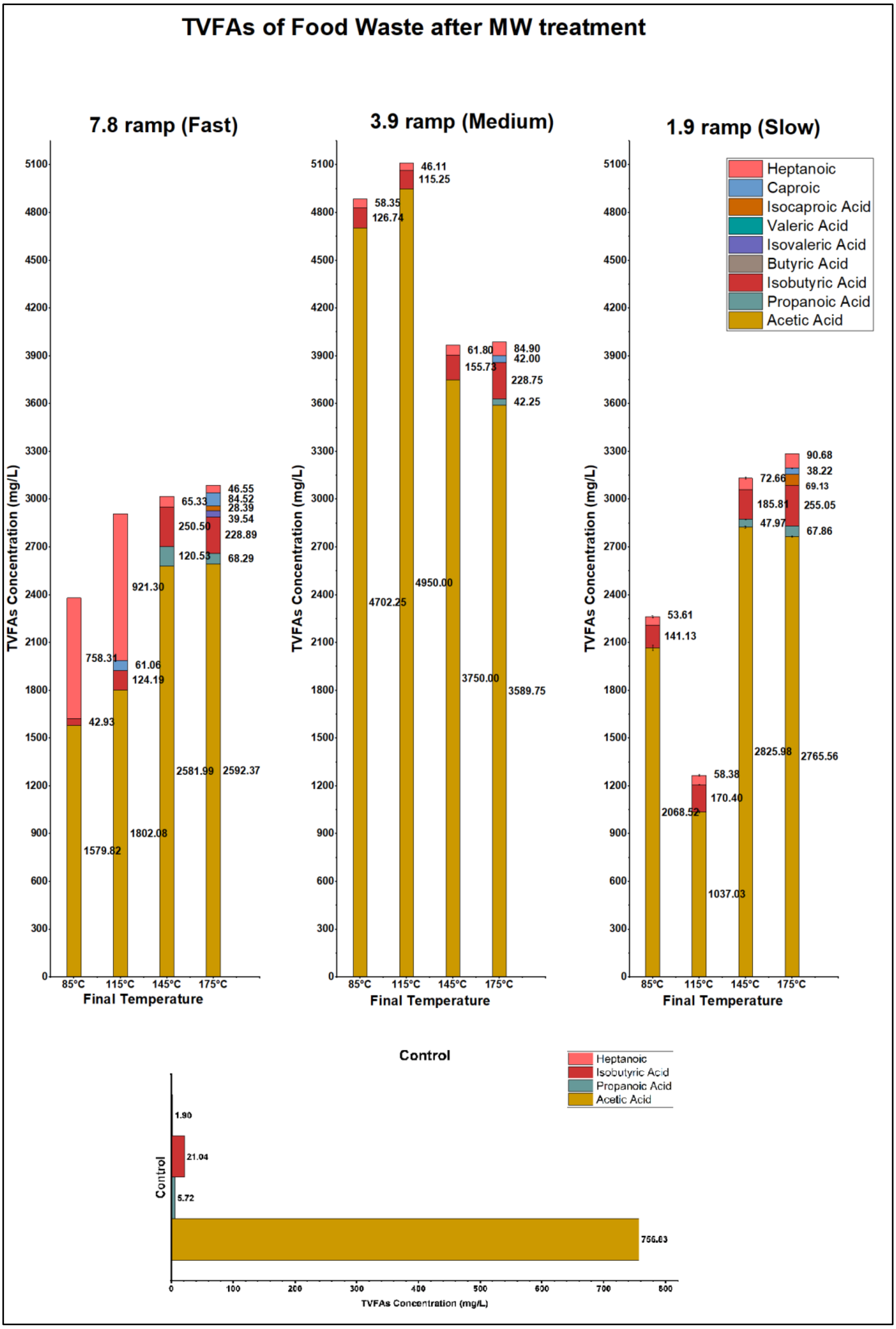


Figure 5.3 Individual VFAs concentrations after microwave pre-treatment of food waste under different operational conditions.

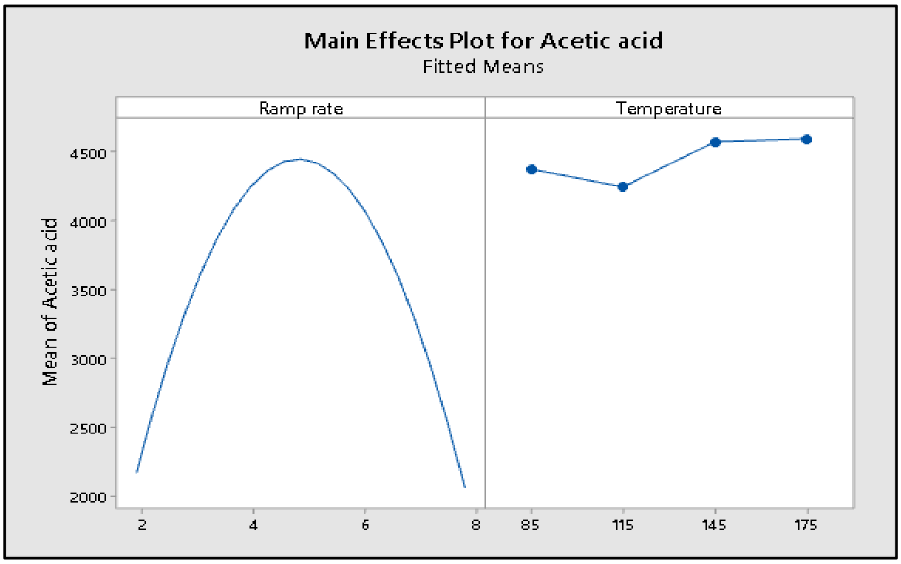


Figure 5.4 main effects (temperature vs. ramp rate) influence on acetic acid concentrations of food waste after MW treatment.

5.2.1.5 MW FW solids

In all tested microwave condition, VS loss was present, especially at higher temperatures and slow heating rate. This happened despite precautionary measures being taken to avoid solids losses such as only opening the microwave vessel's screw caps once the treated samples had reached room temperature.

Solids loss varied between 10.34 and 19.17% for the fast-heating rate, 3.68 - 13.42% at medium heating rates and 16.42-25.64% for the slow heating rate. Consequently, VS/TS content of all treated samples also suffered reduction (Table 5.1).

These findings are in agreement with previous studies of microwave treatment of FW. Shahriari (2011), reported a VS loss for the fast-heating rate ranging from 4.95- 21.42%, and from 2.74 to 25.82% for the medium heating rate, with the maximum VS loss (16.42- 32.41%) occurring for slow heating rates. Marin *et al*., (2010) observed a VS and VS/TS decrease after exposing kitchen waste to fast, medium and slow heating rates for a final temperature of 175°C. The highest loss occurred for the slow heating rate, corresponding to 37% less VS solids than the untreated sample, followed by the medium (12.90%) and fast (4.56%) heating rates.

The loss of solids after the exposure of organic waste to microwave treatment can significantly affect AD performance, since the solids are utilized by the microorganisms for reproduction, growth and biomethane generation (Weiland, 2010). Therefore, the importance of determining, and adjusting if necessary, this parameter before the AD process.

5.2.1.6 Macroscopic aspect of samples after microwave irradiation

Microwave irradiation of FW samples caused a noticeable modification of the final samples. Figure 5.5 shows the distinct colouring of the treated samples as temperature and heating rate were increased. The samples exposed to higher temperature (175°C) had the darkest colour (in some cases completely black), in contrast with those exposed to the lowest temperature (85°C) which were pale yellow. At 115 and 145°C, regardless of the heating rate the samples were different shades of brown with the 145°C being the darker.

The brown colour observed for some of the treated samples is a result of exposure to heat through cooking or caramelizing, and has been associated with the Maillard reaction, first described in 1912 by Louis Maillard. It was not until 1953, however, that the first coherent explanation was put forward by Hodge (Hodge, 1953). Characterized as a non-enzymatic browning reaction, Maillard is a complex network of reactions involving carbonyl and amino compounds, such as reducing sugars and amino acids (Figure 5.6). This is the main process responsible for the transformation of precursors into colorants and flavour compounds during food processing (Davidek *et al.*, 2003; Totlani and Peterson, 2007; Venir *et al.*, 2009).

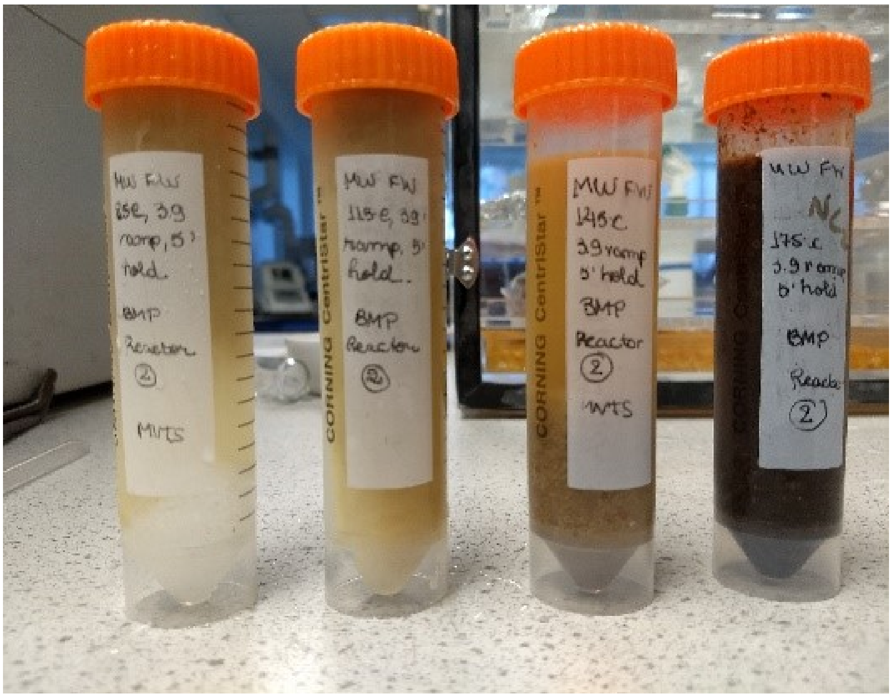


Figure 5.5 Samples treated at medium heating rate and different temperatures (85-175°C). From left to right: 85, 115, 145 and 175°C 3.9 °C/min microwaved FW samples.

According to Ames *et al.* (1999) the Maillard reaction occurs when a reducing sugar, such as glucose, condenses with a compound possessing a free amino group, such as an amino acid, to give a condensation product (Yang *et al.*, 2011). Subsequently, a range of reactions take place, including cyclizations, dehydrations, retroaldolizations, rearrangements, isomerizations and further condensations, which ultimately lead to the formation of brown nitrogenous polymers and co-polymers, known as melanoidins (Coca *et al.*, 2004).

Melanoidins are polymeric and coloured macromolecules primarily formed by interactions between carbohydrates (typically reducing sugars) and compounds with a free amino group, such as amino acids (Montavon *et al.*, 2003). They are present in variety of products such as coffee, bread and roasted malt (Morales and Jiménez-Pérez, 2004; Adams *et al.*, 2005; Echavarría *et al.*, 2012).

Previous studies, such as the one conducted by Shahriari (2011), have also reported a significant change in colour after samples were treated at 175 ºC and different heating rates (20, 40 and 60 minutes). According to the Shahriari, the MW pre-treated samples were dark brown in contrast to the lighter colour of controls and other pre-treated samples, suggesting that it could be indicative of the production of melanoidins.

Melanoidins compounds were not quantified in the current study. The assumption of their presence, however, is plausible as previous studies in the literature have constantly suggested them as a result of food browning (Hodge, 1953; Shahriari, 2011).

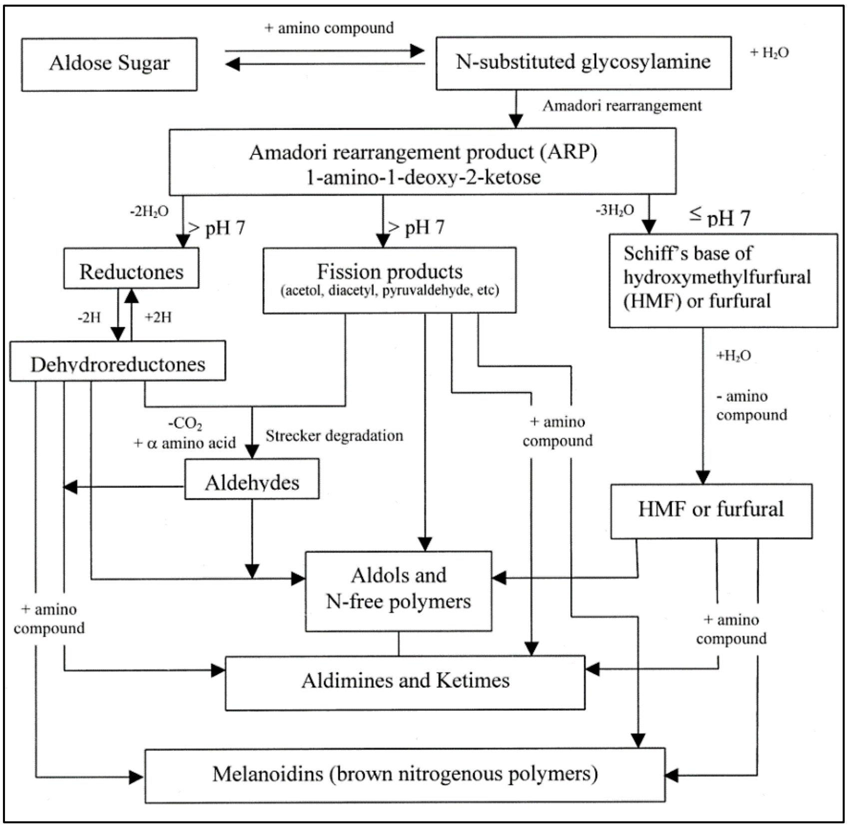


Figure 5.6 Description of Maillard reaction with the formation of melanoidins (Adapted from Hodge, 1953).

5.3 Effect of microwave treatment on MAD of food waste

5.3.1 Ammonium

The ammonium release in the system during the AD for both fast and slow heating rates is shown in Figure 5.7, these were the conditions on which ammonium behaviour was more relevant to explain microwave pre-treatment effects on this ion. At lower temperatures (85 and 115°C), the release of ammonium into the system is slower than at the higher temperatures (145 and 175°C). The peak at day 7 suggests that microwave irradiation is ineffective in breaking proteins under lower temperatures, hence undergoing normal microbial enzymatic metabolization (as previously suggested) taking longer to peak.

Conversely, at higher temperatures, and specifically at 175°C, the ammonium peak occurs earlier (day 4), confirming the effectiveness of the microwave irradiation in encouraging protein hydrolysis under these conditions. Moreover, because most of the protein content is broken and solubilized in the pre-treatment step, the peak observed in this case is lower than at fast heating rate and low temperature.

Despite the higher ammonium content release for the fast heating rate, the final values were within the tolerated threshold for bacteria community- less than 1500mg/L Koster and Lettinga (1988) estimated as being prejudicial for process performance; at 4051–5734mg/L of ammonium), hence not imposing risk of digestion failure.

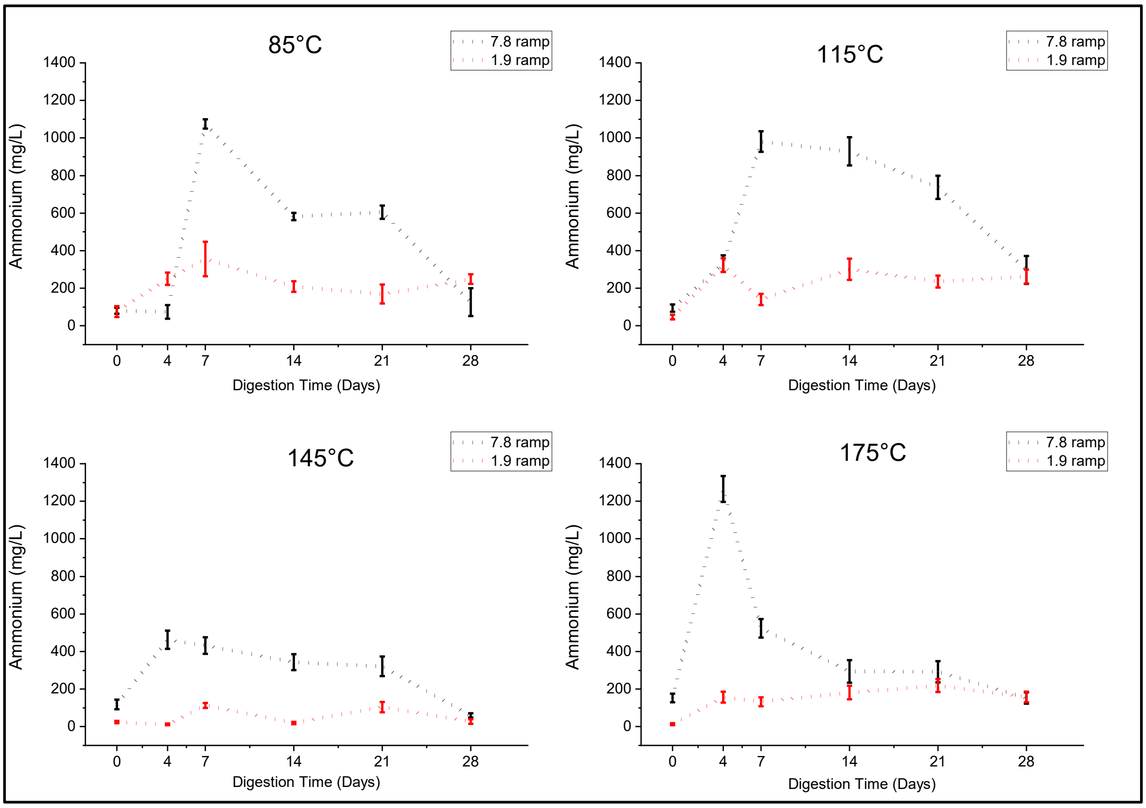


Figure 5.7 Ammonium release from MW treated FW under different temperatures and ramp rates during anaerobic digestion.

5.3.2 Total volatile fatty acids behaviour during MAD of MW FW

5.3.2.1 Volatile fatty acids profile and behaviour at fast heating rate (7.8°C/min)

The total volatile fatty acids behaviour for the fast-heating rate at different final temperatures is shown in Figure 5.8. Regardless of the microwave final temperature, the dominant VFA in all reactors at the beginning of the digestion period (day 0) was acetic acid, representing between 70 and 85% of the TVFAs in the pre-treated samples, as opposed to only 47% of the TVFAs in the control, hence reflecting the MW effect on intermediate products generation. The second most prominent acid in of all reactors was iso-butyric acid, corresponding to between 8 and 15.58%, compared to 12.03% in the control.

For the untreated samples (control), the greatest production of TVFAs occurred between days 2 and 4, during which hydrolysis was intensified. During the hydrolysis step, the microbial enzymes break down complex molecules into smaller ones, which can be converted into intermediate products (VFAs) in the acidogenesis step (Pullen, 2015). There was a 1853.89% increase in

TVFAs concentration on day 2 and a 2036% increase on day 4 (when the VFAs concentration reached its maximum of 2010.44 mg/L) in relation to when the digestion process started (Figure 5.8).

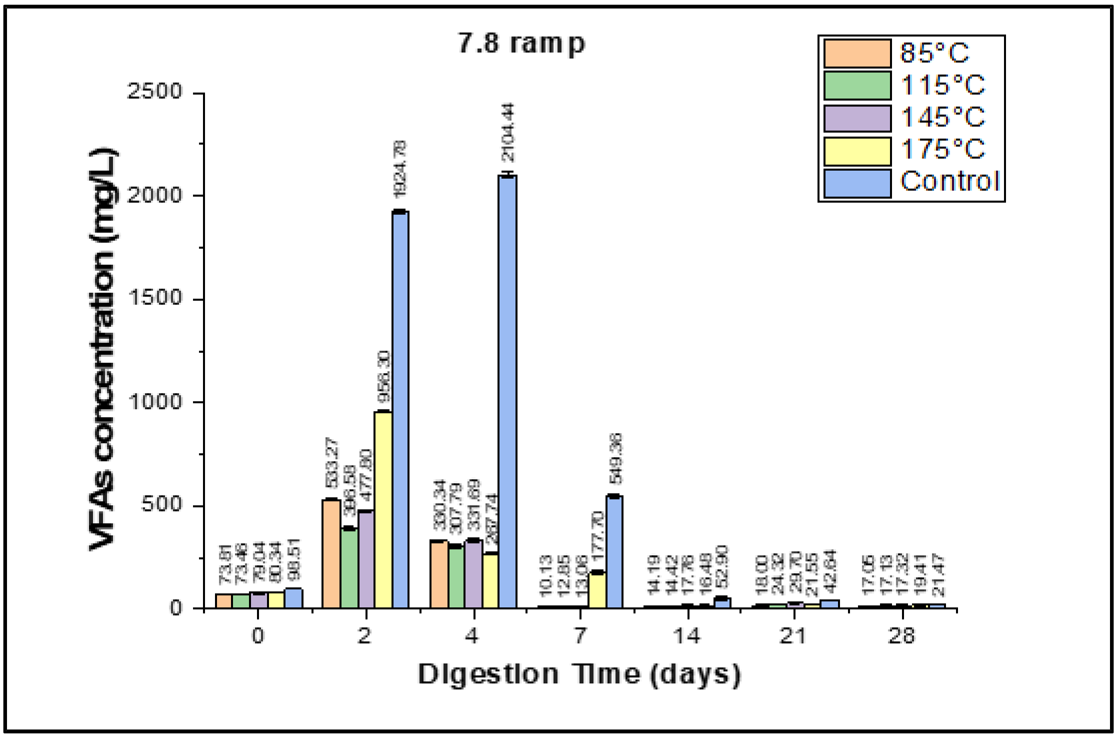


Figure 5.8 TVFAs profile during the anaerobic digestion of microwaved food waste treated at fast heating rate (7.8°C/min)) and different temperatures.

The subsequent accumulation of intermediate products indicates a kinetic imbalance between VFAs production (acetoclastic bacteria) and consumption (methanogenic bacteria). Nevertheless, the intermediate product accumulation did not cause process failure, due to the significant buffering capacity of the system which was able to counterbalance the pH drop (minimum value of 6.50). The observed pH decrease was expected, since during acidogenesis it is common for the pH value to decrease within the range of 5 ‐ 6.5 (depending on the nature of the substrate and organic load) (Jagadabhi *et al.*, 2010).

Similar to the controls, an increase in TVFAs concentration was detected on day 2 of digestion in all the microwaved samples, with the increase ranging from 439.91 to 1,090.00% compared to day 0. The smaller TVFAs increase in relation to the control could have resulted from the thermal treatment breaking down the complex organic molecules (enhancing hydrolysis), thus offering the microorganisms a more readily biodegradable form of substrate, compared to the raw/untreated FW on which organisms would still have to enzymatically solubilise the organic matter. For this reason, VFAs could be consumed at a faster rate avoiding the typical uncoupling between methanogenic and acidogenic populations, which leads to VFAs accumulation in one step-reactors treating FW (Mata-Alvarez *et al.*, 1992).

Faster VFAs consumption for the 7.8 ramp samples resulted in enhanced methane production during the hydrolysis step in relation to the control. The percentages of the total gas generated for the whole digestion period, obtained between days 0 - 2 were as follows: 31.53, 33.53, 30.70 and 22.79% for 85, 115, 145 and 175°C, respectively.

Because of TVFAs accumulation and poor process performance, the controls generated modest methane during the first two days of digestion representing only 10.30% of the total gas generated over the whole digestion period.

Contrary to the control, which exhibited TVFAs accumulation during the first days of digestion, the microwaved treated samples exhibited a continuous consumption of the intermediate products as digestion progressed. This continuous consumption varied significantly between the different final temperatures resulting in significant differences in the volumes of methane generated.

At the lowest temperature tested, 38% of the TVFAs were removed, with acetic acid being the main acid consumed (volume reduced by 86.15%). This resulted in the production of 145.93 ml of methane from a total of 635 ml accumulated since the start of the experiment (Figure 5.9).

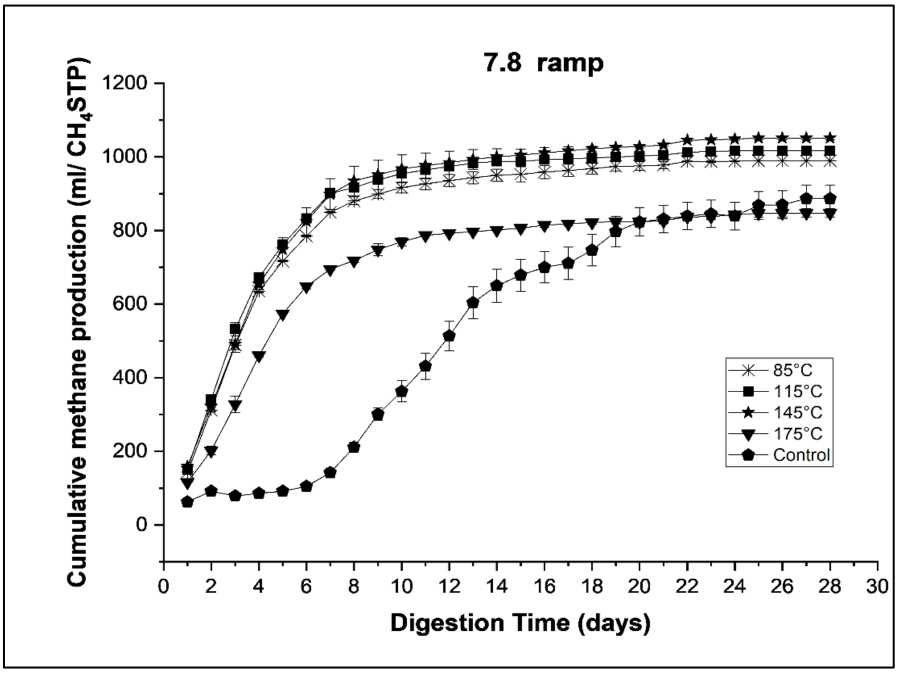


Figure 5.9 Cumulative methane production of food waste samples treated at fast heating rate (7.8°C/min) and temperatures of 85-175°C.

FW samples treated at 115°C exhibited lower TVFAs removal than when samples were microwaved at 85°C. Once again, acetic acid was the main VFA consumed, having its concentration reduced by 77.90%. The volume of methane produced by the microorganisms between days 2 and 4 was 141.16 ml from a total of 672.70 ml accumulated since the start of the digestion. FW samples treated at 145°C showed a 30.57% removal of TVFAs for the same period, with 84% of the acetic acid consumed in this step. The methane volume obtained for this period was 163.43 ml from a total of 654.08 ml produced since the AD process started.

Conversely, the anaerobic digestion of FW microwaved at 175°C, resulted in 72% of the TVFAs consumed between the second and fourth day of digestion. The longer exposure to microwave irradiation, and extreme temperature, apparently favoured acetic acid consumption by anaerobic microorganisms resulting in the greatest acetic acid removal (96.78%). The higher acetic acid removal in turn, resulted in a higher percentage of methane produced: 132.69 ml from a total of 460.10 ml generated since the start of digestion, corresponding to 28% of the total biomethane accumulated since the start of the digestion, compared to an average of 23% at lower temperatures, same heating rate. Nonetheless, the total volume of methane produced during these two days was lower than for other temperatures. This could be explained by inhibitory factors that affect methanogenic activity such as the presence of recalcitrant compounds.

According to Pullammanappallil *et al.* (2001) the presence of certain volatile organic acids and the general increase in TVFAs concentration have a direct correlation with anaerobic process performance. It has been previously observed that when anaerobically treating organic wastes, such as swine manure, food wastes and municipal sludge, the increase of propanoic acid in the system occurred prior to digestion failure (van den Berg and Lentz, 1971; Kaspar and Wuhrmann, 1978; Fischer *et al.*, 1981).

The acetic acid concentration in the system can also be used as a process performance parameter, with levels in excess of 800 mg/L posing a detrimental effect on methanogenic bacteria (Hill *et al.*, 1987). For these reasons, individual VFAs formed during the anaerobic digestion process of MW FW were analysed.

The recommended treshold of acetic acid was not exceded during the hydrolisys/acidogenesis phase (2nd to 7th day), with the maximum value (731.18mg/L) being registered on the second day of digestion for samples treated at 175°C. In contrast, the control samples exhibited high concentrations of acetic acid for the same period, reaching values of 1563.00mg/L during the 7th day of digestion, which could explain, at least in part, the poor process performance for this condition.

Moreover, propanoic acid ratios greater than 1.4 indicates impending digestion failure, and can be used as a tool for the detection of stress in the system Hill *et al.* (1987). All the FW microwaved samples showed increased concentration of propanoic acid in relation to the acetic acid on day 4 of the anaerobic digestion, with values significantly exceeding the 1.4 limit (Figure 5.10).

Values ranged from 9.22 (85°C) to 10.93 (115°C). Samples exposed to longer microwave irradiation and higher temperature (175°) had a second point where the p/a ratio exceeded the recommended threshold:the first one occured on the second day of digestion and corresponded to a p/a of 2.21, and the second one on day 7, corresponding to a p/a of 10.02.

The successive, p/a values significantly higher than the threshold could explain, at least in part, the lower biomethane production for the 175°C samples during this period when compared to other microwaved operating conditions.

The control/untreated samples exhibited an exceeding p/a value (4.61) on the 14th day of digestion. Possible reasons for this are explained in chapter 4. Despite the elevated p/a ratio for all conditions, including control, methane production during this time was not affected. Pullammanappallil *et al.* (2001) also reported an unhindered AD process for a glucose-fed reactor inhibited by the addition of phenol (which later is metabolized to propanoic acid), despite an excessively high p/a ratio of 54.

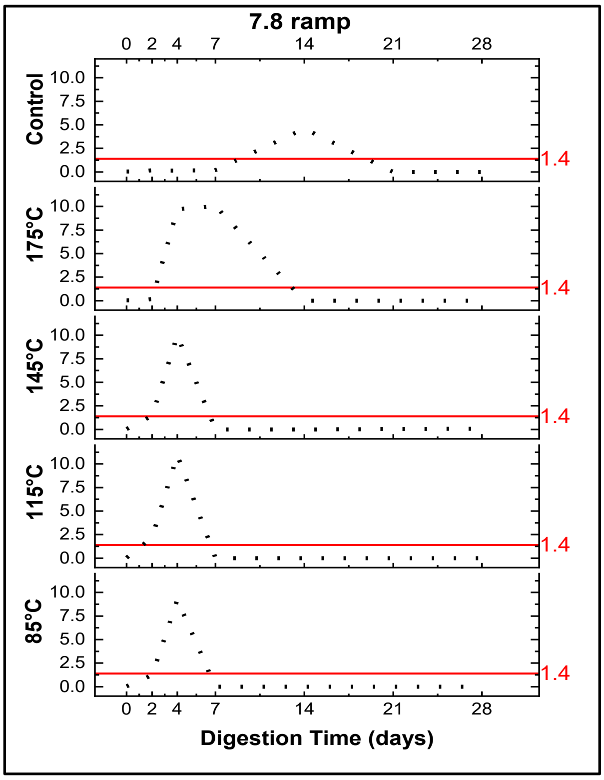


Figure 5.10 Propanoic to acetic acid ratio and its recommended threshold of 1.4 for food waste microwaved samples at a fast-heating rate (7.8°C/min) and different temperatures.

5.3.2.2 Volatile fatty acids profile and behaviour at medium heating rate (3.9°C/min)

Similarly to the fast heating rate, the main VFA on all reactors at the beginning of the digestion period (day 0) was acetic acid, representing between 66.83 and 76.37% of the TVFAs in the pre-treated samples. In contrast to the fast heating rate where iso-butyric acid was the second most prominent acid in the system, propanoic acid predominated in this case, corresponding to values between 3.77 and 5.00% of the TVFAs (Figure 5.11).

In contrast to the 7.8 ramp for which TVFAs production versus consumption rate was balanced throughout the digestion period, there was a TVFAs accumulation for all the temperatures with medium heating rate during the first days of digestion. For this reason, a higher TVFAs concentration at this point was observed, with a minimum of 1453.04mg/L (175°C) and a maximum of 1639.00mg/L (145°C), representing a 943 and 843.11% increase respectively in relation to when the experiment started (day 0).

The significantly higher TVFAs concentration could have been a result of longer exposure to microwave irradiation (with the exception of 85°C 3.9 ramp which had similar exposure time as

145°C 7.8 ramp) compared to when samples were treated using a fast-heating rate. This could have rather or simultaneously promoted: a) enhanced hydrolysis of the food waste organic matter compared to when samples were treated using a fast-heating rate, consequently generating higher TVFAs, and b) the formation of inhibitory compounds, which ultimately affected the methanogenic bacteria, hence reducing the consumption rate of these intermediate products.

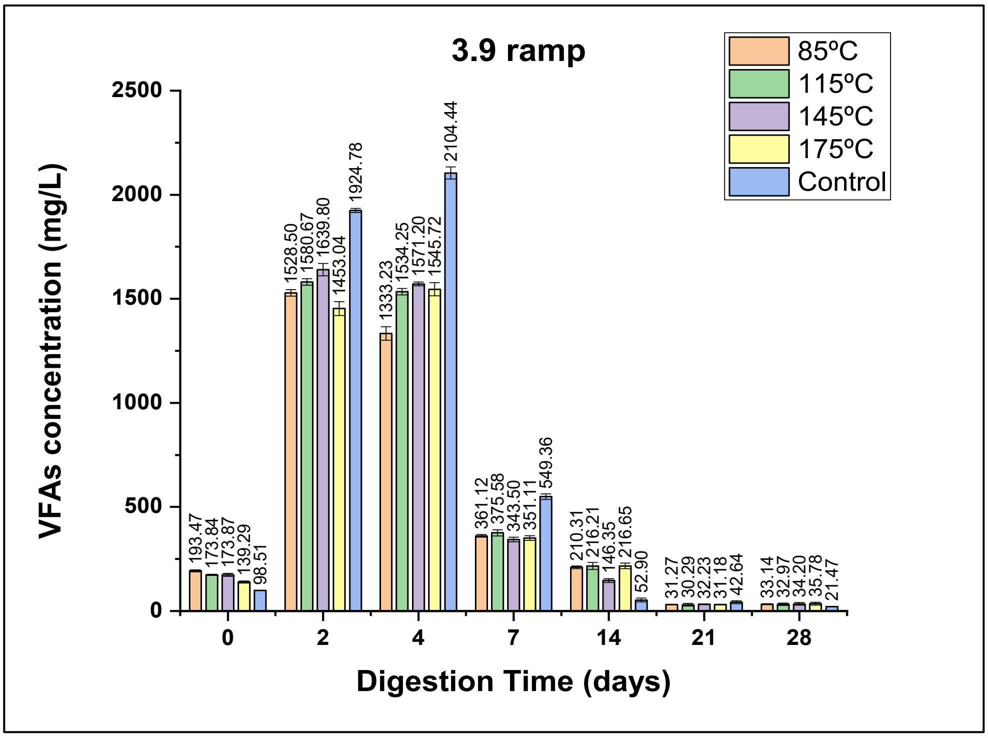


Figure 5.11 TVFAs profile during the anaerobic digestion of microwaved food waste treated at medium heating rate (3.9°C/min) and different temperatures.

The percentages of total gas generated (with respect to the gas generated for the whole digestion period) between day 0 and 2 were: 5.79, 6.33, 7.43 and 5.98% for 85, 15, 145 and 175°C respectively. This is an average of one fifth of the biomethane produced using a fast-heating rate, and supports the hypothesis of methanogenic population inhibition, due to VFAs accumulation.

TVFAs accumulation persisted on the fourth day of digestion for all treated samples. Most of them (85, 115 and 145°C) showed a minor decrease of TVFAs concentration between days 2 and 4 of the BMP test. The decrease of TVFAs concentration was 12.77, 2.98 and 4.18% for the 85, 115 and 145°C samples, respectively and related to a decrease in acetic acid, which was greater at 85°C with a decrease of 16.95%. Acetic acid consumption decreased to approximately half of this amount, 8 and 9%, for 115°C and 145°C respectively.

Because of the poor removal of TVFAs/acetic acid, methane production during this period was significantly suppressed, compared to that for the fast-heating rate. The digestion of FW samples treated at 85°C yielded 64 ml between days 2 and 4 of digestion from a total of 117.52ml accumulated since the start of the process. The values for the 115°C samples were similar, with 65.6ml of biomethane generated from a total of 126.74ml accumulated since day 0. Similarly, the

145°C FW microwaved samples produced 65.02 ml of gas from a total of 131.23ml accumulated since the start of the BMP test (Figure 5.12).

Successive TVFAs accumulation for the medium heating rate (3.9°C/min) at day 2 and 4 caused a decrease in biomethane production compared to that for a fast-heating rate (7.8°C/min). The loss was estimated to be between 40 and 47% depending on the temperature tested.

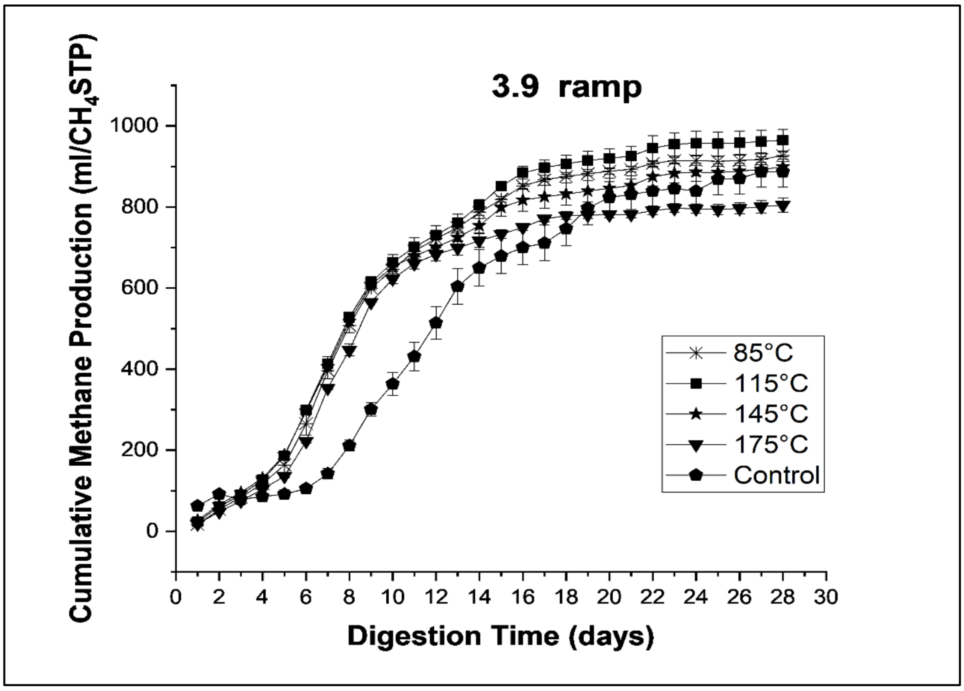


Figure 5.12 Cumulative methane production (at standard temperature and pressure) of food waste samples treated at medium heating rate (3.9°C/min) and temperatures of 85-175°C.

As microwave temperature rose to 175°C and the microwave irradiation exposure time increased, the TVFAs concentration between day 2 and 4 of the BMP increased by 2%. The consecutive TVFAs accumulation during this period affected the process performance and yielded only 55.69ml of biomethane from a total of 103.43 ml accumulated along the BMP test. In this case, the hydrolysis/acidogenesis step of the anaerobic digestion process produced 77% less methane (by volume) than for the same temperature using a fast-heating rate.

The TVFAs accumulation observed for the medium heating rate in the hydrolysis/acidogenesis phase (between days 2 and 4) for all tested temperatures was caused mainly by acetic acid accumulation. The minimum value of this acid (1162.55mg/L) was recorded for 85°C on the fourth day of digestion and the maximum value (1487.17mg/L) was recorded on the second day of digestion for 145°C. All of these values were similar to those for the untreated samples, i.e. raw food waste (maximum value 1563.58mg/L on the 7th day of digestion).

In all cases, the acetic acid concentration significantly exceeded the recommended threshold. The exceeded values represented 45.31 and 85.89% increase in relation to the highest acetic acid concentration observed for the fast heating rate, i.e 731mg/L (175°C). Therefore, the poorer process performance in relation to the fast heating rate could be related to a reduction in the methanogenic activity, hence acetic acid consumption, due to methanogenesis inhibithion caused by a high concentration of this acid in the system.

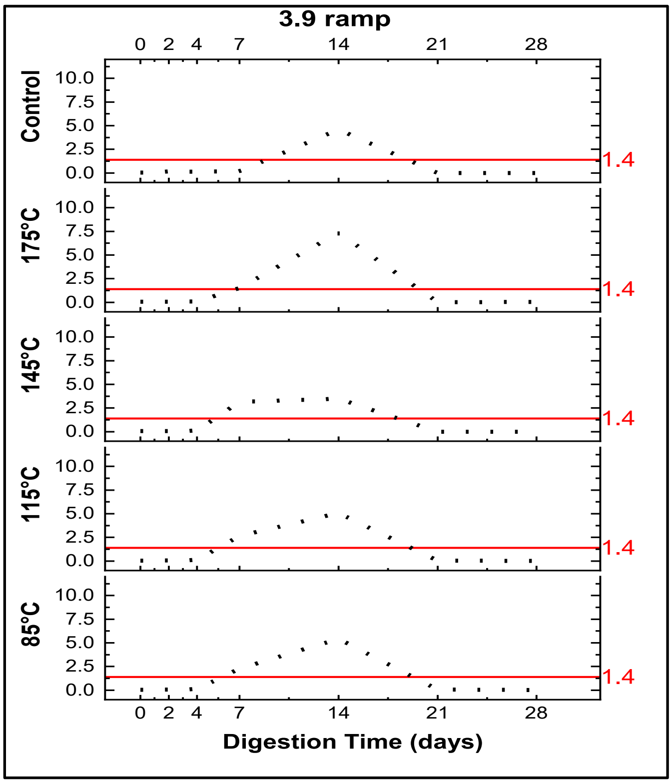


Figure 5.13 Propanoic to acetic acid ratio and its recommended threshold of 1.4 for food waste microwaved samples at a medium heating rate (3.9°C/min) and different temperatures.

For the medium heating rate there were high p/a values observed between days 7 and 14 for all of the temperatures tested (Figure 5.13). On the 7th day the p/a values ranged from a minimum of 1.5 (175°C) to a maximum of 3.14 (145°C), and on day 14 the minimum value was 3.14 (145°C) and the maximum was 7.35 (175°C). Those values were in general lower than the p/a ratios of samples treated using a fast-heating rate, possibly explained by a lower propanoic acid concentration during the digestion. A latter p/a ratio peak in turn, could be explained by the slower removal/metabolization of acetic acid in relation to the propanoic acid by the anaerobic bacteria consortia, possibly due to inhibition of the methanogenic community as a result of TVFAs accumulation between days 2 and 4 of the BMP test (Figure 5.13). Despite the p/a exceeding values, methane production was not affected.

5.3.2.3 Volatile fatty acids profile and behaviour at slow heating rate (1.9°C/min)

Similarly to the fast and medium heating rates, the dominant VFA in all reactors at the beginning of the digestion period (day 0) was acetic acid (78.37 and 90.10%). Propanoic acid was the second most prominent intermediate product in the system, corresponding to values between 3.93 - 7.81%

of the TVFAs (Figure 5.14**).** In contrast to that the medium heating rate, the TVFAs production vs. consumption rate was improved, hence resulting in a lower intermediate products concentration with a maximum value of 1132.84mg/L (85°C) on day 2 and 1488.66mg/L (175°C) on day 4. This represents a 197.41 and 860% increase respectively with respect to when the experiment started (day 0).

Unexpectedly, the TVFAs consumption rate was not significantly decreased by a longer exposure to microwave radiation. Although samples were pre-treated under a longer period (42 minutes more) than the medium heating rate, the microbiological community was better able to process the products of the acidogenesis phase. The accumulation of intermediate products in the system therefore appears to be the result of more than one factor, including inoculum age. This is discussed later in this chapter.

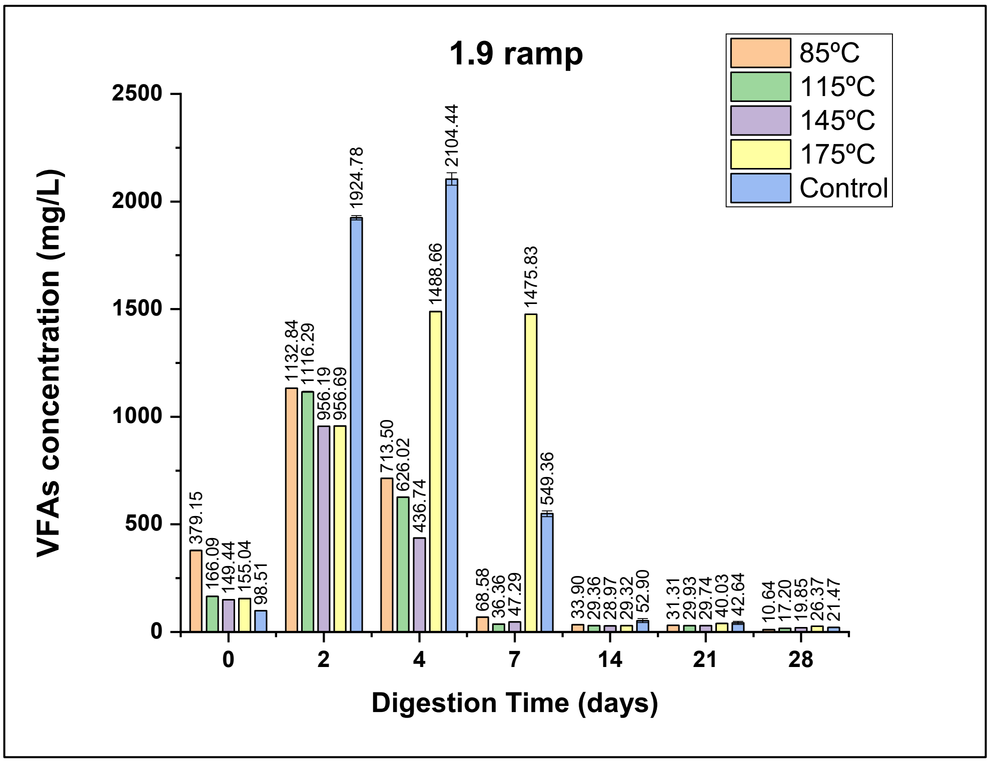


Figure 5.14 TVFAs profile during the anaerobic digestion of microwaved food waste treated at slow heating rate (1.9°C/min) and different temperatures.

The percentage of total gas generated for the whole digestion period obtained between day 0 and 2 were: 25.80, 27.13, 27.33 and 15.55% for the 85, 15, 145 and 175°C treated FW respectively. These values represent an average of 0.8 times less biomethane produced than for the fast-heating rate, and 4 times greater than for the medium heating rate. Therefore, methane production is directly related to the system's acidity.

The TVFAs removal for samples treated at 85°C between day 2 and 4 was 37%, with acetic acid being the principal TVFA consumed (volume reduced by 50.88%), resulting in a 168.34ml of methane produced, from a total of 584.72ml accumulated since the start of the experiment (Figure 5.14). FW samples treated at 115°C exhibited a greater TVFAs removal (44%) than when samples were microwaved at 85°C. Once again, acetic acid was the main VFA consumed, having its concentration reduced by 59.43%. The volume of methane produced was 185.8ml from a total of 616.05ml accumulated since the start of the experiment (Figure 5.15).

Samples treated at 145°C resulted in a 54.32% removal of TVFAs for the same period, with 74.56% of the acetic acid consumed in this step. The methane volume obtained for this period was 201.39ml from a total of 643.89ml produced since the AD process started.

FW samples treated at 175°C exhibited the least TVFAs removal of all the test conditions explored, with intermediate product accumulation between day 2 and 7 of the digestion period. This caused a prolonged elevation of the system acidity, not well tolerated by the methanogenic community. Not surprisingly, biomethane generation was negatively affected during this period, with a total of 57.82ml of biomethane produced between day 0 and 2, and 53.97ml between day 2 and 4 from a total of 322.54ml produced since the start of digestion.

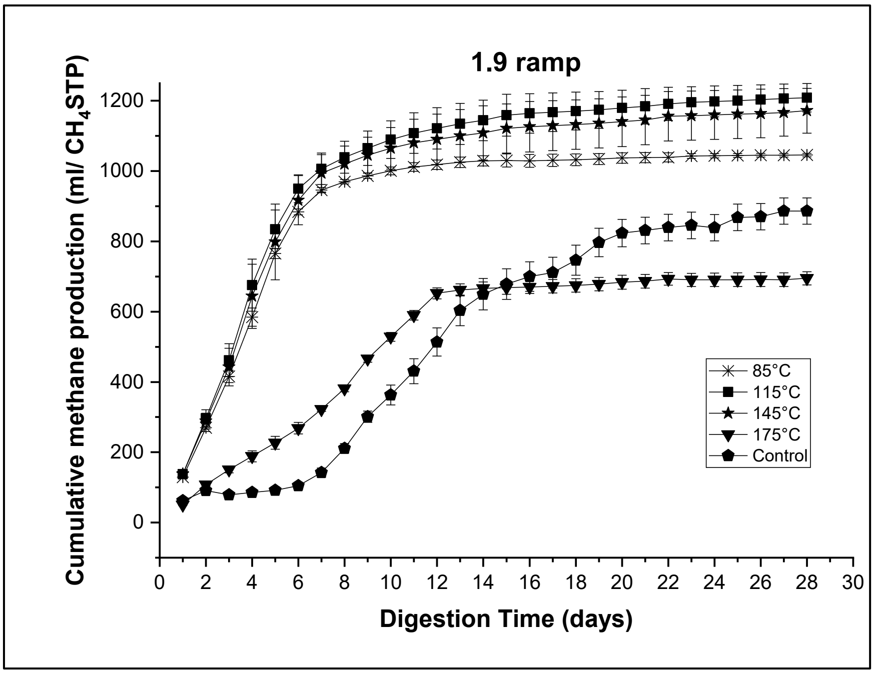


Figure 5.15 Cumulative methane production of food waste samples treated at slow heating rate (1.9°C/min) and temperatures of 85-175°C.

The behaviour of acetic acid concentration during the AD of microwaved FW varied across the various temperatures tested. As expected, there was a peak in acetic acid during the hydrolysis phase, specifically at day 2, were it reached values between 690 and -811mg/L, with the latter marginally exceeding the recommended threshold of 800mg/L, at 85°C. Nevertheless, it was subsequent consumed (and the concentration correspondingly lowered) after the hydrolysis step.

Nevertheless, at 175°C the acetic acid concentration exceeded the recommended threshold both at days 4 and 7 of digestion, with values of 1224.56 and 1272.64mg/L respectively, reflecting the TVFAs accumulation observed for the same period.

As opposed to the other heating rates, p/a threshold was not exceeded at any of the temperatures tested. The highest p/a ratio occurred at 145°C day 4, with a value of 1.2. The lowest p/a ratio occurred for 175°C, with a peak at day 7 of 0.2 (Figure 5.16). Despite the former having the lowest p/a ratio of all tested conditions, this not necessarily result in a better process performance, with for example, higher methane production, hence suggesting that p/a ratio is not the main factor influencing methane production of microwaved FW under high temperatures.

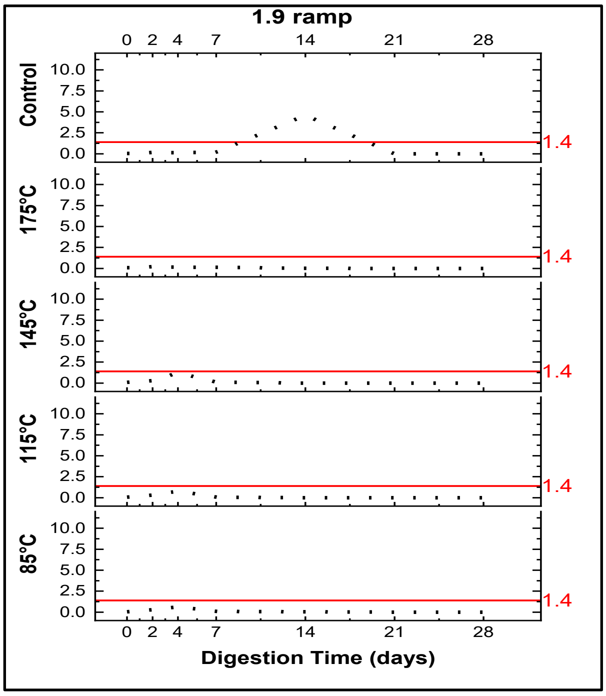


Figure 5.16 Propanoic to acetic acid ratio and recommended threshold of 1.4, for food waste microwaved samples at a slow heating rate and different temperatures.

Differently from the medium heating rate where there was a p/a ratio peak at two consecutive points day 7 and 14 of the digestion, the FW treated at a slow heating rate did not exhibit exceeding values for this period. The later peaks of p/a on the former condition could result from an inhibition of the propanoic degrading syntrophs microorganisms accompanied by a normal acetoclastic activity. On the other hand, the inoculum used for the medium heating rate was recycled from the first set of experiments (7.8 ramp), as opposed to the one used for the slow heating rate experiments, which was otherwise, fresh.

As previously discussed, methane production rate at the beginning of the digestion period, varied significantly amongst the various microwave operational conditions tested. This was mainly related to the presence/absence of fermentative and methanogenic bacteria, which determined the intermediate products (TVFAS) consumption rate. Overall, the anaerobic digestion of microwaved food waste showed improved methane production at the first week of digestion, due to hydrolysis enhancement, improved TVFAs consumption as a consequence of the chosen pre-treatment (section 5.3.2). For most of the conditions tested, methane production started to stabilize from day 14 onwards, with small volumes of gas produced after this period. At day 28 (last day of digestion) the volume of methane obtained for lower temperature range (85-145°C) and different heating rates tested was very similar, with a maximum variation of 17.30% between them (Table 5.4). Nevertheless, when higher temperatures (175°C) were applied, methane production exhibited a decrease in relation to other microwaved food waste samples. The difference in process performance was more evident at a slow heating rate (1.9 °C/min), and it is possibly related to the fermentative and methanogenic bacteria inhibition due to the hypothesized presence of inhibitory compounds such as phenols and melanoidins. In this case, the difference in methane production was more significant, with a 21.95% variation between different heating rates used.

Table 5.4 Methane production at day 28 (end of the AD process) for control/ untreated and microwave pre-treated samples at different ramp rates and final temperatures (85-175°C).

Final temperature (°C)	Heating rate (°C/min)	Methane production (ml) at day 28 (end of the digestion period)
Control	-	
85	**7.8ramp (fast)**	989.46
115		1017.07
145		1051.01
175		847.59
85	**3.9ramp (medium)**	926.66
115		964.79
145		897.99
175		804.34
85	**1.9 ramp (slow)**	1045.63
115		1208.91
145		981.94
175		694.98

It can be speculated, therefore, that the inoculum had a significant influence on the anaerobic digestion of microwaved FW for different operating conditions, affecting the acetoclastic activity and the propanoic degradation, which in turn influenced the degree of waste stabilization and hence methane generation.

5.4 Acidity vs. alkalinity of the system

In addition to the stability indicators previously used (pH and p/a ratio), the acidity (expressed as TVFAs concentration) versus alkalinity ratio (TVFAs/Alkalinity) of the anaerobic digestion process has been widely adopted as an additional tool for evaluating the likelihood of process failure. According to Callaghan *et al.* (2002) there are three critical levels to which one should be attentive when anaerobically digesting organic wastes. These are: 1) VFAs/Alkalinity levels >0.4, indicates process stability; 2) TVFAs/Alkalinity between 0.4-0.8, some instability will occur; and 3) TVFAs/Alkalinity ratio >0.8, then significant instability is present, and process failure is likely to happen. During the anaerobic digestion of microwaved FW treated at the fast-heating rate, for the totality of the digestion period the TVFAs/Alkalinity ratio was significantly below the recommended threshold of 0.4 irrespective of the final temperature applied (Figure 5.17).

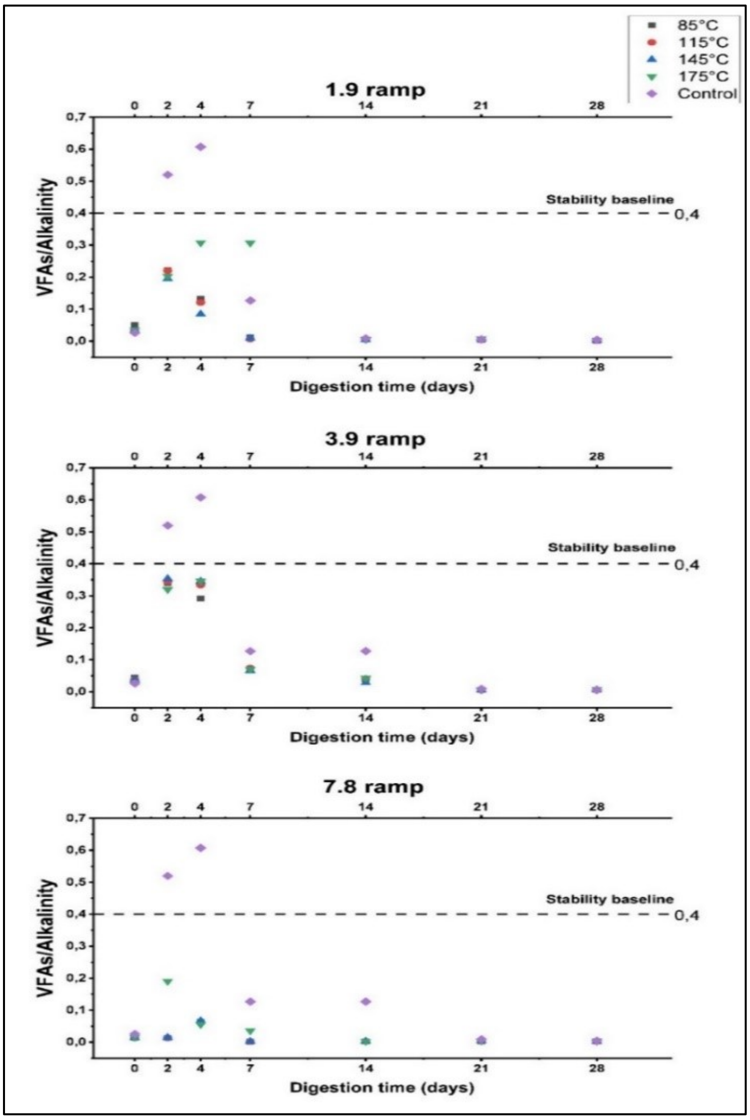


Figure 5.17 acidity versus alkalinity behaviour during the mesophilic anaerobic digestion of microwaved food waste samples at different ramp rates and final temperatures (85-175°C) and controls.

The low value range for the TVFAs/Alkalinity resulted from a better performance of the process, with no VFAs accumulation, which can ultimately raise the ratio to an instability "zone". The highest TVFAs/Alkalinity values occurred on the 2nd day of the digestion and ranged from 0.055–0.067 for the 175°C and 145°C treated samples respectively.

Not surprisingly, the control samples (which did not undergo any thermal pre-treatment) showed a high TVFAs/Alkalinity ratio during the digestion period. This is the result of poor process performance related to the accumulation of TVFAs at the hydrolysis and acidogenesis phase. The threshold was exceeded both on the 2nd and 4th of the BMP test, with values of 0.50 and 0.60 respectively.

Although the samples treated using the medium heating rate showed TVFAs accumulation on days 2 and 4, the final acidity of the system was not sufficiently high to reach a point where the TVFAs/Alkalinity ratio surpassed the recommended threshold. The maximum value observed was 0.35 for a temperature of 145°C on day 2 of the digestion. Therefore, for the medium heating rate, the process was stable thorough the digestion period for all temperatures.

For the slow heating rate, despite the slower TVFAs consumption between the first four days of digestion (for temperatures between 85-145°C), the recommended threshold was not exceeded. The highest values of this ratio were observed for a temperature of 175 °C, possibly due to the accumulation of TVFAs between day 4 and 7, which ultimately caused this ratio to peak at 0.306 and 0.307 respectively. The ratio did not, however, exceed the stability baseline. Therefore, it can be said that microwave pre-treatment of FW improved the process stability in relation to the control, moving the digestion from an instable zone to a stable one.

5.5 Anaerobic biodegradability of control and microwaved food waste

Biodegradability can be used as an indicator of process performance. It compares the volume of methane produced during the BMP assays against the theoretical volume, thus offering an indirect measure of the assimilative methanogenic capacity of the seed, the feasibility of the waste as a feedstock to AD, as well as the effectiveness of the thermal process in terms of methane production enhancement (Neves *et al.*, 2004).

In the present study the theoretical methane potential (TMP) was calculated based on Buswell and Mueller, (1952) and (Li *et al.*, 2013), which takes into consideration the elemental composition (C, H, O, N, S) of the feedstock. The value of the TMP of the composite FW sample was estimated to be 515.65NmlCH_4/gVS. This method does not differentiate between biodegradable and non-biodegradable matter. Therefore, in practical terms, and in most cases, the theoretical method will overestimate the BMP methane production.

Figure 5.18 depicts the biodegradability percentage achieved for each set of microwave operating parameters tested. Amongst the samples treated using a fast-heating rate (7.8°C/min), those heated to 145°C showed the greatest biodegradability (40.76% of the TPM achieved). This result represents an 18.14% improvement in relation to the control/untreated samples. For the medium heating rate (3.9°C/min), samples treated at 115°C exhibited the highest biodegradability, with 37.42% of the TMP achieved. In this case, the improvement in relation to the control was 8.48%.

For the slow heating rate (1.9°C/min), the lowest temperature, 85°C showed the best biodegradability performance, 40.56% of the TMP produced, representing a 13.44% improvement in relation to the untreated samples. This was negligibly different to that for 145°C using a fast-heating rate. One would therefore minimise costs by choosing the fast-heating rate at a higher temperature (7.8 ramp at 145°C) over the slow heating rate at lower temperatures (85°C at 1.9 ramp).

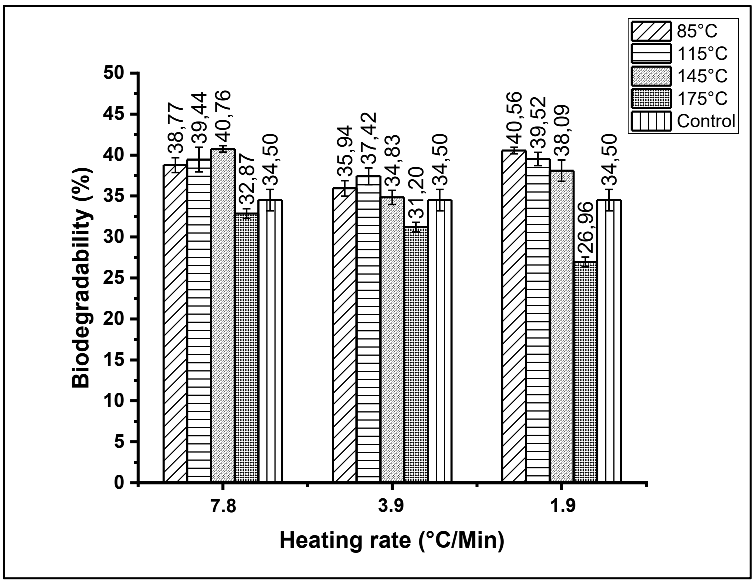


Figure 5.18 Percentage of biodegradability for mesophilic AD of microwaved food waste at different operational conditions.

A possible explanation for the increased biodegradability when employing either 145°C, fast heating rate or 115°C, medium heating rate, would be an observed common factor, i.e. the presence of higher TVFAs concentrations after microwave pre-treatment in relation to other samples. As previously shown, TVFAs composition after microwave irradiation was dominated by acetic acid, which has the potential to be transformed by the methanogenic microbial community into methane gas (assuming a balanced environment, free from inhibitions factors). Conversely, samples treated at 85°C using a slow heating rate (3.9°C/min) did not have the highest TVFAs concentration compared to the other temperatures. Another factor, therefore, must have determined its higher biodegradability.

Samples treated at 175°C regardless of the heating rate consistently showed lower biodegradability, including lower values than the untreated samples. The decrease in the biodegradability was dependent on microwave irradiation time. Therefore, samples exposed to fast heating rate was 4.72% less biodegradable than the control, as opposed to 9.56% and 21.85% less biodegradable than the untreated samples from the medium and slow heating rates. These results strongly suggest that the longer exposure to irradiation promoted the formation of recalcitrant and possibly inhibitory compounds (e.g. phenols, melanoidins) which affected process performance, and hence biodegradability.

These results are in agreement with previous work from Marin *et al.* (2010), who accessed the biodegradability of microwaved kitchen waste heated to a temperature of 175° using same heating rate as the present study. The authors observed that after longer exposure to microwave radiation (using medium and slow heating rates) biodegradability (and biogas production) of the whole fraction samples would decrease. Similar to the present study, the decrease was proportional to the microwave exposure time, with a 1.9 ramp yielding poorer results. The authors attributed this finding to the presence of substances that are difficult to digest such as humic acids.

5.6 Methane yield and kinetic assessment

Figure 5.19 shows that all the temperatures (85-175°C) exposed to the fast heating rate (7.8 ramp) resulted in a significantly shorter lag phase (λ) than the control. Amongst the final temperatures tested, 85 and 145°C had the shortest lag phase (0.123 and 0.125 days respectively), which supports the hypothesis that the improvement in hydrolysis rate is a consequence of enhanced substrate solubilization promoted by microwave pre-treatment. These results represent the most abreviated lag phase of all the microwave operating parameters studied. The lag phase values reported here for the digestion of the whole fraction of FW agree with those reported in the literature. Liebetrau *et al.* (2004) for example, reported a value of 0.34 days for kitchen waste. Similarly, Vavilin and Angelidaki (2005) reported λ value of 0.55 days for the AD of FW.

In turn 175°C had the longest lag phase (0.341 day), with an average of 161.53% increase in relation to the other temperatures tested. Even though the extreme temperature reactors had a poor hydrolysis rate, its lag phases represent an improvement with respect to the control (lag phase of 4.47 days), occuring 12.10 times faster than untreated samples.

The theorethical methane yields (A) obtained from the MGompertz model for all temperatures for a fast heating rate were similar to the cumulative methane yield (B) obtained experimetally (Table 5.5). For most of the temperatures A/B was 97%, hence suggesting a good fitting with the best agreement for 115°C (Figure 5.19).

The reactors containing FW samples treated in the temperature range 85 - 175° with the medium heating rate demonstrated a prolonged lag phase, with values ranging from 2.4 – 3.38 days at 145 and 175°C respectively (Table 5.5). This represents the most extensive delayed hydrolysis step of all the conditions studied. In this case, the average lag phase was 18 times greater than for the fast heating rate and 3 times greater than for the slow heating rate (Figure 5.19). The prolonged fermentation is most likely due to VFAs accumulation during the hydrolysis step. Moreover, these results suggest that there was no inverse relation between the exposure time to microwave radiation and the duration of the lag phase. For the medium heating rate, the A/B was on average 98%, as opposed to 99 % of the slow heating rate, suggesting a good fitting for both conditions.

The samples treated at 175°C, regardless of the heating rate, consistently showed a longer lag phase compared to the samples treated at other temperatures. Although higher temperature promotes higher solubilization (SCOD concentrations), it does not necessarily yield the best hydrolysis rate and lag phase. This may be due to the formation of hard-to-digestion compounds.

Factor(s) other than the formation of inhibitory/hard-to-digest compounds might have had a direct effect on the hydrolysis rate of FW samples treated at 175°C, one hypothesis being the inoculum's age. As suggested by Braguglia *et al.* (2006) and Tomei *et al.* (2008), hydrolysis rate depends on feed/inoculum ratio of the BMP test as well as inoculum quality, including age (Bougrier *et al.*, 2006).

The hypothesis that the inoculum's "age" interfered with the hydrolysis rate, arrived from the observation that both fast and slow heating rate reactors were assembled with fresh inoculum (which was a maximum of 7 days old) exhibiting better lag phase/hydrolysis rate. Conversely, the reactors containing the samples treated using a medium heating rate were assembled with inoculum that was 60 days old and showed a poor performance with prolonged lag phase. The reactors containing FW subjected to 175°C and medium heating rate had the poorest lag phase from all combinations of heating rates and temperatures tested. The lag phase was estimated to be approximately 9 times and 1.3 times greater than for the fast and slow heating rates respectively, and could have been the result of a prejudiced metabolization rate of the organics due to a low bacteria activity associated with the old age of the seed.

The lag phase of the control/untreated samples had the greatest duration (4.47 days). The increased duration could be because microorganisms still had to break hydrogen bonds and further solubilize the organic matter in contrast to the microwaved treated samples.

Table 5.5 depicts the final methane yield for each combination of temperature and heating rate. The operating conditions which resulted in the highest methane yield were: 145°C fast heating rate (210.21mlCH_4/gVS added) and 85°C slow heating rate (209.12mlCH_4/gVS added). To assess if the difference between experimental methane yields for the various operating parameters were statistically significant an analysis of variance (ANOVA) one-way was conducted.

The statistical results reveal that there was no significant difference between the yield produced by the lowest temperature (85°C) using different heating rates. Therefore, from an economic point of view, it would be advantageous to treat the FW at low temperature with fast heating rate, since the duration of the process is then shorter (24 minutes less than the slow heating rate in the lab-scale experiments reported here) and the energy input requirement is, therefore lower.

When comparing the two extreme conditions, i.e. lower temperature with the fast-heating rate and higher temperature with the slow heating rate (85°C 7.8°C/min and 175°C 1.9°C/min) the statistical analysis performed revealed a significant difference between them ($p < 0.05$) (Appendix D). A process optimized to obtain the best methane yield for the least input energy would use a lower temperature and a shorter exposure to MW (85°C 7.8°C/min). In the lab-scale experiments reported here the processing time saved is 81 minutes and the final methane yield is increased by 42.37%.

Table 5.5 Kinetic assessment of the mesophilic anaerobic digestion of the microwave treated food waste samples at different temperatures and heating rates.

		Predicted methane yield (ml) (A)	sdt	Experimental yield /Specific methane yield (ml) (B)	std	λ (lag Phase)
Ramp	Temperature	Value	Std Error	Value	Std Error	Value
7.8 ramp (Fast)	85°C	193.82	0.98	197.89	0.01	0.12
	115°C	199.67	0.88	203.41	0.01	0.15
	145°C	205.17	1.04	210.21	0.01	0.12
	175°C	165.36	0.86	169.51	0.01	0.34
3.9 ramp (Medium)	85°C	182.88	1.25	185.33	0.09	2.86
	115°C	191.50	1.38	192.95	0.00	2.55
	145°C	175.90	1.44	179.59	0.01	2.40
	175°C	158.56	1.15	160.86	0.01	3.38
1.9 ramp (Slow)	85°C	207.55	0.67	209.12	0.01	0.68
	115°C	202.54	0.06	203.79	0.01	0.60
	145°C	195.50	0.54	196.38	0.01	0.63
	175°C	141.29	1.79	138.99	0.01	1.47
Control		181.17	3.22	177.92	0.01	4.47

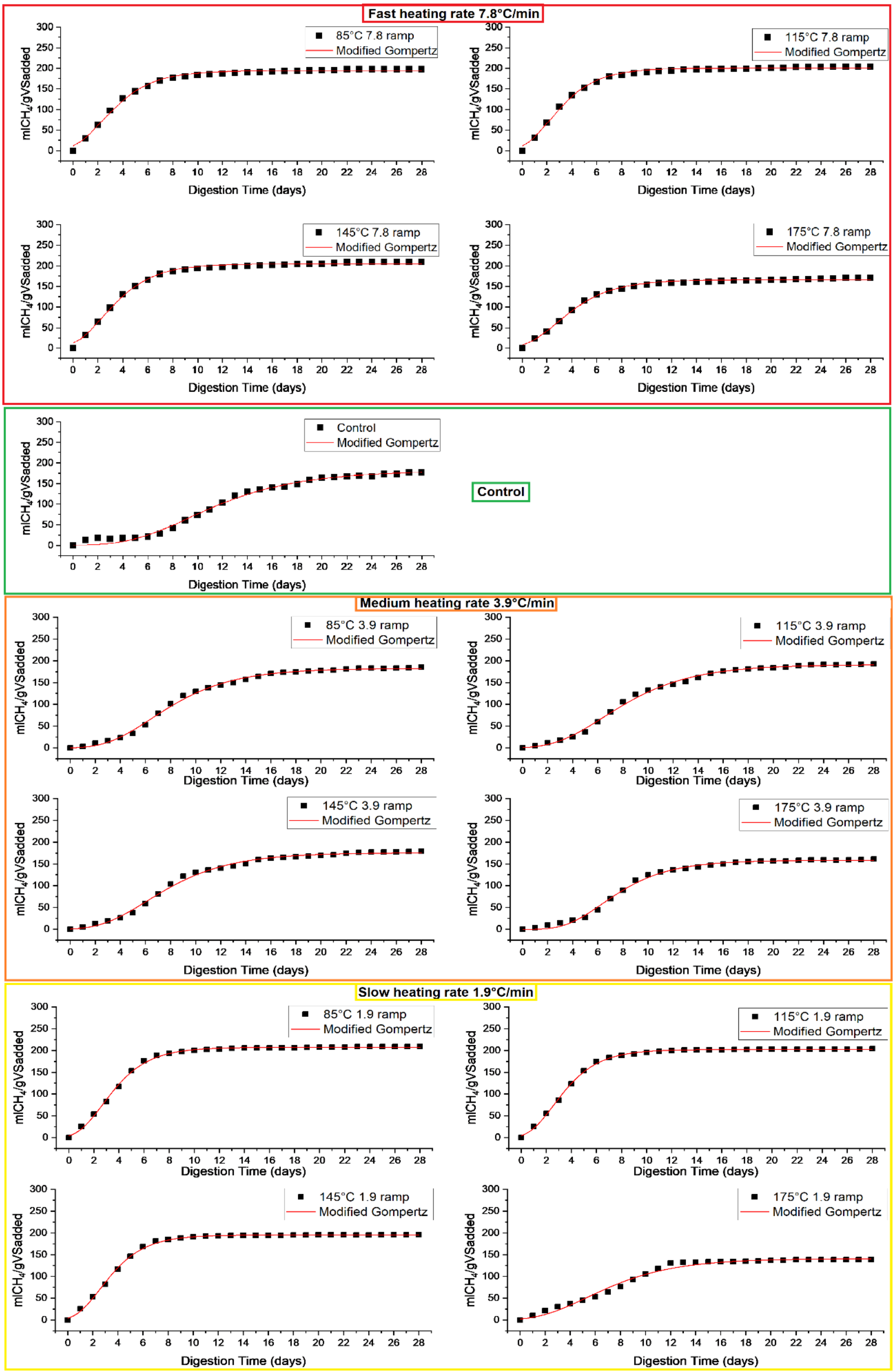


Figure 5.19 Specific methane yield with the MGompertz fitting curves for the food waste samples treated at 85-175°C and different heating rates (7.8, 3.9 and 1.9°C/min).

Figure 5.20 shows the principal influence on final methane yield from FW microwaved at different temperature and heating rates. It is demonstrated that the final temperature has greater influence on final methane yield than ramp rate, with values of methane tending to drop as final temperature rises. A 34.28% drop is observed on the methane yield when temperature rises from 85 to 175°C suggesting an inversely relation. When the heating rate is changed from fast (7.8 ramp) to medium (3.9 ramp), there is also a significant decrease in methane yield, however less significative than when temperature is manipulated. In contrast to final temperature, however, methane yield rises again when the heating rate decreases (1.9 ramp-slow). The postulated explanation for this is the age of the inoculum as discussed previously.

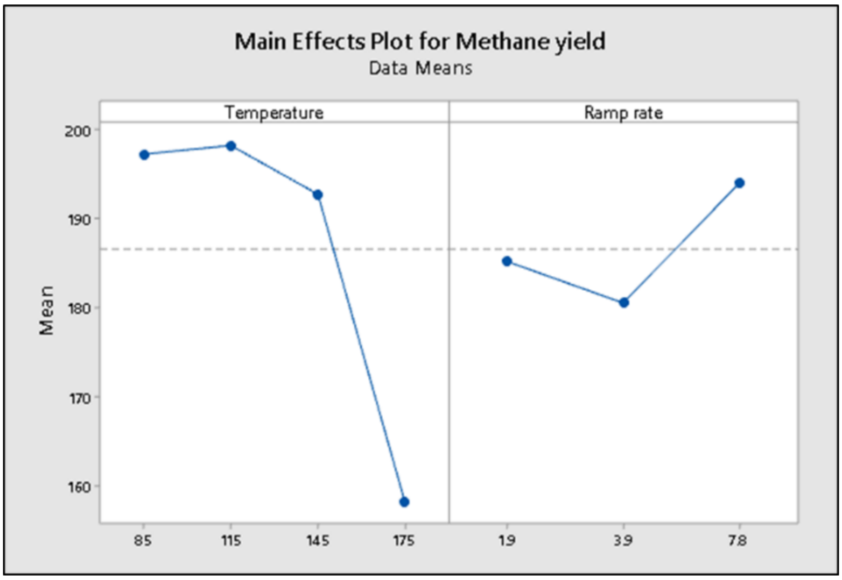


Figure 5.20 Main factor influencing final methane yield: temperature vs. heating rate. The units are not represented in the axes, due Mini-tab software default schematics. Instead they (temperature and ramp rate) are located at the top part of each graph.

A two-dimensional (surface) regression was conducted for cumulative methane yield versus final temperature and heating rate, using yields obtained from the 3^2 factorial DOE ($n = 18$) to establish Equations (12 – 18). These equations were then used to predict the cumulative methane yield at the higher heating rate of 8°C/min (slightly higher than the greatest heating rate tested here of 7.8°C/min) to evaluate the feasibility of applying longer microwave exposure time on methane yield (Table 5.6).

Figure 5.21 demonstrates that if the heating rate was to be increased further, there would be an increase in methane yield for 175°C (to approximately 170mlCH_4/gVS added). This increase would not be enough, however, to justify the costs of microwave pre-treatment at higher temperatures. On the other hand, a higher heating rate of 8°C/min would increase methane yield significantly for the samples treated at 145°C. In this case, the increase in final methane yield would be similar to that for the fast-heating rate and low temperature (85°C). Once again this does not appear to be an economically feasible option for the microwave pre-treatment of FW.

Table 5.6 Response surface regression for the cumulative methane yield versus the final temperatures and heating rates.

Ramp Rate	Equation
Fast (7.8 ramp)	72.2 + 2.304 Temperature - 0.00986 Temperature · Temperature (12)
Medium (3.9 ramp)	Methane yield = 56.2 + 2.323 Temperature - 0.00986 Temperature·Temperature (13)
Slow (1.9 ramp)	Methane yield = 123.9 + 1.839 Temperature - 0.00986 Temperature·Temperature = 123.9 + 1.839 T – 0.00986 T2 (14)
Final Temperature	**Equation**
85°C	Methane yield = 222.24 - 11.29 Ramp rate + 0.985 Ramp rate·Ramp rate (15)
115°C	Methane yield = 213.48 - 9.14 Ramp rate + 0.985 Ramp rate·Ramp rate (16)
145°C	Methane yield = 195.03 - 6.28 Ramp rate + 0.985 Ramp rate·Ramp rate (17)
175°C	Methane yield = 156.99 - 5.50 Ramp rate + 0.985 Ramp rate·Ramp rate (18)

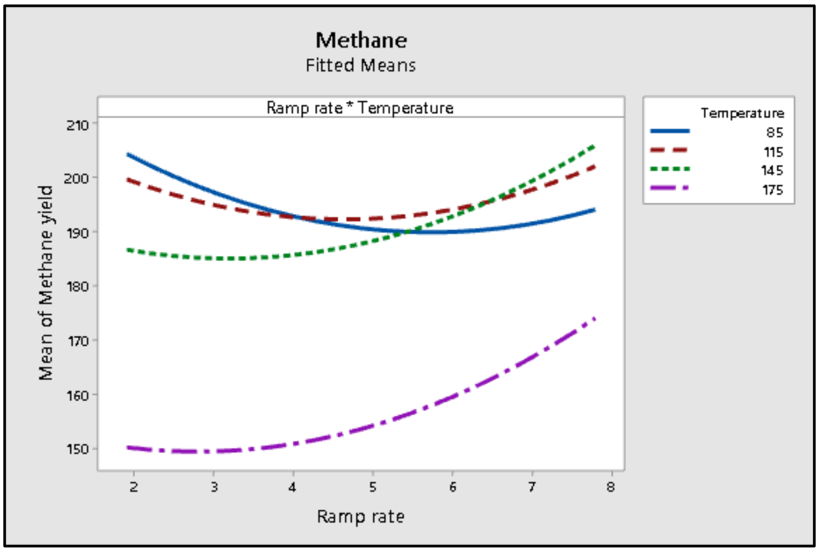


Figure 5.21 Methane prediction at higher heating rate (8°C/min).

5.7 Elemental composition (C, H, N, S) of microwaved food waste

Microwave irradiation promoted some significant changes in the composition of the FW that are shown on Table 5.7. Regardless of the ramp rate or final temperature, there was a significant decrease in the elements C, H, N and S in relation to the control/untreated samples.

After irradiating FW the carbon content suffered an average of 9% reduction compared to the untreated sample. The largest reduction (11.60%) for the fast-heating rate occurred at 85°C, for the medium heating rate at 145°C (11.79%) for the slow heating rate at 85°C (12.34%).

The carbon loss is clearly related, at least in part, to the loss of organic matter (volatile solids) which was observed for all operating parameters after irradiating the FW.

Reduction in nitrogen concentration after microwave treatment is expected due to the ammonium volatilization of the samples (for the medium and slow heating rate). Unexpectedly, however, the fast-heating rate was the only operating parameter resulting in a decrease in nitrogen. A larger decrease (20.61%) occurred at 85°C and a smaller decrease (7.21%) occurred at 145°C.

Nevertheless, little existing work is reported in the literature relating to the effects (thermal and athermal) of microwave irradiation on the elemental composition on this type of organic waste.

Abbey *et al*. (2017) evaluated the effects of microwave irradiation on the nutrient concentration (nitrogen and phosphorous amongst others) of the vermicast composition. According to the authors, the microcosm of vermicasts bears some resemblance to that of soil and, to perhaps a lesser extent, with that of food. It is plausible that there could be similarities in chemical alterations in vermicasts upon exposure to microwave irradiation. The authors observed that when applying a power output level of 0 to 400W, the total N concentration (i.e. the sum of ammonium-nitrogen, NH_3-N; nitrate-nitrogen, NO_3eN; nitrite nitrogen, NO_2-N; and organically-bound nitrogen) was gradually reduced, but remained unchanged from 400 to 800W. This contrasts with the results reported here.

The decrease in carbon and nitrogen content of the FW after microwave irradiation ultimately caused a subsequent decrease on the C/N ratio, compared to the control samples. For the fast-heating rate, the average C/N ratio was 10.63% less than the control, as opposed to the medium heating rate, where the average C/N ratio was 18.40% lower than the control. The slow heating rate in turn, showed an average decrease of 17.48% in the C/N ratio compared to the untreated samples. A decrease on the C/N ratio could compromise the AD performance, since the bacteria requires both sufficient amounts of carbon and nitrogen for feeding and growing/reproducing (Zhang *et al*., 2007).

There was also a post-treatment loss in hydrogen content. The loss (49.48%) was greater for the slow heating rate and high temperature (175°C) and less (12.70%) for 85°C and the fast-heating rate.

Oxygen content after microwave treatment increased in relation to the control for all microwave parameters tested. The greatest increase (41.31%) occurred at 85°C and the slow heating rate and the smallest increase (11.93%) occurred at 85°C and the medium heating rate. Lastly, sulphur also exhibited a decrease for all the conditions tested. In no cases did the samples show any sign of this component after the thermal treatment.

The changes in elemental composition (C, H, N, S, O) has a significant impact on the theoretical methane potential estimated according to Boyle (1977). Because of the significant decrease in the concentration of these elements, especially carbon compared to the control, the final theoretical methane yield was reduced by a maximum of 36.59% at 175°C using the medium heating rate.

The changes in elemental composition of the FW after microwave irradiation suggests that care will be required if treated substrate is used to generate further methane via anaerobic digestion. As previously discussed, loss of carbon may result in lower methane yield (demonstrated and estimated via Boyle's equation) and adjustment of solids might be required as part of a commercial process set-up.

Table 5.7 Elemental Composition, Carbon and Nitrogen ratio, Stoichiometric formula and theoretical methane potential of untreated and microwave treated food waste.

	Carbon (wt%)	**Nitrogen (wt%)**	**Hydrogen (wt%)**	**Sulphur (wt%)**	**Oxygen (wt%)**	**C/N**	**Stoichiometric Formula**	**Theoretical Methane Potential**
7.8 ramp (Fast heating rate)								
85°C	46.90 (0.03)	4.36 (0.01)	6.80 (0.37)	0.00 (0.00)	41.94 (0.02)	10.75	$C_{12.58}H_{21.93}O_{10.21}N$	542.02
115°C	47.00 (0.20)	4.41(0.04)	4.59 (0.03)	0.00 (0.00)	44.00 (0.03)	10.65	$C_{12.41}H_{14.57}O_{8.87}N$	468.84
145°C	47.60 (0.56)	4.50 (0.18)	6.33(0.07)	0.00 (0.00)	41.57 (0.05)	10.57	$C_{12.33}H_{19.71}O_{8.06}N$	484.50
175°C	47.50 (0.06)	4.49 (0.01)	4.39 (0.08)	0.00 (0.00)	43.62 (0.07)	10.89	$C_{12.34}H_{13.71}O_{8.50}N$	455.17
3.9 ramp (Medium Heating rate)								
85°C	49.47 (0.19)	5.51(0.02)	6.76 (0.03)	0.00 (0.00)	38.26 (0.02)	8.97	$C_{10.48}H_{17.20}O_{6.08}N$	373.31
115°C	47.16 (0.06)	5.20 (0.00)	4.51 (0.25)	0.00 (0.00)	43.13 (0.01)	9.06	$C_{10.59}H_{14.01}O_{7.25}N$	379.77
145°C	46.80 (0.14)	5.15 (0.07)	4.44 (0.19)	0.00 (0.00)	43.61 (0.18)	9.08	$C_{10.62}H_{12.09}O_{7.41}N$	372.90
175°C	50.54 (0.40)	5.85 (0.02)	4.36 (0.03)	0.00 (0.00)	39.25 (0.47)	8.63	$C_{10.26}H_{10.63}O_{5.82}N$	326.52
1.9 ramp (Slow Heating rate)								
85°C	46.51 (0.46)	5.19 (0.04)	4.47 (0.36)	0.00 (0.00)	48.30 (0.33)	8.96	$C_{10.45}H_{14.02}O_{8.13}N$	391.14
115°C	47.61 (0.17)	5.59 (0.06)	4.90 (0.21)	0.00 (0.00)	41.90 (0.17)	8.52	$C_{9.79}H_{14.01}O_{6.54}N$	343.88
145°C	49.58 (0.26)	5.76 (0.04)	5.40 (0.12)	0.00 (0.00)	39.26 (0.18)	8.60	$C_{10.04}H_{14.01}O_{5.96}N$	340.63
175°C	49.22 (0.20)	4.89 (0.03)	3.95 (0.02)	0.00 (0.00)	41.94 (0.08)	10.06	$C_{12.05}H_{13.97}O_{7.7}N$	433.84
Raw Food Waste (Control)								
Untreated Food waste/control	53.06 (0.37)	4.85 (0.07)	7.79(0.10)	0.13 (0.03)	34.18 (0.51)	**10.95**	$\mathbf{C_{13.97}H_{24.81}O_{6.73}N}$	515.65

5.8 Summary

Microwave irradiation resulted in important changes in the FW parameters. These include an increase in Total and Soluble Chemical Oxygen demand concentration in all the treated samples, regardless of the final temperature and heating rate used. A Tukey test demonstrated that the difference between the SCOD obtained from the samples treated at 175°C and all other temperatures (85 - 145°C), regardless of the heating rate, was statistically significant. This is evidence that longer irradiation exposure time and higher temperature promotes greater solubilization of the organic matter. It was found that heating rate was the main factor affecting SCOD solubilization and acetic acid production.

For most of the microwave operational conditions tested (medium and slow heating rates at all temperatures) a significant loss of ammonium in relation to the untreated samples was observed, ranging from 92.51 to 98.82%. This could be explained by the combination of two factors: a) the volatilization of ammonium as a result of the thermal effect of the microwave irradiation, and b) volatile losses due to the poor condition of the screw caps used in the microwave treatment. If validated, this finding can be rather promising when digesting FW with high protein content.

Microwave irradiation of samples caused a noticeable modification to the colour of the final solutions. The colours varied between black (175°C) to pale yellow (85°C) and brown (115 and 145°C). The browning of the samples is the result of cooking or caramelizing and has been associated with the Maillard reaction, which promotes the formation of methanogenic inhibitory compounds, such as melanoidins. Their presence could explain, at least in part, the poor TVFAs consumption rate, biodegradability, methane production and yield for the samples treated at higher temperatures (175°C).

Process stability for all tested conditions was analysed using propanoic to acetic ratio (p/a ratio), pH and VFAs/alkalinity ratio. Although most of these parameters (in particular the p/a ratio) exceeded the predetermined threshold, the anaerobic digestion process proceeded unhindered for the microwave operating parameters studied.

The microwaved samples exposed to 145°C and the fast-heating rate, and those exposed to 115°C and the medium heating rate, had the highest biodegradability of all the conditions studied. A common factor was the presence of higher TVFAs concentration (principally acetic acid) after microwave pre-treatment in relation to the other samples. The higher concentration of acetic acid increases the potential for biomethanation, thus enhancing biodegradability.

For most of the operational conditions studied (fast and slow heating rates and temperatures between 85 - 145°C) microwave irradiation proved effective in enhancing process performance via hydrolysis and methane generation amelioration during the first week of digestion compared to the controls. It was found that all of these parameters were greatly improved at 85°C 7.8 ramp, with no intermediate products accumulation for any of the final temperatures tested at this ramp, up to 77% more methane produced in the first week of digestion compared to the other conditions tested and reduction of 96.36% on the lag phase, compared to the control. Temperature was found to be the main factor influencing final methane yield.

Microwave irradiation promoted some important changes in the elemental composition of the FW, with a significant decrease of C, H, N and S in relation to the control. This was true for all the treated samples, regardless of the final temperature and heating rate. The changes in elemental composition after microwave irradiation means that attention will need to be paid when applying treated substrate for further methane generation via commercial anaerobic digestion. The loss in carbon content, due to loss of volatile solids, can result in a lower methane yield.

The samples treated at 175°C, regardless of heating rate, consistently showed poor process performance compared to other temperatures. Although it promoted higher solubilization (SCOD concentrations), this did not necessarily yield the best hydrolysis rate or lag phase, possibly due to the formation of hard-to-digest compounds.

There was no statistically significant difference between the yields produced using different heating rates for the lowest final temperature (85°C). It is concluded that a lower temperature and shorter exposure time (85°C 7.8 ramp) is likely to increase final methane yield and reduce energy input requirement, and for this reason, classified as the optimum condition for biomethanation of FW.

CHAPTER 6

6. INFLUENCE OF MICROWAVE IRRADIATION ON THE BIOMETHANATION OF THE SOLUBLE FRACTION OF FOOD WASTE UNDER MESOPHILIC ANAEROBIC CONDITIONS

6.1 Introduction

After performing BMP tests on the microwave FW, it was noticed that samples treated at 175 °C regardless of the heating rate exhibited the poorest process performance with lower methane yields, despite higher organic matter solubilisation rates (SCOD). The poor process performance could have been a consequence of the formation of inhibitory compounds (such as melanoidins and humic acids) for the bacterial community, especially the methanogenic population (Marin *et al.*, 2010).

Previous studies have looked at the relative improvement in methane potential of the supernatant/soluble fraction of Kitchen Waste (KW) following thermal or chemical pre-treatment, evidencing that this fraction can be digested faster and produce less sludge compared to the treatment of the whole fraction of this waste (Sawayama *et al.*, 1997; Lee *et al.*, 2009).

Henceforth, in this chapter the biomethane potential originating from the soluble fraction of microwaved FW at 175°C at different heating rates (7.8, 3.9, 1.9°C/min), will be evaluated for potential improvements in process performance after solids removal, and compared against MW FW whole fraction under the same conditions (175°C, 7.8, 3.9, 1.9°C/min). Moreover, the effects of solid fraction removal on the 85°C 7.8 ramp (identified as the optimum condition for methane production – chapter 5) will also be evaluated and compared against 175°C, 7.8, 3.9, 1.9°C/min, as well as the controls (FW without microwave pre-treatment) at both whole (with no solids removal) and soluble (with solids removal) fractions.

A detailed description of important changes on FW characteristics as well as in the biomethanation process, including TVFAs behaviour, pH, methane production and yield are contained here. This information will not just validate the soluble fraction as being the most feasible one for methane production, but also help to elucidate the origin of the inhibitory compounds (soluble or whole fraction).

6.2 Results and discussion

6.2.1 Effect of microwave irradiation on the soluble fraction of FW characterization

FW microwaved at 175°C under different heating rates (fast (7.8°C/min), medium (3.9°C/min) and slow (1.9°C/min) as well as 85°C 7.8 ramp had their solids removed by centrifugation and filtration to determine the effects of thermal pre-treatment followed by phase separation on their main parameters such as: pH, Soluble Chemical Oxygen Demand (SCOD), Ammonium, TKN, amongst others. The effects of microwave irradiation and phase separation of the FW are show in Table 6.1.

6.2.1.1 pH and TVFAs after MW pre-treatment and phase separation

All of the soluble fraction FW samples exhibited a decrease in the pH in relation to the control soluble fraction (FW without MW pre-treatment, but with solids removal) because of microwave pre-treatment, therefore becoming more acid. The soluble fraction samples thermally treated at 85°C 7.8 ramp exhibited the highest pH decrease, hence the lowest pH (3.17) from all tested conditions. In contrast, soluble fraction samples irradiated at 175°C and fast heating rate (7.8°C/min) showed the most discreet pH drop (3.95) in relation to the control soluble fraction from all tested conditions. These results are in consonant with the ones obtained for the whole fraction, where the highest pH drop, and hence the lowest pH value from all tested conditions occurred at 85°C and 7.8 ramp.

A possible explanation for the pH drop of the soluble fraction is the increase in intermediate products concentration (TVFAs) after phase separation, as show in Table 6.1

These results contrast with the ones presented by Marin *et al.* (2010) who studied the effects of microwave irradiation on the soluble phase of kitchen waste. The authors tested a final temperature and heating rates similar to the ones here, and observed a less acid pH of the soluble phase (5.2-4.5) in relation to the whole one (5.9-3.9). The less acidic pH of the soluble fraction on their study might have been the result of a lower concentration of TVFAs in relation to the whole fraction. A possible explanation for the difference in TVFAs distribution between whole and liquid fraction here and in Marin *et al.* (2010) could arise from a more intense thermal treatment of the present study, comprised of 5-minute holding time, as opposed to 1 minute in their case, hence promoting a higher solubilisation of organics to the soluble fraction.

Thermally treated samples at 175°C and in a soluble form, exhibited an increase in the TVFAs concentration in relation to the control soluble fraction. Nevertheless, this increment was not linked to the ramp rate used. The highest TVFAs of the soluble fraction occurred at 175°C and medium heating rate (4150mg/L), which represents a 325.20% increase in relation to the control soluble fraction. This finding is in consonant with the pattern for the whole fraction, on which the highest TVFAs also occurred at the medium heating rate (3987.65mg/L) amongst the 175°C final temperature FW samples. These results suggest that medium heating rate and 175°C is the optimum condition for intermediate products generation in both solid and liquid phases.

The lowest TVFAs concentration for the soluble fraction was observed at 85°C 7.8ramp with 2523.61mg/L, representing a 158.56 % increase in relation to the control soluble fraction, and 221.41% increment in relation to the same conditions under the whole fraction.

Table 6.1 Composite Food Waste characteristics before and after microwave irradiation and phase separation (whole and soluble).

	175°C						85°C			
Heating rate	Fast		Medium		Slow				Control (FW without MW pre-treatment)	
Ramp (°C/min)	7.8		3.9		1.9		7.8	7.8	-	-
Fraction (units)	Whole (g/kg)	Soluble (g/L)	Whole (g/kg)	Soluble (g/L)	Whole (g/kg)	Soluble (g/L)	Whole (g/kg)	Soluble (g/L)	Whole (g/kg)	Soluble (g/L)
Parameters										
pH	4.45 (0.09)	3.95 (0.07)	3.98 (0.02)	3.66 (0.03)	4.02 (0.06)	3.86 (0.05)	3.36 (0.01)	3.17 (0.01)	4.20 (0.01)	3.96 (0.01)
SCOD	313.40 (29.60)	365.62 (28.63)	384.60 (25.00)	434.25 (29.57)	395.41 (21.00)	474.85 (29.67)	235.20 (13.67)	283.66 (24.49)	228.30 (21.00)	242.74 (23.00)
TKN	25.63 (1.18)	13.55 (0.41)	11.00 (0.25)	8.50 (0.40)	8.71 (0.15)	6.96 (0.75)	16.45 (0.50)	11.55 (0.30)	16.44 (0.50)	14.56 (0.60)
Ammonium	24.50 (0.78)	11.41 (0.50)	1.00 (0.02)	0.515 (0.07)	1.69 (0.03)	0.575 (0.08)	13.30 (0.35)	9.85 (0.04)	13.36 (0.94)	11.41 (0.07)
TVFAs	3088.16 (113.31)	3278.10 (82.90)	3987.65 (124.41)	4150.00 (97.30)	3286.49 (95.00)	3450.00 (54.05)	2381.00 (64.48)	2523.61 (79.24)	785.49 (3.85)	976.00 (78.54)
VS/TS (%)	83.90 (0.38)	78.22 (0.48)	91.10 (0.45)	80.30 (0.52)	81.00 (0.26)	76.80 (0.45)	81.45 (0.35)	79.30 (0.50)	92.91 (0.55)	88.70 (0.47)
VS	249.62 (2.55)	157.22 (2.30)	262.50 (2.38)	180.43 (2.61)	220.10 (2.00)	168.50 (2.30)	239.25 (2.38)	200.15 (2.20)	296.00 (4.05)	255.54 (2.62)

These results are in consonant with Marin *et al*. (2010) who also reported a TVFAs increase in relation to the control for most of the soluble fraction KW samples, with 60.11% increment for the 175°C 3.9 ramp and 173.44% for the 175°C 1.9 ramp. The exception was 175°C 7.8 ramp, which had 18.38% lower TVFAs concentration than the untreated ones.

The TVFAs results here shown are also in agreement with the ones from Shahriari *et al*. (2013) who reported lower VFAs concentration in the whole fraction compared to the soluble one (1205mg/L less), as well as a higher VFAs (on average 1000-1500 mg/L greater) of MW treated samples than untreated ones.

Figure 6.1 depicts the different VFAs present at the soluble fraction of FW treated at 175°C and different heating rates, as well as for the 85°C 7.8 ramp and controls. Regardless of the pre-treatment condition, acetic acid was the most abundant intermediate product. For the samples treated at 175°C, the increase varied from 6.22-16.10%, with the greatest one at fast heating rate (7.8°C/min).

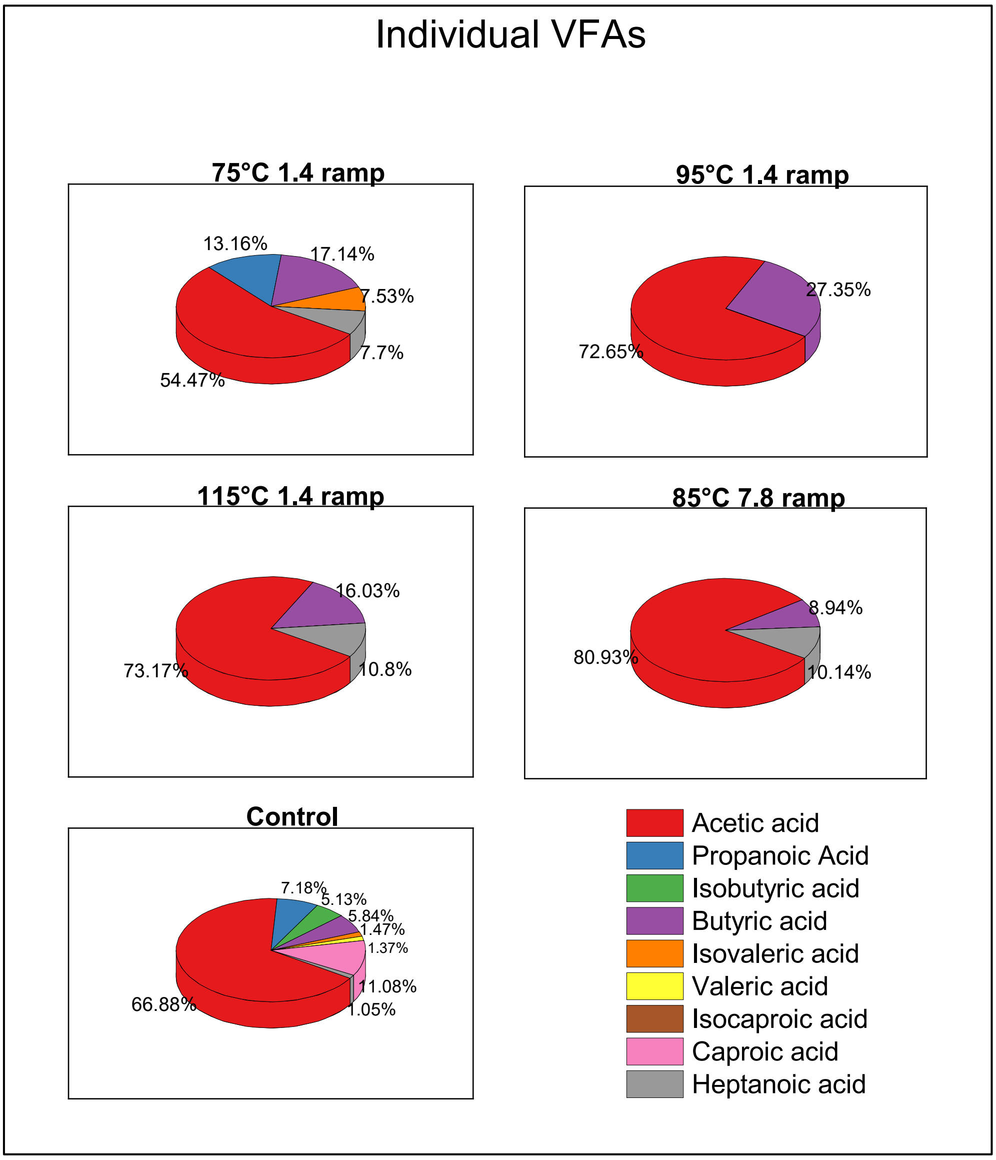


Figure 6.1 Individual VFAs yield after microwave pre-treatment of food waste under different operational conditions at soluble and liquid fraction.

FW treated 85°C 7.8 ramp at the soluble fraction showed a 45% increase on the acetic acid concentration in relation to the solid fraction, representing the most significant increase of this acid amongst the soluble samples.

The untreated samples/controls at both solid and liquid fraction also followed the same pattern as the microwaved samples, with acetic acid prevailing amongst the intermediate products, hence suggesting that the VFAs distribution within the phases is not just a result of the thermal treatment but also suffers influence of the separation method-centrifuging step). In the soluble phase, there was a 20.87% increase in the acetic acid concentration in relation to the solid phase. The increased acetic acid concentration on the soluble phase could potentially favour the production of biomethane, since this intermediate product is the main precursor of this gas (Feng *et al*., 2013).

6.2.1.2 Soluble Chemical Oxygen Demand after MW pre-treatment and phase separation

Regarding the soluble fraction of FW, there was an increase in the SCOD in relation to the control soluble fraction for all studied conditions. Microwave treatment at 175°C, at fast, medium and low heating intensities, resulted in SCOD increase from 242.74 g/kg (untreated sample) to 365.52, 434.25 and 474.85 g/kg, representing a 50.58, 78.89 and 95.61% increase, respectively. On the other hand, samples treated at 85°C 7.8 ramp, showed a 16.85% increase in relation to the soluble fraction control.

Regarding the SCOD behaviour at the two different fractions, it was observed that: at 175°C 7.8 ramp there was a 16.35% increase of the SCOD content in relation to the whole fraction, as opposed to 12.90 and 20.09% of 3.9 and 1.9 heating rates, respectively. These results suggest that longer exposure to microwave irradiation coupled with phase separation leads to a higher soluble organic matter content (Table 6.1).

Under the final temperature of 175°C, the solubilisation degree varied significantly amongst the different rates applied. Nevertheless, all of the soluble fraction samples exhibited an improvement on the amount of soluble organic matter (SCOD/TCOD) in relation to the whole fraction.

Samples pre-treated at a fast-heating rate (7.8 ramp) showed a solubilisation degree of 37.54%, which represents a 44.21% increase in relation to the whole fraction (Figure 6.2). At a medium heating rate (3.9 ramp), there was a 28.79% increment in relation to the whole fraction. On the other hand, at the slow heating rate (1.9 ramp) this increment was more significant at 38.90%. Solubilisation degree increment of the soluble fraction in relation to the whole one was strongly related to the microwave exposure time, with a slow heating rate exhibiting a more pronounced solubilisation degree (70.91%) than the fast-heating rate (37.54%).

Although the solubilisation rate of the soluble fraction presented an amelioration in relation to the whole fraction, the results obtained here were not as improved as the ones from Marin *et al.* (2010). The authors showed 82, 68 and 99% improvement of the solubilization degree at 175°C and heating rates of 7.8, 3.9 and 1.9°C/min, respectively in relation to the whole fraction.

Nevertheless, it can be agreed that the longer exposure to MW irradiation (1.9°C/min) is the optimum condition for enhancing organic matter in the soluble fraction when dealing with FW.

Despite a significant increase in solubilization degree (SCOD concentration) obtained for the soluble fraction 85°C 7.8 ramp in relation to the whole one (339%), the final value of 16.76% is considerably lower than any of the values observed at 175°C under different ramps. Nevertheless, an overall increase of the soluble organic matter of the soluble fraction of FW could be potentially favourable towards enhancing biomethanation compared to the whole fraction.

The COD results of the soluble fraction are in consonance with the ones reported by Shahriari *et al*. (2012). The authors evaluated the effects of microwave (MW) heating (under different temperatures 115-175°C) on municipal solid waste (OFMSW) characteristics. Changes in COD content were observed for both liquid and whole fraction, with an increase in both cases in relation to the control samples. The increment in organic matter content showed a straight relation with temperature increase, with the exception of 175°C whole fraction on which there was an actual decrease in this parameter. On the other hand, for the soluble fraction, the highest increase occurred at 175°C, with 34.76% more COD than the controls, hence corroborating that extreme conditions are more favourable for solubilization of organic matter.

ANOVA and t-student test were performed to evaluate statistical differences between the SCOD content of both soluble and whole fractions. Table 6.2 summarizes the main conditions where a significant statistical difference was found, as well as the SCOD and energy input increment (kWh) for each case.

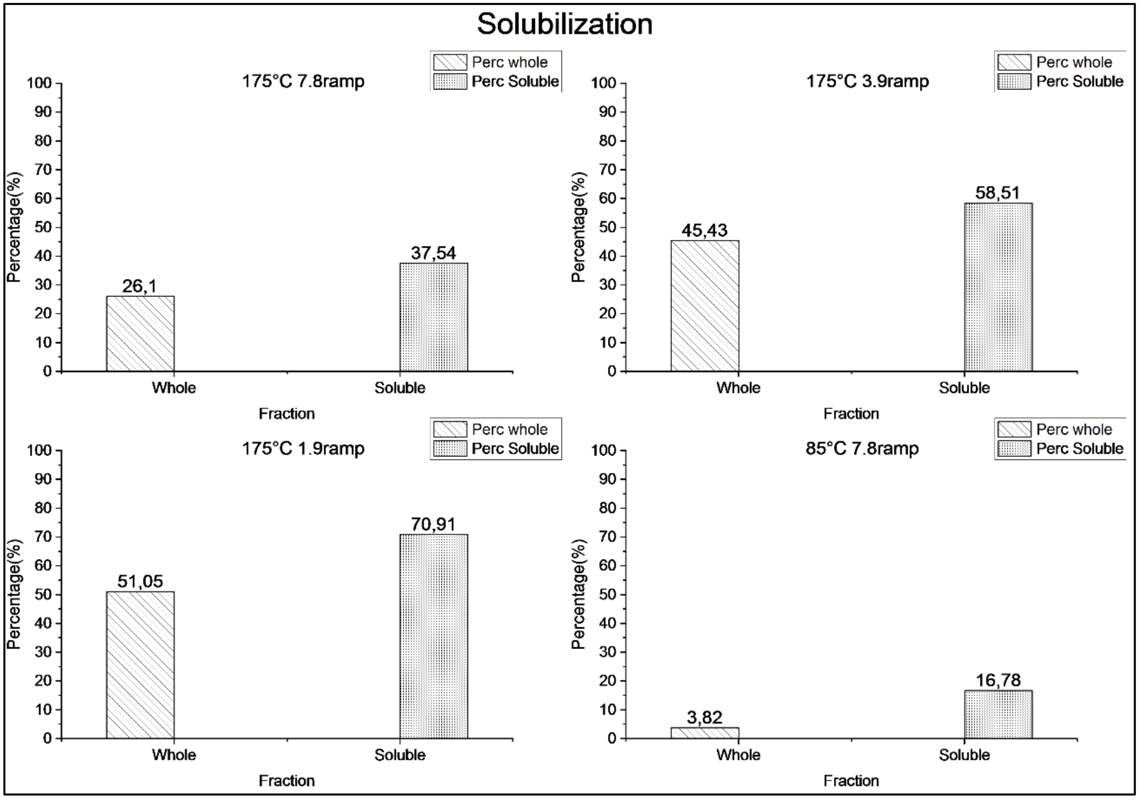


Figure 6.2 Solubilisation of food waste sample after microwave irradiation at different temperatures and heating rates, as well as phase separation (whole and soluble).

The greatest SCOD increase occurred between the 175°C 1.9-ramp soluble 85ºC 7.8 ramp whole samples and between 175°C 3.9 ramp soluble 85ºC 7.8 ramp whole. Both conditions were statistically different. In the former case, the increase in the organic matter content was accompanied by an additional 0.212 energy input and in the latter, by 0.269kWh. Considering the Energy Efficiency (SCOD increase (%) per kWh Increase), the former condition was the best one in terms of solubilization. Nevertheless, the least recommended operation condition in terms of Energy Efficiency for SCOD increase is 85ºC 7.8 ramp soluble, which exhibited a E.E of 0.064 (Table 6.2).

Table 6.2 Anova and t-test for SCOD from whole and soluble fraction of microwaved food waste.

Samples	Statistical Value (*p* value) ANOVA	Statistical Value (p value) t-test	SCOD increase (%)	Energy kWh increase	kWh Increase per gSCOD increase
175°C 3.9 ramp soluble/ 85ºC 7.8 ramp whole	$p = 0.0000$	$p = 0.0001$	80.34	0.269	0.298
175°C 1.9 ramp soluble/ 175°C 7.8 ramp whole	$p = 0.0000$	$p = 0.0000$	50.51	0.212	0.238
175°C 1.9 ramp soluble/ 85ºC 7.8 ramp whole	$p = 0.0000$	$p = 0.0000$	97.20	0.212	0.458
85ºC 7.8 ramp soluble/ 85ºC 7.8 ramp whole	$p = 0.0011$	$p = 0.0167$	17.80	0.278	0.064
Control whole/ 175°C 3.9 ramp whole	$p = 0.0000$	$p = 0.0000$	74.77	0.556	0.134
Control whole/ 175°C 1.9 ramp whole	$p = 0.0000$	$p = 0.0000$	85.39	0.499	0.171

6.2.1.3 Ammonium and TKN after MW pre-treatment and phase separation

Ammonium concentration behaved very similar in the soluble fraction treated at 175°C different ramp rates with most cases showing a decrease in relation to the control soluble fraction. For the fast-heating rate (7.8 ramp) the final value of ammonium was identical to the control soluble fraction (11.41mg/L).

At a medium heating rate (3.9 ramp), there was a significant decrease in relation to the control soluble fraction (95.48%). As previously explained, during the microwave irradiation of FW under the whole fraction there was a volatilization of the ammonium content as from the medium heating rate onwards, hence causing the ammonium values to decrease significantly in relation to the untreated samples as temperature and microwave exposure time rose. Moreover, phase separation (centrifuging and filtering) of the samples played an important role in reducing even further the ammonium content, hence suggesting that this component was closely connected to the solids in the solution. The further reduction of the ammonium content in the soluble phase could be beneficial for the methanogenic community, since methanogenic activity can be inhibited by its high concentrations in the system (Hansen *et al.*, 1998).

At a slow heating rate (1.9 ramp) the decrease of the ammonium content in relation to the control (soluble fraction) was also expressive; 94.96%, due to the reasons previously explained.

FW samples treated at 85°C 7.8 ramp on the other hand, showed a less prominent ammonia drop in relation to the control after phase separation; 13.62% decrease. The reduced decrease in relation to the control compared to samples treated at a higher temperature (175°C) can be attributed to the absence of ammonia volatilization, with the phase separation being the only factor affecting ammonium loss.

These results are almost on its totality in agreement with the ones from Marin *et al*. (2010), who also applied similar pre-treatment conditions as the ones here (175°C at 7.8, 3.9 and 1.9°C/min) when microwaving kitchen waste. However, in their study, the decrease in ammonium concentration for the soluble fraction occurred only of the fast and medium heating rate with a 11.31 and 49.3% reduction respectively.

Regarding the TKN concentration, it was observed a drop in relation to the control soluble fraction for all the conditions tested, with the 175°C 3.9 ramp exhibiting the greatest decay, equivalent to 41.62%.

Contrary, Marin *et al*. (2010), observed a much more discreet drop in the TKN concentration on the fast, medium and slow rate of 6.52, 20.65 and 36.95% in relation to the control soluble fraction.

6.2.1.4 Volatile Solids after MW pre-treatment and phase separation

Regardless of the final temperature and heating rate, a loss of VS content was noticed for all the soluble fraction samples. This finding can be attributed to solids removal by centrifugation and filtration steps. MW FW treated at 175°C and fast heating rate (7.8°C/min) soluble fraction showed the greatest VS loss (38.47%) in relation to the control soluble fraction from all tested conditions.

Sawayama *et al*. (1997) performed a study on thermochemical liquidization of kitchen garbage and also noticed a reduction on the VS content (8.76%) of the filtrate in relation to the solid fraction. Pérez-Elvira *et al*. (2016) found that after the thermal pre-treatment of secondary sludge (170ºC, 50min) there was a significant reduction on the VS content of the soluble fraction in relation to the solid one, with mass balance showing that only 34% of the VS remained in the soluble fraction after separation step.

Marin *et al*. (2010) reported an actual increase of the VS content of the supernatant fraction of kitchen waste by 42.85, 55.84 and 100% for the fast, medium and slow heating rate in relation to the control, respectively. The contrasting findings on solids presented in the literature and herein shows that the waste type/composition as well as the thermal treatment choice are important factors regulating this parameter.

The results found here suggest that the solids removal by filtration and centrifugation step is more accentuated than the one originating from the volatilization process as a consequence of the microwave treatment (highest value of VS loss of 25.64% for the slow heating rate).

6.3 Effect of microwave treatment on MAD of FW using soluble fraction

6.3.1 Total volatile fatty acids profile and behaviour

The TVFAs behaviour for soluble samples treated at 175°C and different heating rates, as well as for 85°C 7.8 ramp and controls are described in Figure 6.3. Acetic Acid was the main VFA on all reactors at the beginning of the digestion period (day 0) regardless of the microwave final temperature and heating rate applied, representing between 59.18 and 70% of the TVFAs. For the soluble fraction control acetic acid concentration corresponded to 75%, as opposed to the whole fraction control on which this acid represented only 47% of the TVFAs. These results suggest that phase separation of microwaved FW with solids removal favours high acetic acid concentration compared to when FW is left untreated and with the solids. The former scenario can be positive influence methane production.

As expected, the greatest TVFAs production occurred between days 2-4 of the digestion for all the soluble phase samples under different ramp rates and final temperature of 175°C. TVFAs concentration incremented by 822.00, 960.12, 640.20% in relation to when the digestion started (day 0) for the fast, medium and slow heating rates, respectively between day 0-2. The maximum intermediate products values registered were of 876.08mg/L, 840.78mg/L and 775.89mg/L for the fast-heating rate, medium and slow heating rate.

These results contrast with the ones obtained for the whole phase, where there was a much higher increment on the TFVAs concentration at 175°C, especially between days 0-4 of the digestion. This was equivalent to: 1090% increase for the fast-heating rate, with maximum value of 956.30mg/L, 943.17% increase for the medium heating rate with maximum value of 1453.04mg/L, and 860% increase with a maximum value of 1488.66 mg/L for the slow heating rate (between day 0-4). The higher TFVAs increment at the beginning of the digestion observed for the FW whole fraction could have been the result of higher concentration of solids at the beginning of the digestion. Moreover, these results suggest that phase separation, despite removing solids did not prevent kinetic unbalance between VFAs production and consumption.

These results are in consonance with the ones from Shahriari *et al*. (2013). The authors evaluated the intermediate products behaviour when anaerobically digesting the free-liquid fraction of kitchen waste after microwave pre-treatment (145°C) under mesophilic conditions and different scenarios: single and dual stage systems and different HRT: 20, 15, 12 and 9 days. The authors concluded that microwaving of the SKW (synthetic kitchen waste) followed by phase separation had no positive impact in terms of avoiding acidification, since TVFAs values were as high as in the controls (which also showed TVFAs accumulation).

For this reason, the liquid fraction originating from thermal treatment of FW under high temperatures (175°C) needs to be considered differently from the untreated samples and applied with restrictions on the digestion process, as to avoid possible TVFAs accumulation on the start-up phase.

85°C 7.8 ramp soluble FW also showed greater TVFAs concentration on the second day of the digestion, with a total of 578.03mg/L (equivalent to 515.64% increase in relation to when the digestion started). Nevertheless, differently from the 175°C there was no kinetic unbalance, with intermediate products accumulation (Figure 6.3).

The soluble fraction controls had a small increase on the TVFAs concentration on the second day of digestion, equivalent to 50.33%, and the maximum TVFAs concentration of 161.96mg/L, hence less prominent than the other conditions. Moreover, there was no TVFAs accumulation after the second day of digestion, contrasting with the control whole fraction (maximum TVFAs concentration of 2010.44mg/L at 4th day of digestion) (Figure 6.3).

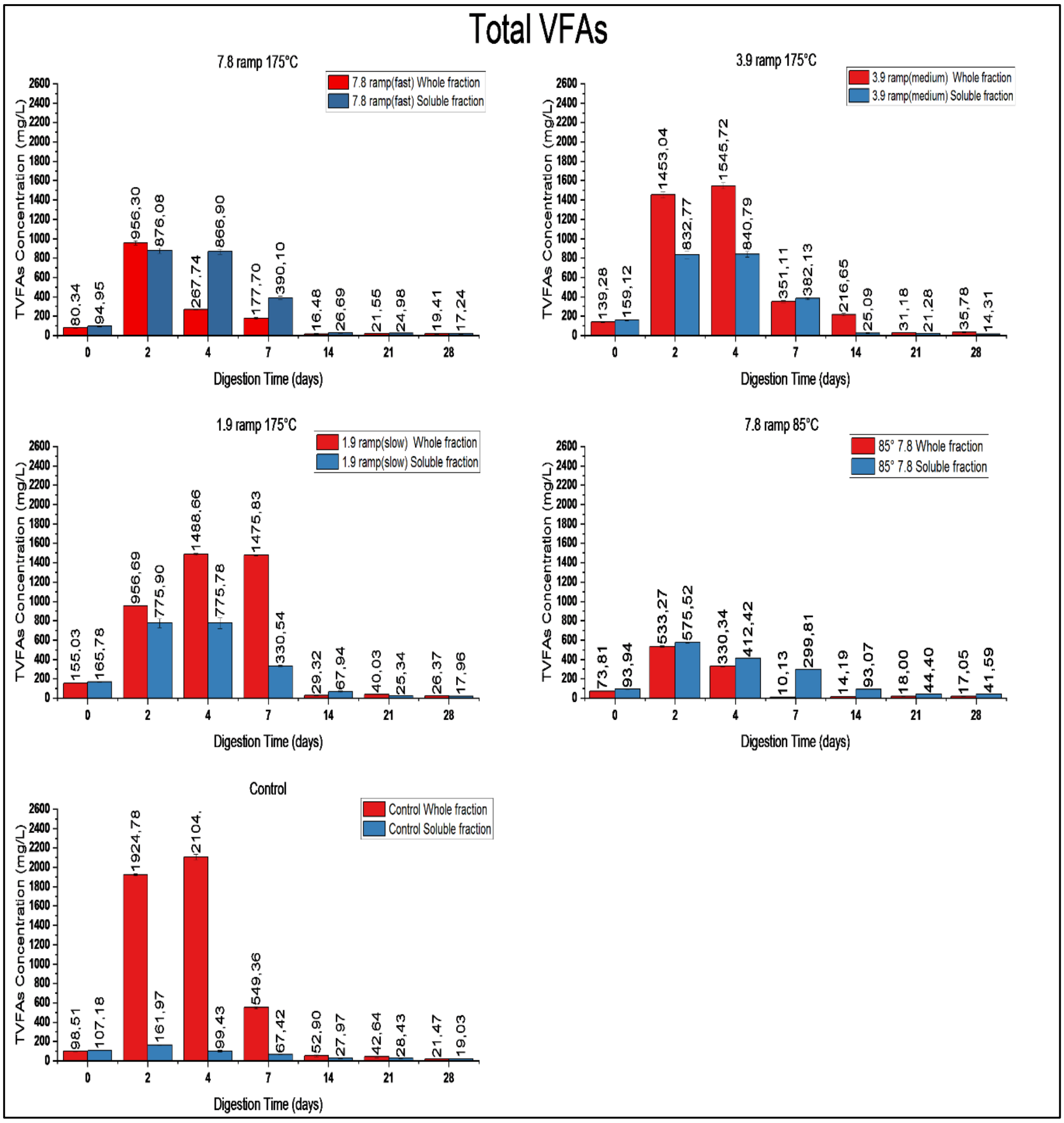


Figure 6.3 Total volatile fatty acids profile during the digestion of soluble fraction and whole fraction of microwaved food waste under various temperatures and ramp rates, as well as controls.

Despite the intermediate product accumulation observed for some of the soluble fraction samples, process failure did not occur, and methane production was not negatively affected. This might have been the result of a less sharp pH drop compared to the whole fraction samples (Figure 6.4).

For the 175°C soluble samples regardless of the heating rate the lowest pH occurred at the fourth day of digestion, with values of 7.61, 7.63 and 7.61 for the fast, medium and slow heating rate, respectively, as opposed to 6.64, 7.00 and 6.80 for the same conditions under the solid fraction. The lower pH of the latter samples could arrive from either: a) the presence of Maillard reaction products- melanoidins. The chemical properties of these refractory organic compounds resemble humic substances, which are acidic, polymeric and dispersed colloids, therefore contributing to the acidity of the system (Dwyer *et al*., 2008) or b) lower solids concentration, leading to lower TVFAs and hence less sharper pH drop.

For the samples treated at 85°C 7.8 ramp soluble fraction, the pH drop on the hydrolysis and acidogenesis phase (between days 0-4) was very small; from 8.64 to 8.32, compared to the whole fraction (8.22 to 6.67), possibly reflecting the higher TVFAs concentration of the latter.

Due to the lack of intermediate products accumulation at the hydrolysis/acidogenesis step, the soluble fraction control exhibited a basic pH (8.18-8.09), contrasting with the whole fraction control which had an acid pH between 6.50-6.84 during this phase.

The MW FW whole fraction samples showed an acidification of the system due to a higher concentration of TVFAs and sharper decrease of the pH, as opposed to the MW FW soluble fraction samples on which, despite TVFAs accumulation for most cases, the pH showed a steadier behaviour. These results are in consonance with the literature as discussed next.

Kawai *et al*. (2014), anaerobically digested kitchen waste under mesophilic conditions, using two different fractions: whole (substrate 1 rich in LOF) and soluble fraction (substrate 2 poor in LOF) as well as distinct S/I ratios: 0.33, 0.5, 1.0, 2.0 and 4.0. The authors observed that during the AD of Substrate 2, mainly propanoic acid was detected and the pH remained at 7.0 to 8.3 at S/I ratios of 0.33 and 0.5, hence suggesting that no acidification occurred. Nevertheless, under higher S/I ratios of 1.0, 2.0 and 4.0, acetic acid and n-butyric acid accumulated and the pH dropped below 6, indicating acidification. Still according to the same authors, under low S/I ratios the total VFA concentrations never exceeded 3g/L when digesting the soluble fraction of FW, as opposed to when the whole fraction of FW was treated, where the concentration of intermediate products significantly exceeded this value. This suggested that the appropriate inoculum quantity necessary to avoid acidification for the soluble fraction is lower than for the whole one. Moreover, the better consumption of TVFAs of substrate 2 was attributed to a small LOF.

FW treated at 175°C under a fast-heating rate and in a soluble fraction exhibited a biomethane production of 59.03ml between day 0-2, which represents 14.05% of the total gas produced during the digestion period. FW samples treated under medium heating rate in turn, had 58.00 ml obtained between day 0-2, representing 14.48% of the total gas produced during the digestion period. For the slow heating rate, 53.53ml of gas was generated between day 0-2, equivalent to 13.09% of the total gas produced during the 28 days of digestion.

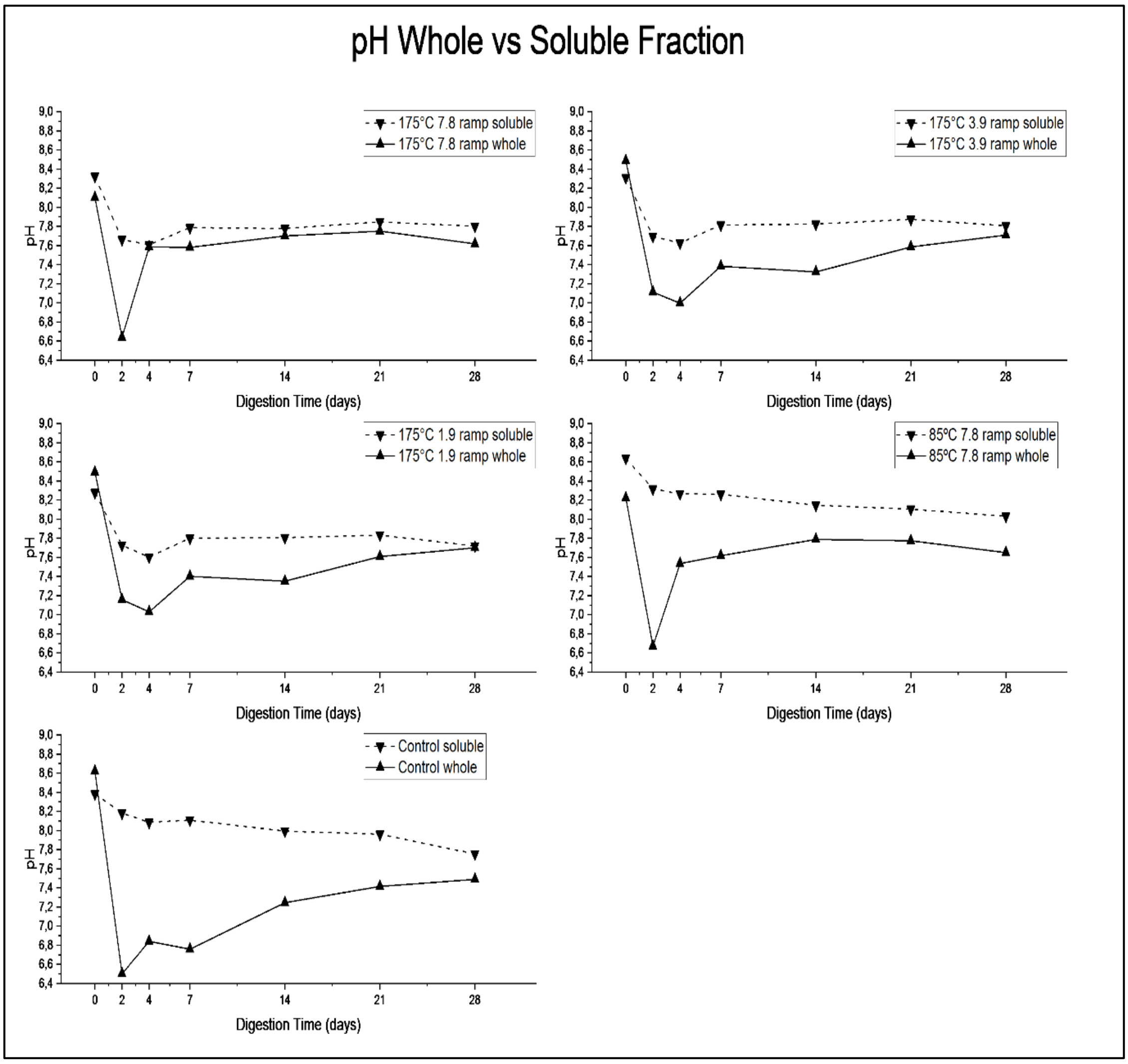


Figure 6.4 pH range during AD of microwaved food waste under different temperatures, ramp rates and phases, as well as controls.

These values contrast with the ones from the whole fraction. Under 175°C and a fast-heating rate, 22.79% of the total gas produced during the digestion period was obtained, hence representing an improvement in relation to the soluble fraction. This finding is most likely related to the improved consumption of TVFAs during this period in the whole fraction, compared to the soluble one. On the other hand, at a medium heating rate, whole fraction, the gas produced between days 0-2 was as low as 5.98%, corresponding to 41% less gas than in the soluble fraction for the same period. A better methane production rate of the latter condition could have been the result of a less pronounced acidification, and or the effective removal of inhibitory compounds after phase separation.

Samples treated at 175°C, slow heating rate showed similar gas production for the soluble (13.09%) and whole (15.55%) fraction between days 0-2. This occurred despite the former having 6.93% higher concentration of TFVAs (especially acetic acid) at the beginning of the digestion (day 0). These results suggest either that: a) inhibitory compounds were not removed together with the solid fraction, b) if inhibitory compounds were removed with the solid fraction; its removal was not as effective as in the medium heating rate.

On the other hand, FW samples treated at 85°C 7.8 ramp exhibited a poor biomethane production during the first two days of digestion, with only 6.19% of the total gas produced during the digestion period, hence representing the worst TVFAs-biomethane conversion rate of all the soluble fraction conditions. In contrast, the whole fraction had 31.53% of the total gas produced for the same period. These highly contrasting results could originate from: a) the higher VS content of the latter in relation to the former, and/or b) the use of inoculum with different ages. For the whole fraction experiments, the seed had been recently collected, as opposed to 3 weeks old in the soluble fraction experiments. Again, these results point to the process performance as being extremely dependant on bacterial activity.

The soluble fraction controls exhibited a very low biomethane production rate for the first two days of digestion with only 4.26% of the total gas generated for the whole digestion period being obtained. Nevertheless, unlike the controls whole fraction where the poor biomethane generation is attributed to the TFVAs accumulation and possible methanogenic inhibition, the soluble fraction controls did not have any kinetic unbalance. Therefore, a more plausible explanation for the low biomethane generation is the lower solid content (16.34% less than in the former) at day 0.

Despite all of the soluble fraction samples at 175°C presenting a TVFA accumulation between day 2-4, after this period there was a continuous consumption of the intermediate products as the digestion progressed, with a further methane generation which varied discretely amongst the different ramp rates tested (Figure 6.5).

At the highest ramp rate, the TVFAs removal between days 4-7 of digestion was equivalent to 55.00%, with acetic acid being the main consumed one (volume reduced by 62.14%) resulting in 172 ml of methane produced, of a total of 400.17ml accumulated since the start of the experiment (Figure 6.5). At a medium heating rate TVFAs removal was equivalent to 54.00%, with acetic acid having its concentration reduced by 63.41%. The volume of methane produced by the microorganisms between days 4-7 in this case was 174.08ml of a total of 400.47ml accumulated since the start of the experiment.

No differently, samples treated at a slow heating rate had a 57.39% TVFAs removal, with acetic acid being the main one transformed into gas (volume reduced by 69.37%) resulting in 171.28ml of methane, of a total of 390.70ml accumulated since the start of the experiment.

Nevertheless, for the 85°C 7.8 ramp soluble samples TVFAs were consumed throughout the digestion process, with no intermediate products accumulation. The TVFAs consumption profile since day 0 was as follows: 28.34, 27.30, 68.95, 52.29% and 6.34% between 2-4, 4-7, 7-14, 14-21 and 21-28th days of digestion. Acetic acid was reduced by 27% between days 4-7, resulting in

84.83ml of methane produced, of a total of 437.98ml accumulated since the start of the experiment.

The soluble fraction control also exhibited a constant TVFAs removal throughout the AD process, as follows: 38.61, 32.19, 58.51, 1.66 and 33.05% between 2-4, 4-7, 7-14, 14-21 and 21-28th days of digestion. Similar to the other soluble fraction conditions, acetic acid was the main one removed from the solution, with 41.89% consumed between day 2-4 of digestion, resulting in 22.60ml of methane produced, of a total of 186.45ml accumulated since the start of the experiment.

In spite of having greater initial concentrations of acetic acid as a result of microwave irradiation and phase separation, the 175°C soluble fraction samples exhibited a lower volume of methane generated between days 4-7. These results suggest that acetic acid concentration was not the main factor affecting methane production during the first week of digestion, but rather volatile solids initial concentration. Therefore, it is important to mention that the better methane production of the whole fraction samples in relation to the soluble ones, does not necessarily mean a better process performance, but instead could be the result of a straight relationship with the initial solids concentration of each condition (Figure 6.5).

On the other hand, it was also observed that samples treated at 175°C medium heating rate soluble fraction, showed improved gas production between days 0-2 compared to the same condition whole fraction, despite the lower VS content. This could be related to the amount of inoculum in the reactor and/or the specific activity of acidogenic and methanogenic bacteria (Kawai *et al*., 2014). Nevertheless, because all the reactors had the same amount of inoculum, it can be postulated that at the medium heating rate, the methanogenic bacteria were able to better assimilate the volatile acids, possibly due to an enhanced removal of inhibitory substances (phenols, melanoidins).

Yin *et al*. (2019) evaluated the influence of melanoidins on the acidogenic fermentation of FW, specifically in the TVFAs production. The melanoidins were obtained by pre-treating the FW with hydrothermal treatment operated at final temperatures of 150, 160 and 170°C, which heralded specific concentrations of 4, 8 and 16g of melanoidins, respectively. According to them, the different melanoidins concentration caused distinct effects over the TVFAs accumulation. Under low and medium melanoidins concentration, TVFAs accumulation was very similar to the control (no addition of melanoidins), hence suggesting a negligible effect towards reducing intermediate products concentration. On the other hand, under higher dosage of melanoidins (16g), there was a decrease in TVFAs concentrations by 11.9% compared to the control, with a positive effect towards avoiding TVFAs accumulation. The authors suggested that the lower VFAs production in the fermentation when high dosages of melanoidins are present could be the result of acetogenic and acidogenic microorganisms growth inhibition due to the chelation of metal ion (Hiramoto *et al*., 2004; Ćosović *et al*., 2010). The adverse effects of melanoidins can arrive from their ability of cross –linking polypeptide chains and sequestering essential multivalent metal cations compounds, which inhibits microbial growth by sequestering ammonia and amino acids (Painter, 1998). Moreover, Yin *et al*. (2019) also explained that the TVFAs loss/reduction can also occur due to substrate consumption which can be translated into the loss of proteins and sugars (two main precursors of TVFAs in FW) due to melanoidins formation, during the Maillard reaction.

Still according to the authors, these results contrast with the ones reported in literature, where it has been shown that because of a chemical similarity of the melanoidins with some specific humic acids, these molecules would be expected to act as an electron acceptor, improving VFAs yield from wastes such as the activated sludge (Arfaioli *et al*., 1997; Liu *et al*., 2015). Regarding the specific VFAs present as a consequence of melanoidins concentration in the fermenter, the authors observed that propanoic and butyric acid content were very similar in the low and medium concentrations of melanoidins, as in the control samples. Nevertheless, under high concentrations of these substances, acetic acid was reduced in concentration. Therefore, the decrease in VFAs production associated with high dosages of melanoidins were mainly related to acetic acid consumption.

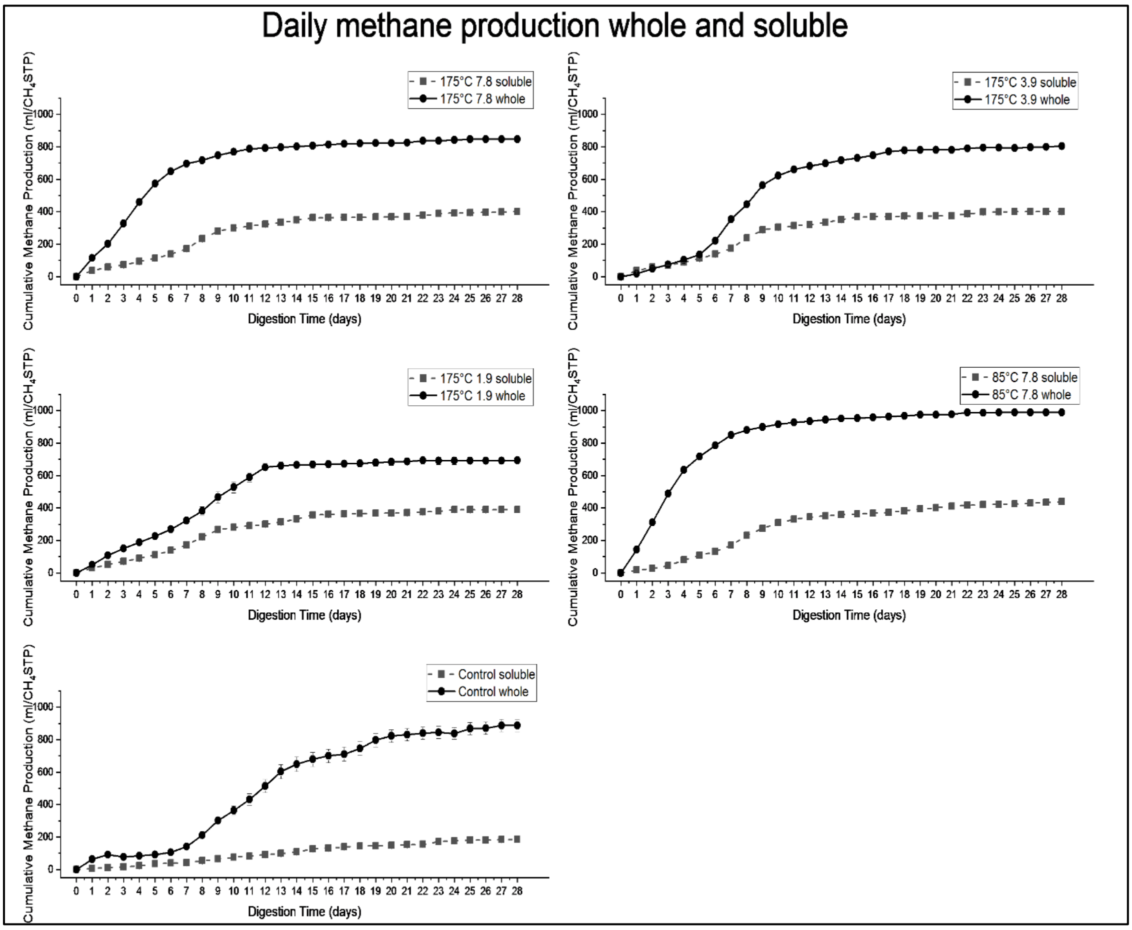


Figure 6.5 Daily methane production of microwaved food waste at different temperatures, heating rates and fractions (whole and soluble).

The TVFAs accumulation observed for the soluble fraction samples at 175°C and different heating rates during the hydrolysis/acidogenesis phase (between days 2-4) was caused mainly by acetic acid accumulation. The minimum value of this acid (628.82 mg/L) was registered at the slow heating rate at the fourth day of digestion and the maximum value (778.94 mg/L) occurred at the second day of digestion of the medium heating rate. These results suggests a similar behaviour as the one reported by Yin *et al*. (2019), where lower acetic acid concentration was associated with higher melanoidins concentration. It corroborates the hypothesis that samples treated at 175°C medium heating rate had a higher removal of inhibitory compounds than the low heating rate ones.

Still regarding process performance, it is important to consider the propanoic to acetic acid ratio (p/a ratio). As discussed in other chapters, this is a relevant indicative of an imminent process failure, and therefore should be strictly monitored throughout the process, with values above 1.4 indicating process instability (Hill *et al*., 1987).

In general, the soluble samples did not exceed the p/a recommended threshold, with values significantly lower than the whole fraction (Figure 6.6).

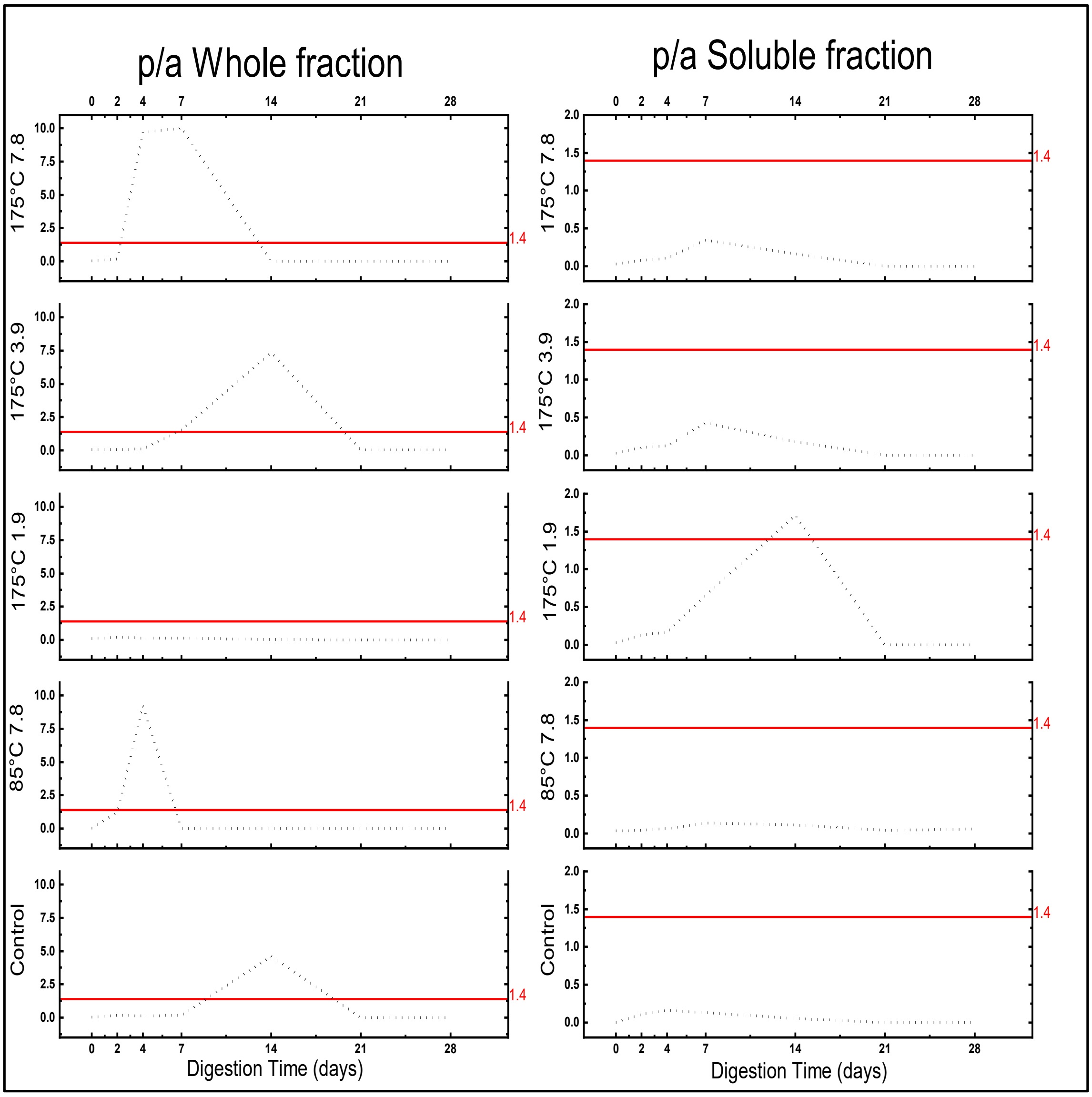


Figure 6.6 p/a ratio of the soluble and whole fraction of microwaved food waste under different temperatures and ramp rates.

The exception occurred when FW samples treated at 175°C and slow heating rate (1.9°C/min) showed an opposite behaviour to the other soluble fraction samples, being the only one with a p/a peak above the recommended threshold (1.71) at day 14 of the digestion. Another contrasting result was that under the same conditions at whole fraction there was no p/a peak throughout the duration of the digestion, hence being an exception amongst the whole fraction samples (Figure 6.6). Regarding the soluble fraction, a balanced consumption of acetic acid coupled with a lower consumption of propanoic acid between days 7-14 of digestion with only 67% of this acid being removed, compared to an average of 97% in the other ramps at a same temperature for the same period, could help explain the exceeding p/a peak.

These results suggest that under this scenario the propanoic syntrophs may have been affected by an intrinsic factor other than pH which was on a basic range as previously described. In contrast, the whole fraction samples of MW FW at 175°C 1.9°C/min showed an acetic acid accumulation between day 4 and 7, bringing the average concentration of this acid to twice as much as the soluble phase. During this period, propanoic acid was removed by 99%, thus resulting in very low p/a ratios.

The absence of TVFAs accumulation for the 85°C 7.8 ramp soluble samples and a lower concentration of TVFAs at the beginning of the digestion (in relation to the whole fraction) for the 175°C samples at different heating rates, helped the maintenance of the basic pH (8.18-8.09). This ultimately contributed to a healthy functioning of propanoic degrading syntrophs (e.g. *Syntrophobacter wolinii)* (Griffin *et al*., 1998), hence avoiding trespassing the recommended p/a ratio threshold, contributing to keeping the process stable.

6.4 VFA to alkalinity ratio during MAD of soluble fraction of MW FW

Figure 6.7 depicts the acidity versus alkalinity behaviour during the mesophilic anaerobic digestion of microwaved FW samples at different ramp rates (fast, medium and slow) and final temperatures (175 and 85°C) soluble fraction, as well as for the control/untreated samples from the whole and soluble fraction.

During the anaerobic digestion of the soluble fraction of microwaved FW treated at 175°C at different heating rates, the values did not exceed the recommended threshold for process stability. For the fast-heating rate, the maximum TVFAs/Alkalinity ratio value was 0.15 at the 4th day of digestion, as opposed to 0.14 at the 4th day of digestion for the medium heating rate and slow heating rates.

Shahriari *et al*. (2013) evaluated the effects of microwave pre-treatment of kitchen waste in continuous reactors (both single and dual-stage), under two fractions (whole and liquid) and different HRT on the rate and extent of KW stabilization. The reactors were denominated as follows: SFt (Single stage system treating liquid fraction of MW KW), DmFt (Dual stage reactors treating liquid fraction of MW KW) DmWt (two-stage system treating microwaved KW whole fraction), DmWnt (Dual stage system treating KW whole fraction without pre-treatment) and DmFnt (Dual stage system treating liquid fraction of KW without pre-treatment).

Differently from the results here shown, the authors reported that for HRTs of 15 and 20 d the TVFAs/Alkalinity ratio was below the established threshold for all the reactors. Nevertheless, at a reduced HRT of 12 days the ratios increased to the equivalent to 0.53, 0.47, 0.55 and 0.63 for reactors SFt, DmWt, DmFnt and DmFt, respectively. Nevertheless, by reducing even further the HRT to 9 days, the ratio results for almost all the totality of the reactors were in an unfavourable range, apart from DmWnt, which showed a ratio below 0.4, and therefore a better performance of the AD process. This result is contrary to the whole fraction untreated samples in this study which had elevated ratios at the first week of digestion.

In contrast to the VFAs/Alkalinity ratio results of the soluble phase of MW FW presented here, the authors showed that DmFt (reactors treating soluble fraction of MW KW) failed at an HRT of 9 d and had a VFA/alkalinity ratio of 0.94. The authors attributed this finding to the microbial consortia in the two-step reactor not being in equilibrium when treating the free liquid which resulted in VFA accumulation in the second reactor with concomitant reduced methane production.

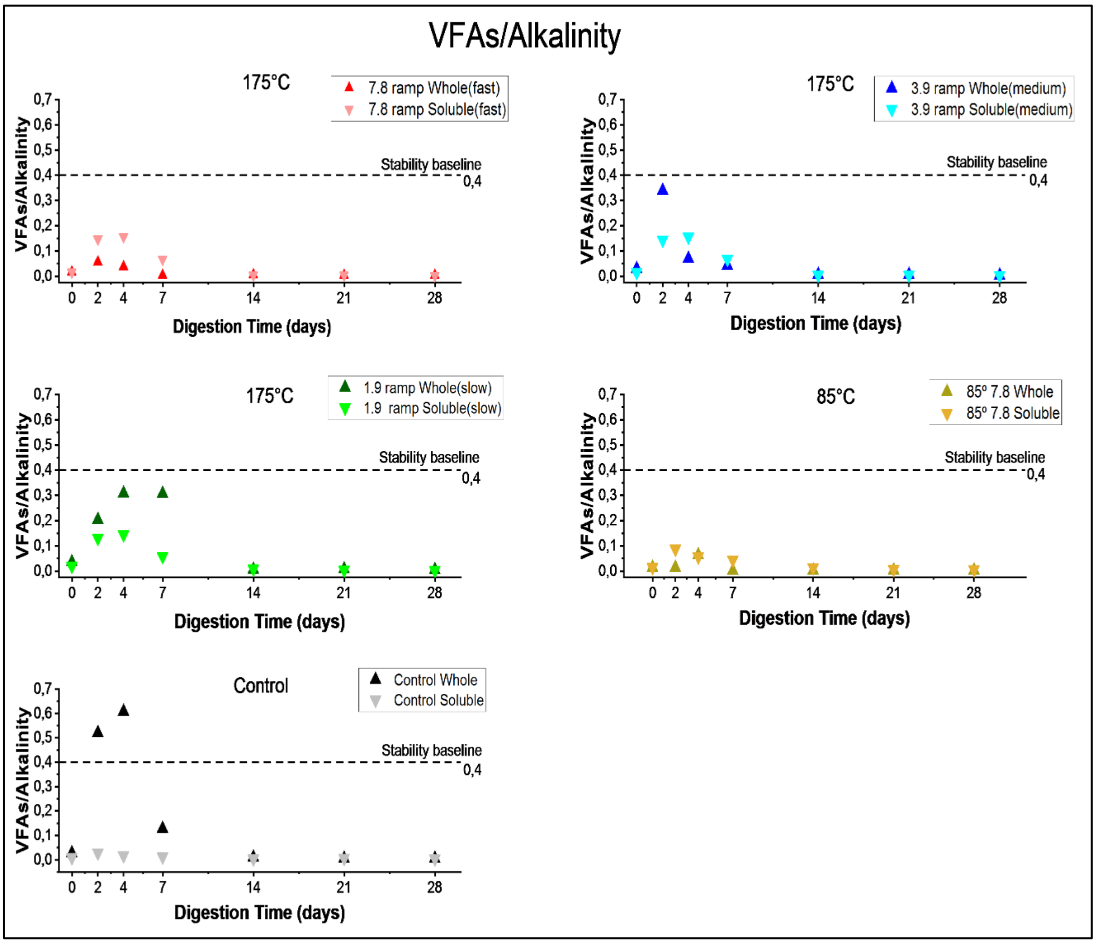


Figure 6.7 VFAs/Alkalinity ratio of microwaved food waste samples at different heating rate and different temperatures under soluble and whole fraction.

Soluble FW samples microwaved at 85°C 7.8 ramp had TVFAs/Alkalinity ratio significantly below the recommended threshold of 0.4 during the AD. Moreover, under this condition, the lowest acidity/alkalinity ratios amongst all of the soluble fraction samples were obtained, with a maximum value of 0.08 at the second day of the AD process. This finding is most likely related to a lower concentration of TVFAs in relation to 175°C soluble samples, coupled with a higher alkalinity (on average 16% higher). The increased alkalinity, could have originated from the presence of certain intermediate products such as propanoic, butyric, iso-butyric and valeric acids after the 4th day of digestion, as opposed to the 175°C soluble samples on which most of these acids were completely metabolized becoming absent after the 7th day of digestion.

The aforementioned acids have a relatively low pKa, signifying that they are relatively strong acids requiring higher concentrations of buffering agent/alkalinity to neutralize the dissociated H^+ (Table 6.3) (Sun *et al*., 2015). In this context, the longer presence of propanoic, butyric, iso-butyric and valeric acids in the reactors of the 85°C 7.8 ramp soluble phase could help explain the higher alkalinity concentration and therefore, the lower TVFAs/Alkalinity ratios (Figure 6.8).

Table 6.3 pKa values of the main compounds in anaerobic digester. Adapted from Sun *et al*. (2015).

ACID	DISSOCIATION AND EQUILIBRIUM EQUATIONS	PKa
Acetic	CH3COOH > CH3COO– + H+	4.75
Propanoic	C2H5COOH > C2H5COO– + H+	4.87
Butyric	C3H7COOH > C3H7COO– + H	4.82
Iso-Butyric	(CH3)2CHCOOH > (CH3)2CHCOO– + H+	4.84
Valeric	C4H9COOH > C4H9COO– + H+	4.82
Iso-Valeric	(CH3)2C2H3COOH > (CH3)2C2H3COO– + H+	4.77

The soluble phase controls also showed a very low TVFAs/Alkalinity ratio, with values significantly below the recommended threshold. The maximum value obtained was of 0.028 at the second day of digestion, contrasting with the whole fraction control which had trespassing values (0.50 and 0.60) due to a poor process performance related to the accumulation of TVFAs at the hydrolysis and acidogenesis phase.

Overall, the AD of microwaved FW under both soluble and liquid fractions showed good process stability with values never exceeding the recommended literature threshold, with the exception of the whole fraction control. The soluble fraction samples had lower TVFAs/Alkalinity ratios values than the whole ones, indicating that there could be an improvement of the process performance once solids are removed from the solution.

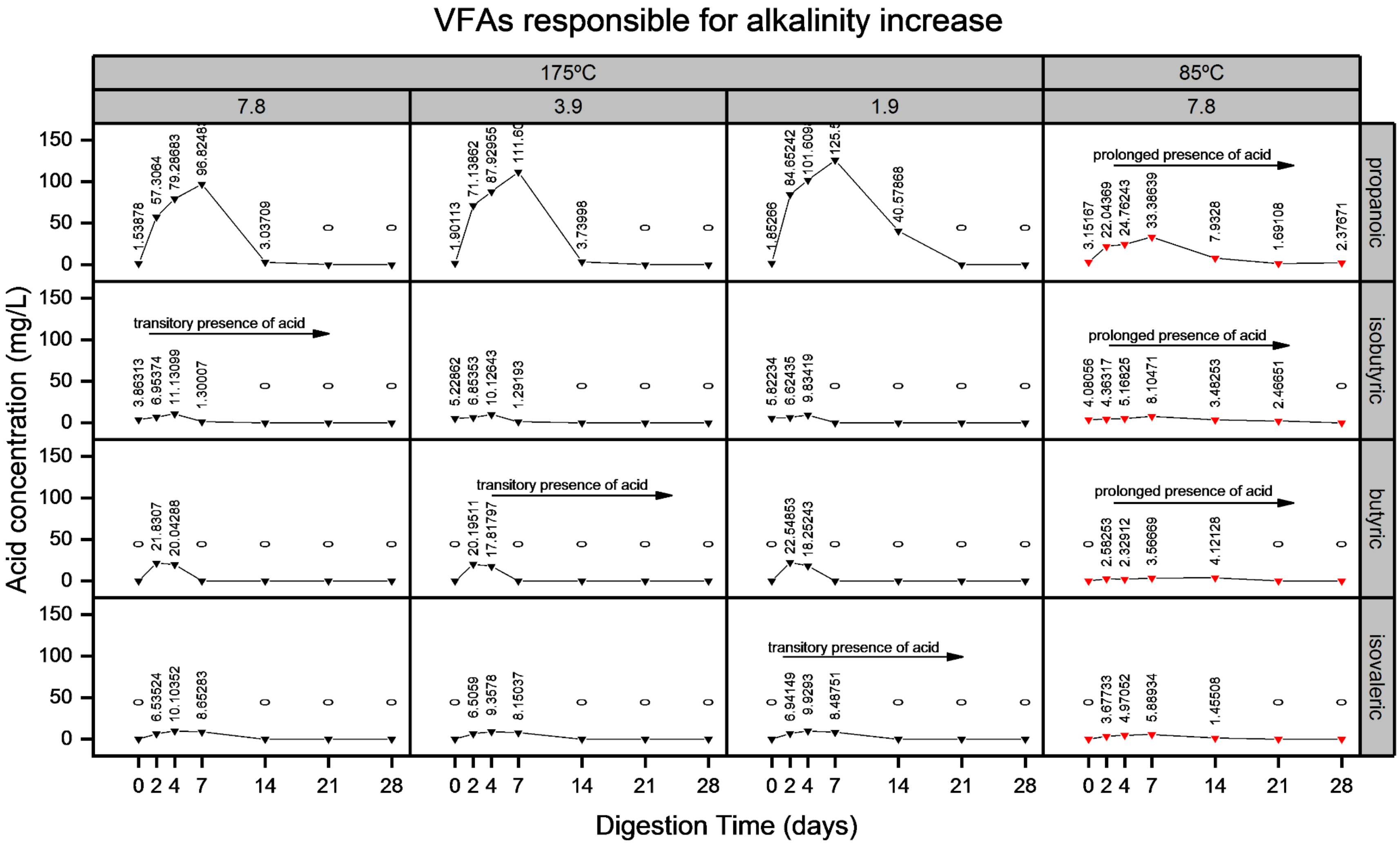


Figure 6.8 Influence of VFAs on the alkalinity of food waste samples treated at different temperatures and heating rates at a soluble fraction form.

6.5 Ammonium behaviour during MAD of soluble fraction of MW FW

Despite the abundance of information on the ideal concentration of ammonia, ammonium and other forms of nitrogen in the reactor to maintain a healthy functioning of the bacterial population, especially the methanogenic one (Wang *et al*., 2009; Zhang *et al*., 2014) the fate of the ammonium ion during the process of AD is not usually discussed in the literature. Herein, the ammonium behaviour during the AD of soluble and whole fraction of microwaved FW is described in Figure 6.9. This information is useful as a baseline for future AD plants wishing to decide on which fraction of the treated waste to apply in order to optimize the process.

The highest ammonium peak was observed for the whole fraction samples treated at 175°C fast heating rate (7.8°C/min) at day 4 (represented by a 62.76% increase in relation to the beginning of digestion -day 0) (Figure 6.9). This increase does not represent a disturbance factor for the system, since the final value (3000mg/L) was below the prejudicial range for process performance (4051–5734 mg/L) (Koster and Lettinga ,1988). Moreover, the significant increase in ammonium content, coincided with a concomitant increase in pH, suggesting that ammonia could have acted as a buffer agent. Alternatively, the higher peak could have been the result of bacterial activity towards protein breakage during the digestion process.

Conversely, MW FW soluble fraction samples treated at 175°C fast heating rate did not show an initial peak on the ammonium concentration as a result of the typical break down of proteins and release of ammonium to the solution during the hydrolysis step (4th day of digestion), with lower ammonium values than the whole fraction throughout the digestion period. A possible explanation for lower ammonium values of the soluble phase could be related to solids removal, and hence, ammonium reduction, as previously explained. On the other hand, it also suggests that the soluble fraction of MW FW applied to AD, avoids peaks, and delays in the metabolization of nitrogen by bacteria, most likely due to the nitrogen forms being already solubilized.

On the other hand, both soluble and whole fraction samples treated at 175°C medium heating rate (3.9°C/min) showed a small (8.25%) ammonium release during the first four days of digestion (hydrolysis step) not forming any significant peaks, so did the slow heating rate. This could reflect the lower ammonium content of these conditions, or/ and be the result of harsher pre-treatment MW conditions, known for affecting protein metabolization as suggested by Yin *et al*. (2015).

The authors performed hydrothermal carbonization pre-treatment on FW under different final temperatures: 100-220°C for 30min without addition of chemicals. The authors observed that ammonium concentration linearly increased with fermentation time after 4 days. However, later in the digestion (day 15) the release of this ion differed greatly amongst the final temperatures used. At 140, 160°C, and the control all showed ammonium release above 50 mg/L per day. On the other hand, 180 and 200°C had only 35.61 and 20.48mg/L per day, respectively. This further indicated that the microbial activity was negatively affected at higher temperatures possibly due to the presence of inhibitory compounds, causing an unbalance between dissolution and consumption of proteins (Wang *et al.*, 2014).

FW samples treated at 85°C 7.8 ramp soluble fraction showed an increase on ammonium's concentration in relation to day 0, on days 4 and 7 of digestion, equivalent to 6.58% and 5.86 respectively, possibly indicating protein breakage. Conversely, the whole fraction showed a more pronounced peak of ammonium between days 4-7 of digestion, with a 29.34% increase, equivalent to the second most prominent peak of ammonia of all the whole fraction samples. The higher ammonium peak in relation to the 175°C samples (medium and slow heating rates) could be the result of a higher concentration of ammonium in the reactors, which differently from the 175°C (medium and slow heating rates) samples did not suffer expressive ammonium loss during MW treatment (Section 6.2.1.3). Additionally, less harsher pre-treatments conditions could have promoted lower concentration of inhibitory compounds or even their complete absence, favouring a balanced dissolution and consumption of proteins .

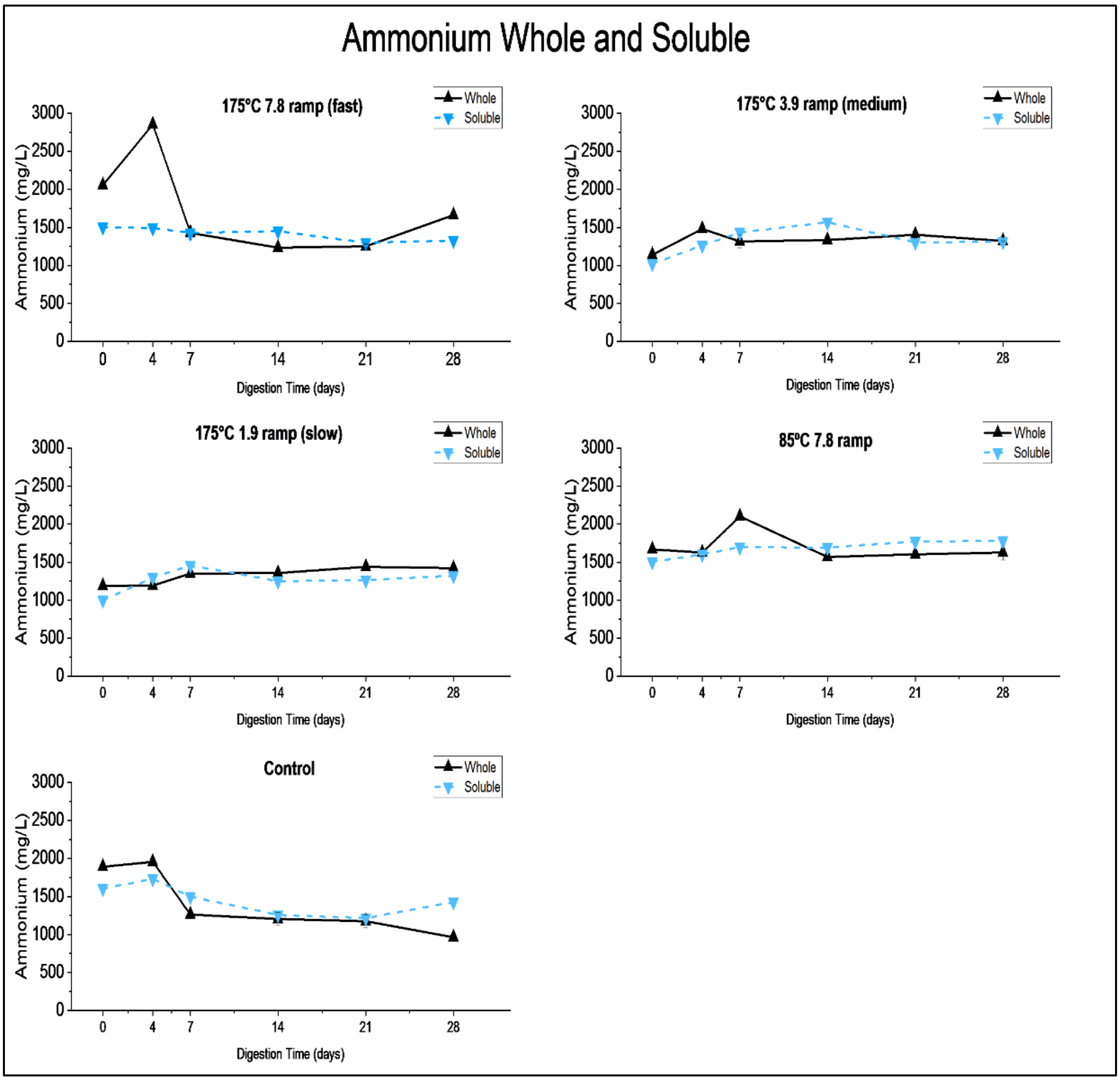


Figure 6.9 Ammonium behaviour during AD of MW FW under different temperatures and ramp rates.

It can be postulated that if the AD optimum conditions were to be chosen based solely on the ammonium release behaviour during the process, 175°C fast heating rate (7.8°C/min) whole fraction would be the ideal one. This is because under this condition, the initial ammonium peak suggest either good protein breakage by microorganisms (without ammonium reaching inhibitory levels), and good buffer capacity of the system. Both are important for a stable AD environment.

6.6 Volatile solids removal and methane production during MAD of soluble fraction of MW FW

The total and volatile solids in the soluble and whole fraction were plotted against the methane production. After phase separation, the solids in the soluble fraction were not adjusted, and for this reason, its concentration varied amongst the different samples.

For the soluble fraction MW FW samples treated at 175°C 7.8 ramp, the consumption of TS and VS for the first four days of digestion was equivalent to 18.36 and 2.15% respectively, against 22 and 9.88% TS and VS of the whole fraction. The difference in solids content and consumption rate ultimately resulted in distinct volume of methane obtained for this period. There was a significant methane volume obtained during the first day of digestion for the whole fraction heralding 115 ml of gas, as opposed to 37ml of the soluble fraction (Figure 6.10). The second most prominent peak in methane production of the whole fraction occurred at the fourth day of AD, with 132ml of gas generated after a further reduction on the solids content equivalent to 9.00% and 6.82% on the TS and VS, respectively.

For the soluble fraction, the second peak occurred at the 8th day of digestion, after a further volatile solid reduction of 9.94%, producing a total of 61.87ml of methane. Again, this peak was not as high as in the whole fraction, and does not necessarily indicate a poorer processes performance, but can rather reflect the difference in initial solids concentration of both fractions.

For the soluble fraction MW FW samples treated at 175°C medium heating rate, the TS and VS consumption during the first four days of digestion were equivalent to 5.69 and 2.50%, as opposed to 13.69 and 18.05% of the whole fraction. Despite the higher reduction in solids for the whole fraction, the methane produced in the first day of digestion (17ml) was lower than in the soluble fraction (37ml) (Figure 6.10). This finding could be the result of a combination of factors, such as: a) the VS content was being consumed to bacteria growth instead of methane production, as well as b) the inoculum's methanogenic activity of the former condition was negatively affected by its age (Figure 6.10).The second most prominent peak in methane gas for the soluble fraction occurred on the 7th day of digestion, with a total of 64.50ml produced, after a further VS removal of 13.04% between days 4-7, hence representing an improvement in relation to the 175°C 7.8 °C/min soluble fraction samples.

For the soluble fraction MW FW samples treated at 175°C 1.9 ramp, the consumption of TS and VS for the first four days of digestion was very similar for both fractions: 21.20 and 12.14.% TS and VS of the soluble compared to 20.30 and 12.62% TS and VS of the whole fraction. Notwithstanding, the methane generation for each fraction differed, with 32.53 and 50.28ml of gas obtained for the first day of digestion for the soluble and whole fraction, respectively. These results confirm that the difference in initial solids concentration and solids removal rate were the main factor influencing methane production, especially in the first days of digestion (Figure 6.10).

Whole and soluble fraction samples of FW treated at 85°C and 7.8 ramp showed a different consumption of TS and VS for the first four days of digestion. For the former 16.44 and 27.12% of TS and VS were removed, as opposed to 13.22 and 18.01% for the latter. The VS removal for the whole fraction for this period was considered the greatest of the whole fraction conditions tested. Not surprisingly, the volume of methane produced for the whole fraction was 143.31ml as opposed to only 17.94ml for the soluble one (Figure 6.10).

The significant methane production for the whole fraction MW FW treated at 85°C and 7.8 ramp on the first day of digestion was followed by a further high methane generation for three consecutive days, as opposed to the whole fraction same temperature, on which there was a decay in methane production after the 4th day. The soluble fraction, had an increase in methane production between the 4 and 7th day of AD, with 61ml obtained after a 9.24% removal of the VS content. The period corresponding to 9-13th day of digestion the volume of methane generated on the soluble fraction was an average of 30% higher than in the whole one, as a result of a further 4.5% reduction of the VS content, compared to an actual increase of the VS and TS content of the whole fraction for this period.

The control samples had the most contrasting solids removal results between the two fractions of FW. The TS and VS behaviour for the first four days of digestion was very different for both fractions: 14.04% TS and 10.51% VS reduction in the whole fraction, as opposed to an increase of 1.44% and 2.10% on TS and VS of the soluble fraction. This major difference in solids consumption led to significant discrepancy in methane production between the two controls. During the first day of digestion the methane production was very low for the soluble fraction with less than 10ml of methane generated, as opposed to 62.20ml from the whole fraction (Figure 6.10).

Nevertheless, after the third day of AD, the methane production in the soluble fraction started to increase significantly, surpassing the values of the whole fraction for a period of 8 consecutive days. The percentage of extra methane produced for the soluble fraction during this period was on average 85% higher than for the whole fraction. The peak on methane production occurred at day 10 of digestion, with 115.3 ml of gas. The higher gas production was most likely related to a higher volatile solids removal between days 4-7, corresponding to 13.78% as opposed to 6.67 from the whole fraction. Moreover, other factors previously shown and discussed can have possibly positively contributed for the enhanced methane generation of the soluble fraction, such as the solubilized organic matter in relation to the whole fraction, as well as the absence of TVFAs accumulation and enhanced acidification of the system, which caused methanogenic inhibition. The improved solids removal of the soluble fraction control in relation to the whole fraction control, ultimately lead to a higher methane yield, as discussed next.

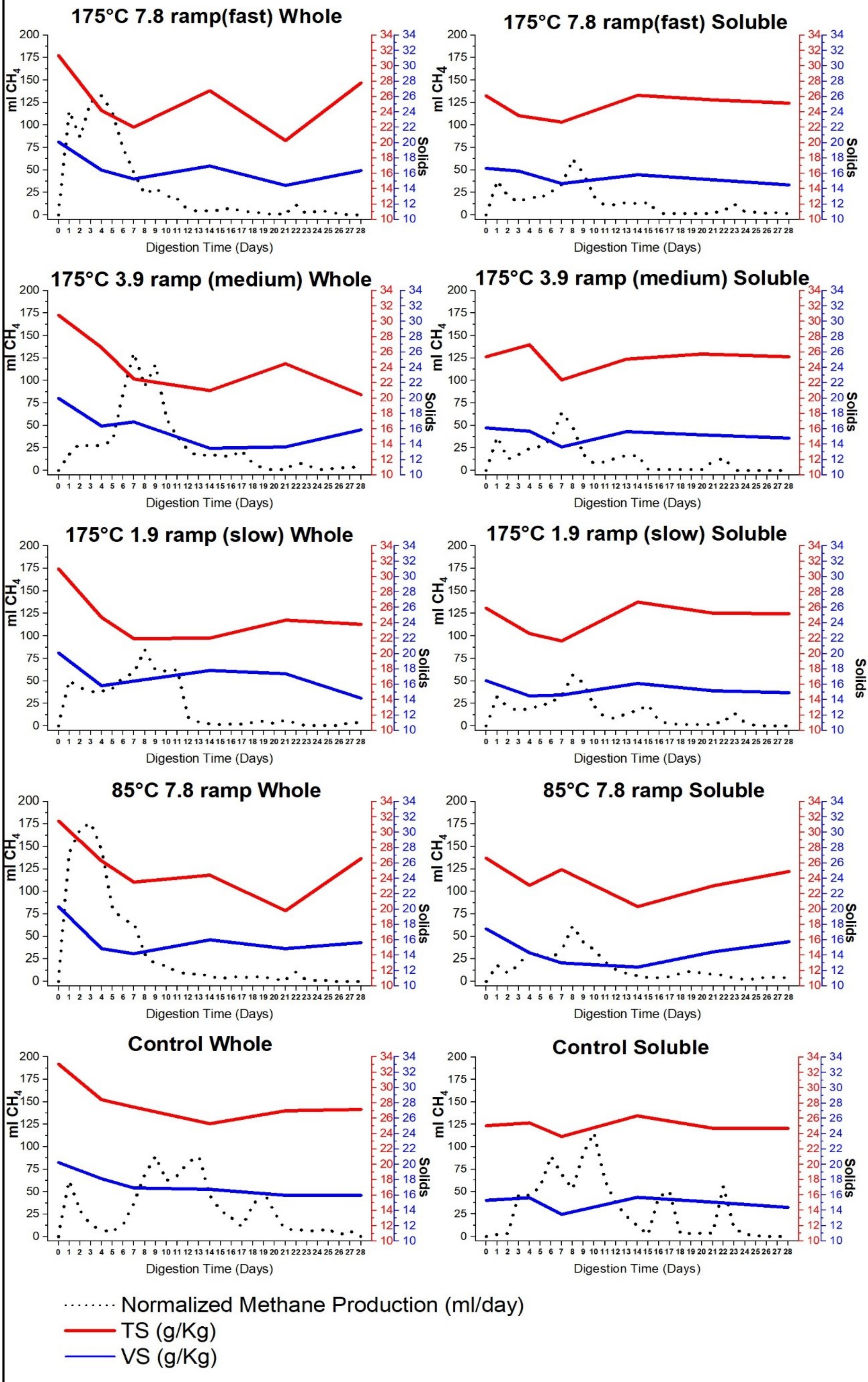


Figure 6.10 Soluble removal vs. Methane production during AD of Soluble and Whole fraction of FW.

Because no inhibitory compounds (melanoidins, humic acids, phenols, etc) measurements were performed in this study, it is not possible to confirm their presence, hence the efficiency (%) of the phase separation on their removal. However, by comparing the process performance (methane production on the first day of digestion and the highest volume of gas produced during AD of the soluble fraction samples treated at a higher temperature (175°C) and different heating rates with the whole fraction same conditions, it is possible to have better indicatives of possible success of inhibitory compounds removal.

The results presented in Table 6.4 are a summary of the AD process performance of the different soluble fraction samples (175°C), including initial solids concentration, volume of methane produced on the first day of digestion and the volume of methane produced at the highest peak, expressed as means. The volume of methane produced at the beginning of the digestion and the one produced at the highest peak, should be related to the amount of solids in the reactor, bacterial activity and concentration of inhibitory compounds in the solution.

Several t- student tests were performed for both volume of methane produced during the first day of digestion and for the volume of methane produced on the highest peak to determine statistical differences amongst the various samples. Regarding the volume of methane produced for the first day of digestion, the t-test showed that there was a statistical difference between most of the samples tested; 175°C 7.8 ramp vs. 175 °C 1.9 ramp ($p = 0.0031$), 175°C 3.9 ramp vs. 175°C 1.9 ramp ($p = 0.0083$), with the exception of 175°C 7.8 ramp and 175°C 3.9 ramp ($p = 0.58$) (Appendix E).

These results suggests that despite having a lower initial solids concentration in relation to the other samples, the amount of gas produced by the 175°C medium heating rate samples (3.9°C/min) in the first day of AD was significantly and statistically higher than in slow heating (1.9°C/min) rate and yet very similar to the one produced by the fast-heating rate (7.8°C/min) (Table 6.4). Therefore, it can be postulated that phase separation was effective in removing inhibitory compounds for the medium heating rate.

Similar to the volume of methane produced in the first day of digestion, it was postulated that the ramp rate with a higher concentration of solids and/ or with a successful removal of inhibitory compounds (believed to be in the solid fraction), would have the highest methane peak during the first days of digestion. In this case, the 175°C fast heating rate should be the condition heralding the highest methane peak, since the initial solids concentration was on average 21% greater than the other heating rates (Table 6.4).

Table 6.4 AD process performance parameters utilized to stablish possible process improvement regarding inhibitory compounds removal.

Sample's Name	VS content (%) in relation to the whole fraction	Volume of methane (ml) day 1 of digestion soluble fraction	Peak of methane (ml) Soluble fraction
175°C 7.8 ramp sol.	16 less	37.78	61.87
175°C 3.9 ramp sol.	19.45 less	37.30	64.50
175°C 1.9 ramp sol.	17.94 less	32.53	56.87

The t-student test for the peak of methane showed a significant statistical different between the fast-heating rate and medium heating rate (*p* = 0.02), corroborating the hypothesis of the effective removal of inhibitory compounds in the medium heating rate. Further specific tests would need to be performed in order to confirm the hypothesis here postulated and the effectiveness of inhibitory compounds removal for the soluble fraction samples (Appendix F).

6.7 Methane yield and kinetic assessment during MAD of soluble fraction of MW FW

Soluble samples treated at a final temperature of 175°C and different heating 7.8, 3.9 and 1.9°C/min) rates showed a similar lag phase (λ), equivalent to 1.85 day for the fast (7.8°C/min) and medium (3.9°C/min) heating rate and 1.91day for the slow (1.9°C/min) heating rate respectively. In general, these values represent an increase of the lag phase duration, and consequently, hydrolysis step in relation to the whole fraction. For the fast-heating rate, the soluble fraction had a 1,441% increment on the hydrolysis step duration in relation to the whole fraction.

Nevertheless, samples treated at 175°C at a medium (3.9°C/min) heating rate there was an actual shortening of the lag phase in relation to the whole fraction equivalent to 54% less time need to complete this step. In fact, of all the soluble fraction conditions tested, this was the only one which heralded an improvement in the lag phase in relation to the whole fraction.

For the samples exposed to 175°C slow heating rate (1.9°C/min), there was an increase in the lag phase duration in relation to the whole fraction same condition, representing 29.93% more time needed for the hydrolysis step to be completed.

Similarly, samples treated at 85°C 7.8 ramp soluble fraction showed an increment on the duration of the lag phase and hydrolysis in relation to same temperature under the whole fraction. Nevertheless, under this operational condition the increase was significantly higher than the aforementioned ones, equivalent to 28 times longer (Table 6.5).

Regardless of the final temperature or heating rate used, the soluble fraction of microwaved FW showed an improvement of the lag phase, in relation to control soluble fraction. The greatest improvement occurred for FW treated at 175°C fast and medium heating rate (3.9°C/min), with the equivalent to 58% and 26% less time spent on the lag phase respectively. Although very small, the soluble fraction control showed a deterioration of the lag phase in relation to the whole fraction, with a 5% increase on the time spent on the hydrolysis step.

The delayed hydrolysis step and lag phase of the soluble fraction is a rather contradictory finding. This is because the soluble form of the organic matter in this fraction, which is expected to me metabolized faster by the microorganisms. Nevertheless, the poor initial process performance of the soluble fraction in relation to the whole one could have been the result of several factors. The most important and critical one is the TVFAs accumulation observed for all the soluble fraction samples despite solids removal. Although the concentration of the intermediate products was smaller than in the whole one (Section 6.3) it was sufficient to cause a significant delay on the hydrolysis phase.

On the other hand, the improved lag phase of the 175°C medium heating rate soluble fraction in relation to the whole one could have been the result of the effective removal of inhibitory compounds.

Shahriari *et al*. (2011) studied the effect of high temperature and pressure microwave (MW) irradiation on the AD of OFMSW. Different temperatures (175, 145 and 115 ºC), MW intensities (20, 40 and 60 minutes) and supplemental water additions (SWA) of 20% and 30% were evaluated. As opposed to the values reported here, the lag phase values of whole and liquid fraction of OFMSW were similar, in the range of 0.25-0.30 day for SWA20 and SWA30. They concluded that 145 ºC is the optimum condition for MW pre-treatment of the OFMSW, attributed to a shorter lag phase and better methane yield.

The maximum methane yields obtained from the MGompertz model (A) for temperatures of 175°C at different heating rate were similar to the cumulative methane yield obtained experimentally (B). For the fast-heating rate, A/B was equivalent to 85.41%. On the other hand, for the medium heating rate, A/B corresponded to 97.17%, thus suggesting a good fitting. The slow heating rate showed A/B value of 86.54% (Table 6.5). These results represent a retrogress in relation to the fitting of the samples under the same temperature and heating rates at a whole fraction, which in turn had values of A/B of approximately 98%. The poorest fitting of the soluble fraction could reflect the state of the process at the beginning of digestion characterized by a slower lag phase and hydrolysis rate (Figure 6.11).

On the other hand, the reactors containing FW samples treated at 85°C 7.8 ramp had similar A/B values for both soluble and whole fraction, at an average of 97.13%. The soluble fraction control in turn, showed an A/B value of 92.29%, as opposed to the whole fraction control which had a theoretical methane yield higher than the experimental methane yield. This later finding could have been a consequence of the poor process performance associated with an accentuated acidification of the system as a consequence of significant TVFAs accumulation, at the start-up phase of the digestion.

The control soluble fraction heralded the greatest final methane yield of all soluble fraction samples, corresponding to a total of 326.00ml CH_4/gVSadded. The second highest methane yield occurred for the 85°C 7.8 ramp sample, equivalent to 294.74mlCH_4/gVSadded. Amongst the high temperature (175°C) samples, the ones treated at a medium heating rate (3.9ramp) showed the highest methane yield, representing a 16 and 5% increase in relation to the fast and slow heating rate, respectively. These results corroborate the hypothesis of efficient inhibitory compounds removal, most likely associated with the solids in the solution.

Table 6.5 Kinetic assessment of the mesophilic anaerobic digestion of the microwaved Food Waste soluble fraction at different temperatures and heating rates and controls.

		Predicted methane yield (ml) (A)	sdt	Experimental yield Specific methane yield (ml) (B)	std	λ (lag phase)	Statistics	
Temperature	Heating rate	Value	Std Error	Value	Std Error		Reduced Chi-Sqr	Adj R-Square
175°C	Fast (7.8°C/min)	186.78	2.45	218.67	0.88	1.85	56.17	0.98
	Medium (3.9°C/min)	246.82	3.37	254.00	0.98	1.85	103.48	0.98
	Slow (1.9°C/min)	209.37	2.44	241.83	0.97	1.91	50.64	0.99
85°C 7.8 ramp		286.30	2.78	294.74	1.05	3.46	92.95	0.99
Controls	Whole	181.17	3.22	177.92	0.01	4.47	35.42	0.99
	Soluble	320.87	3.63	326.45	2.45	4.72	56.49	0.99

The highest improvement of the soluble fraction in relation to the whole one occurred for the control, with 83% increment on the methane yield. The second most prominent increase happened at 175°C slow heating rate (1.9°C/min), with a 74% in relation to the same condition whole fraction. The least significant enhancement in the methane yield occurred at 175°C 7.8 ramp with only 29% in relation to the same condition whole fraction (Table 6.6).

Table 6.6 Comparison of methane yield of FW under different scenarios.

Soluble Samples Names/Methane yield	175°C 7.8 ramp	175°C 3.9 ramp	175°C 1.9 ramp	85°C 7.8 ramp	Control
Final Methane yield ($mlCH_4/gVSadded$)	218.67	254.00	241.83	294.74	326.45
Improvement in relation to the whole fraction (%)	29.00	57.90	74.00	48.94	83.22
Improvement relative to Control (whole) (%)	22.90	42.76	35.92	65.65	83.22
Reduction of the lag phase/hydrolysis	–	+	–	–	–

On the other hand, when comparing the soluble fraction samples yield against the whole fraction control (sample without exposure to microwave irradiation or phase separation), the amelioration was not as high. In this case, the highest increment occurred for the control soluble fraction (83.22%), followed by the 85°C 7.8 ramp, which showed 65.65% improvement in the yield.

If process operational conditions were to be chosen based solely on the final methane yield, the soluble fraction control would be the optimum condition, hence implying in cost savings, since the microwaving step would be absent, and the ones associated with solid fraction removal (e.g. centrifuging) is not economically costly/unfeasible. Nevertheless, under these conditions, the AD plant operators would have managed the environmental and economic impacts of solids disposal.

Shahriari *et al*. (2013) evaluated the effects of microwave pre-treatment of kitchen waste on the methane production utilizing continuous reactors (both single and dual-stage) and under two fractions (whole and liquid). The authors observed that the whole fraction control under a dual-stage system heralded the greatest methane production (60% greater) compared to the single-stage reactor treating control or single-stage reactor with pre-treated substrate. Therefore, the authors found that mesophilic AD staging without MW pre-treatment of whole KW was deemed the best and most economical configuration to achieve the greatest gas production. Nevertheless, the greatest increment in methane production (1.97-fold) was observed when the dual-stage system treating soluble fraction from treated kitchen waste was put against the single-stage reactor treating soluble fraction control, hence suggesting the soluble fraction of organic wastes such as KW is better digested when different bacterial populations are spatially segregated.

Anova test for soluble and whole fraction samples was performed in order to evaluate possible statistical differences in the final methane yield, and most feasible option for scaling up the process. The operational conditions with the highest methane yield (85°C 7.8 ramp and control soluble fraction), as well as the one with the most improvement in terms of process performance (less TVFAs accumulation in the hydrolysis step, shorter lag phase) – 175°C 3.9 ramp, were compared against each other.

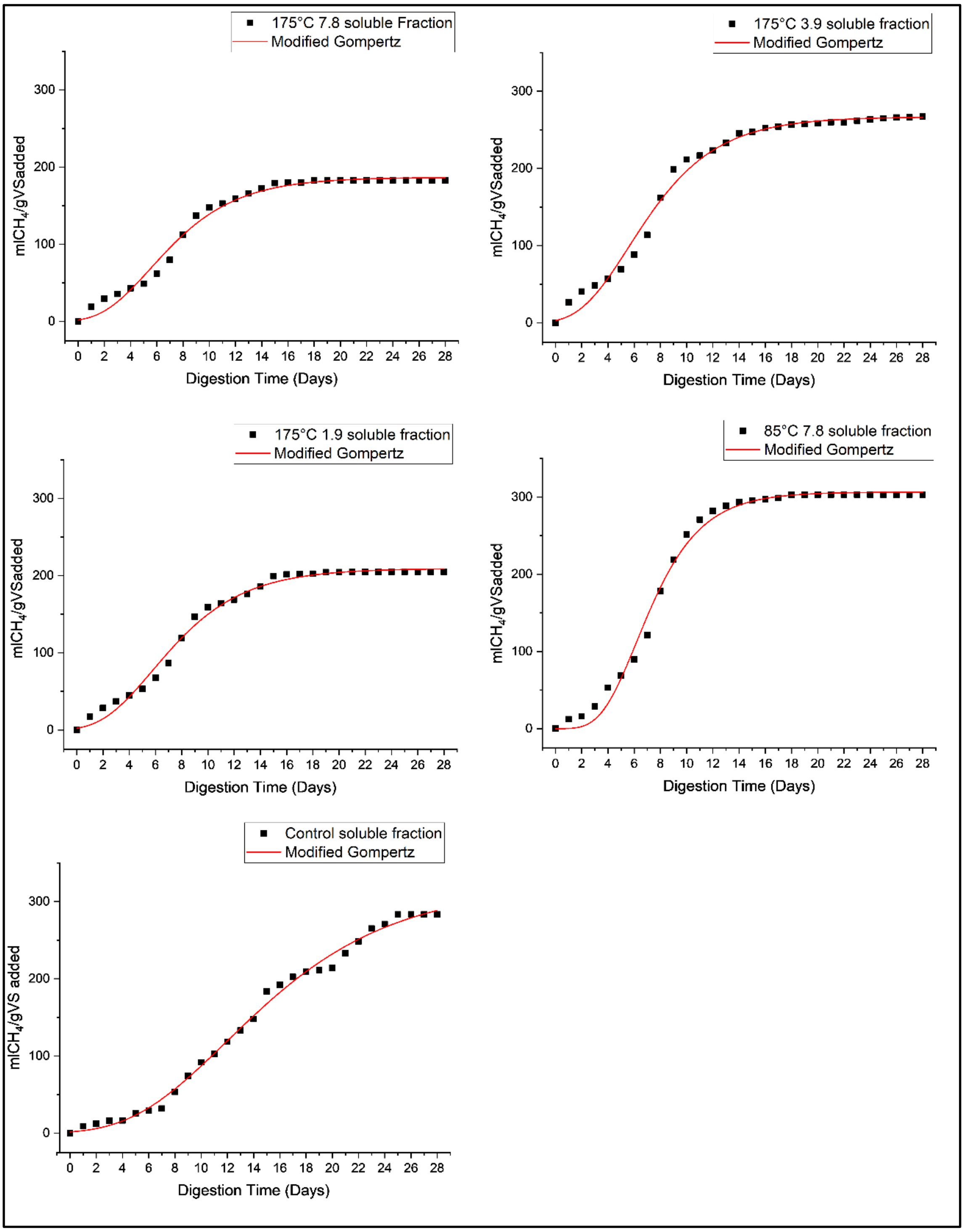


Figure 6.11 Specific methane yield with the MGompertz fitting curves for the food waste samples microwaved at 85-175°C and different heating rates, soluble fraction, as well as control.

The statistical results showed that there was a significant difference between the 85°C 7.8 ramp soluble and control soluble fraction (Table 6.7). Moreover, no statistical difference between control soluble fraction and 175°C 3.9 ramp soluble was observed. There was no statistical difference when 85°C 7.8 ramp soluble and 175°C 3.9 ramp soluble samples were compared.

Therefore, based on these results, it is possible to assert the control soluble fraction as the optimal condition for methane production (Appendix G).

Table 6.7 Anova test for methane yield soluble fraction.

Microwave operational condition	Final Methane yield ($mlCH_4/gVSadded$)	p value
85°C 7.8 ramp soluble vs. control soluble	294.74	*p* = 0.0000
control soluble vs. 175°C 3.9 soluble	326.45	*p* = 0.3579
85°C 7.8 ramp soluble vs. 175°C 3.9 ramp soluble	254.00	*p* = 0.4539

6.8 Technical digestion time (T80) of soluble and whole fraction of FW

Overall, the technical digestion time of the soluble fraction samples was longer than the whole ones. The better process performance of the microwaved whole fraction FW samples at the beginning of the digestion (shorter lag phase) for most samples, stimulated a faster methane production, culminating with a shorter T80.

Nevertheless, the time needed to obtain 80% of the biomethane via AD of the microwaved samples (both soluble and whole fraction) was significantly shorter than the untreated ones/controls (whole and soluble) (Figure 6.12). The greatest improvement in relation to the whole fraction control occurred at 85ºC 7.8 ramp whole, on which T80 was reached on the 6th day, reduce by 64%, the time required to produce most of the methane, hence reducing the process costs.

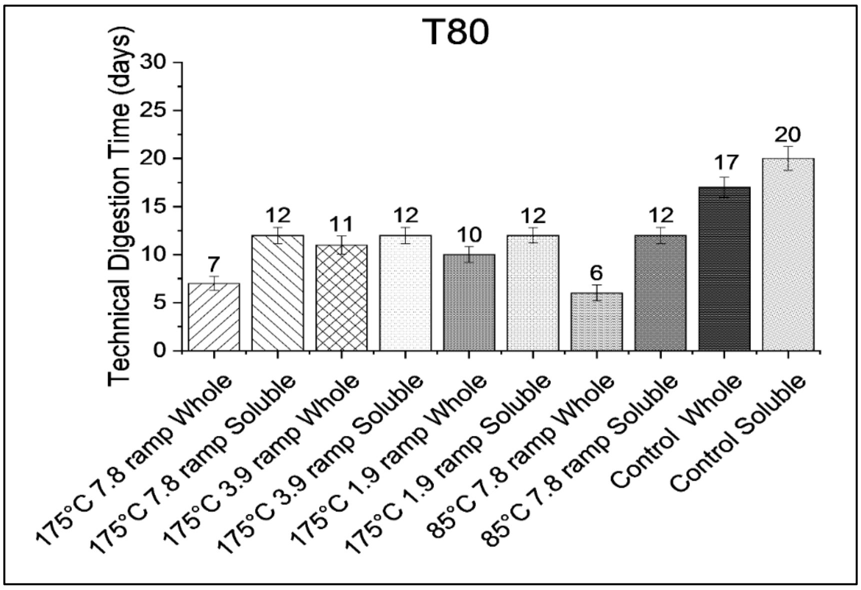


Figure 6.12 Technical digestion time (T80) of the soluble and whole fraction of microwaved food waste sample and the untreated samples.

All the soluble fraction samples showed identical values of T80 (12 days) equivalent to 40% reduction on the time in relation to the control soluble fraction.

Despite having the greatest methane yield, the soluble fraction control exhibited the longest T80 of all the microwaved conditions here tested, hence suggesting that attention is needed to estimate if the energy saved by not applying microwave pre-treatment compensates the longer duration of the process. Henceforth, all the factors denoting process performance should be considered when deciding on scaling up the process, in a manner that the optimum condition is the result of their best combination.

6.9 Summary

Phase separation of MW FW into soluble and whole fraction resulted in important changes to the characteristics of the waste that could ultimately influence the final methane yield, including VS content, acetic acid concentration and solubilization (%). Due to solids removal, all the soluble samples showed a loss in the VS content. Under high temperature (175°C) and fast heating rate (7.8°C/min), the greatest VS loss in relation to the control was observed (38.47%). In turn, the lowest VS loss occurred at 85°C and 7.8 ramp (23.63%).

Moreover, there was a TVFAs increment in relation to the whole fraction and to the control in all cases, with acetic acid prevailing. 175°C medium heating rate (3.9°C/min) had the highest acetic acid increase with 67.49% in relation to the controls. On the other hand, the solubilization (%) was greater (70.91%) at the slow heating rate (1.9°C/min), suggesting that longer exposure to MW irradiation promoted better organic matter solubilization.

Phase separation did not circumvent the typical VFAs accumulation seen in the digestion of FW, however, made it less pronounced, by avoiding a sharper pH drop. Moreover, the soluble fraction in general had lower TVFAs/Alkalinity ratios than the whole one, indicating that there could be an improvement of the process performance as solids are removed from the solution.

In this study, the microwaved FW samples were not analysed for melanoidins due to resources limitation. Nevertheless, it is possible to infer that from the final temperature range and heating rate (exposure time) used for the thermal pre-treatment and the colour change observed at temperatures >115°C, most samples underwent Maillard reaction with possible melanoidins formation, especially the ones treated at a higher temperature (175°C). It has been hypothesized that, phase separation favours melanoidins removal by centrifugation and filtration. Several parameters were adopted as indicators of possible successful melanoidins and other inhibitory compounds removal, including volume of methane produced in the first day of digestion and volume of gas produced in the highest peak. It was observed that the 175°C 3.9 ramp showed a higher biomethane production on the first day of digestion, despite having a lower initial solids concentration in relation to the other samples. Moreover, these samples also showed an actual shortening of the lag phase in relation to the same condition under the whole fraction equivalent to 54% less time need to complete this step. Hence, it can be postulated that phase separation was effective in removing inhibitory compounds in the system on the medium heating rate. Nevertheless, further specific tests would need to be performed in order to confirm the hypothesis here postulated and the effectiveness of inhibitory compounds removal.

In general phase separation caused an improvement of the methane yield in relation to the whole fraction for all the conditions tested, hence validating the soluble phase as being the most feasible fraction for methane production.

In spite of the improvements on process performance at 175°C 3.9 treated FW, this condition did not represent the highest methane yield of the soluble phase samples. In turn, the soluble fraction control heralded the highest methane yield, with a total of 326.00mlCH_4/gVS added, followed by 85°C 7.8 ramp soluble sample, with the equivalent to 294.74mlCH_4/gVS added, with a statistically significant difference between them. Overall, the technical digestion time of the soluble fraction samples was longer than the whole ones. Nevertheless, the time needed to obtain 80% of the biomethane gas via AD of the microwaved samples (both soluble and liquid fraction) was significantly shorter than the controls (whole and soluble).

Despite having the greatest methane yield, the soluble fraction control exhibited the longest T80 of all the microwaved conditions here tested, hence suggesting that attention is needed to estimate if the energy saved by not applying microwave pre-treatment compensates the longer duration of the process. Henceforth, all the factors denoting process performance should be considered when deciding on scaling up the process, in a manner that the optimum condition is the result of their best combination.

CHAPTER 7

7. EFFECTS OF THE RE-CIRCULATION OF MICROWAVE PRE-TREATED DIGESTATE ON THE MESOPHILIC ANAEROBIC DIGESTION OF FOOD WASTE

7.1 Introduction

As a consequence of the increase of AD around the globe as an alternative method for energy and by-products generation, there has been a significant increment in the amount of digestate produced. Nevertheless, due to strict land regulations, land shortages, rising disposal costs (including gate fees) amongst others, digestate management has become a matter of concern (Wei *et al*., 2003; Curry and Pillay, 2012; Zhang *et al*., 2016).

An alternative approach for this product management would be to recover residual energy in the form of biomethane by pre-treating it with microwave irradiation and further applying into AD. It is believed that similar to MW digestion of FW, an enhancement of the hydrolysis step can occur by disruption of tough cell wall hard to digest, thereby increasing biodegradability and enhancing biomethane production (Eskicioglu *et al*., 2008; Zhang *et al*., 2016). Digestate could benefit from these MW pre-treatment effects.

Despite this, in order to optimize the efficiency of the coupled process (microwave irradiation and AD) some corrective measures should be adopted. The most important are the increase in anaerobic biodegradability and the correction of the digestate's low C/N ratio (6 - 8) (Zhang *et al*., 2016). This can be achieved by combining the pre-treated substrate with another organic substrate rich in carbon, such as untreated food waste (C/N 10.9 in this study).

To the best of our knowledge, microwave pre-treatment of digestate and its re-circulation effects in the MAD of food waste has not yet been tested. Adopting this novel re-circulation strategy would result in several advantages namely: 1) reduction of the final volume of digestate to be disposed of, 2) energy recovery from two current substrates of environmental concern (food waste and digestate), 3) alternative option for their management and 4) reduction of fresh water added to raw FW feeding MAD. Moreover, the use of the whole fraction of these substrates may avoid possible environmental impacts and additional costs relating to the disposal of the solid fraction. In this chapter, the effects of microwave irradiation of digestate on its characteristics and the effects of its re-circulation on MAD of FW process performance will be evaluated and discussed.

7.2 Results and discussion

7.2.1 Effects of microwave irradiation on digestate characterization

The effects of microwave irradiation on the digestate characteristics (Oxygen Demand (TCOD, SCOD), Ammonium, TNb, TVFAs, pH, Alkalinity, solids and phosphate) are show in Table 7.1 with results expressed as means and standard deviation in brackets. The novel aspect of this part of the work makes comparison with the literature difficult. Nevertheless, because sewage sludge material is to some extent similar to the digestate, this will be used for comparison wherever it is considered appropriate.

7.2.1.1 pH and VFAs

All the treated digestate samples exhibited an increase in pH in relation to the untreated digestate (control), with 115°C 1.4 ramp exhibiting the greatest pH increase, of all conditions tested, and 95°C 1.4 ramp showing the smallest pH increase in relation to the control There was no easily identifiable or simple relationship between pH increase and final temperature (Table 7.1).

Digestate control had a similar pH values to those reported in the literature for digestate from food waste. De la Rubia *et al*. (2010) reported an average pH of 8.2 with maximum values of 8.5 for food waste digestate collected from a commercial digester treating FW from a mixture of commercial and municipal sources. The pH value reported by Serna-Maza *et al*. (2015) for FW digestate was marginally lower (7.95) than the one reported here, which might be due to differences in food waste composition.

The aforementioned changes in the treated digestate's pH could be related to the sample's alkalinity. Despite there being an increase in intermediate products concentration (TVFAs) for all the operating conditions tested, the acidity of the samples did not decrease significantly. This suggests a good buffer capacity of the MW digestate. Both digestate and sludge materials are known to have high nitrogen content (Zheng *et al*., 2009; Borges *et al*., 2009). Moreover, according to De la Rubia *et al*. (2010), the most common buffer agent in digestate is ammonium, which contributes to the stabilization of the pH value in the reactor. This, in the form of ammonium bicarbonate, buffers the system in case of an increase on the acidity/TVFAs. Herein, changes in the digestate after thermal treatment included a 105.48% (average) increase in alkalinity, 355%.39% (average) increase in ammonium and 162.4 % (average) increase in TVFAs increase in relation to untreated digestate. This supports the hypothesis of the origin of an alkali predominant medium, avoiding significant pH drop. (Table 7.1)

The changes observed in the pH of the MW digestate contrast with those reported by Eskicioglu *et al*. (2006) when pre-treating waste activated sludge with microwave irradiation (MW) and conventional heating (CH), both at 96°C. The authors observed that in both cases there was pH decrease in relation to the control from 7.5 to 6.9 in the former, and from 7.5 to 7.0 in the latter. The change in pH was accompanied by an increase in TVFAs concentration (acidity of the system) at an order of 379%- and 233%, respectively, which most likely caused a pH drop due to a lower buffer capacity (alkalinity). Although alkalinity change before and after MW treatment was not reported, ammonium concentrations revealed a subtle variation after thermal treatment, especially for the conventional heating (1.54% and 0.44% increase for MW and CH, respectively), hence confirming a lower buffer capacity of the sludge in relation to digestate.

All thermally treated digestate exhibited an increase in TVFAs concentration in relation to the control. This increase was not correlated to the final temperature, however. The highest TVFAs concentration (379.90mg/L) occurred at 75°C and 1.4 ramp, which represents a 280.81% increase in relation to the control. The lowest TVFAs concentration (191.05mg/L) was observed at 95°C and 1.4 ramp representing a 91.50% increase in relation to the control.

An increase in TVFAs after microwave irradiation of sludge is a common finding within the literature. Coelho *et al*. (2011) studied the effects of microwave pre-treatment (parameters 14.4ºC/min and 96°C) of activated sludge using different reactor types (mesophilic, thermophilic and temperature-phased AD) and four different sludge retention times (20, 15, 10 and 5 min). Because the sludge fed into the reactors had different characteristic depending on the period of collection, they were characterized prior to AD. The authors reported that regardless of the sludge used, there was an increase in intermediate products concentration in relation to the raw/untreated samples. This increase varied between 154.61 to 256.44%, i.e., similar to those found here.

Figure 7.1 depicts the changes in VFAs composition after the digestate was exposed to different final temperatures and heating rates. Regardless of the pre-treatment condition, acetic acid was the most abundant intermediate product after microwave irradiation. For a 1.4 ramp, acetic acid percentage increase showed a monotonic relationship with final temperature, representing 54.46% of the TVFAs at 75°C, 72.65 % at 95°C and 73.16% at 115°C.

At 85°C 7.8 ramp, acetic acid corresponded to 80.92% of the TVFAs representing the greatest proportion of this acid of all the conditions tested, which could favour the production of biomethane, especially at the beginning of digestion since this intermediate product is the main precursor of this gas (Feng *et al*., 2013). In most cases, acetic acid percentage of the microwaved samples represents an improvement in relation to the control samples in which acetic acid represented 66.88% of the TVFAs.

Eskicioglu *et al*. (2006), reported an increase in TVFAs concentration after thermal pre-treatment of WAS (Waste Activated Sludge). In terms of individual VFAs, acetic acid had the greatest increase, from absent in the raw WAS to an average of 944mg/L in the microwave samples and 778mg/L in conventional heating samples.

Butyric acid was the second most prominent intermediate product in the digestate after MW irradiation. In contrast to acetic acid, its increase (%) was not related to final temperature. At 75°C 1.4 ramp this acid represented 17.14% of the TVFAs, as opposed to 27.34% and 16.03% of the 95 and 115°C, 1.4 ramp, respectively. Digestate treated at 85°C 7.8 ramp showed a smaller increase of this acid, equivalent to 8.93%, with similar values to the controls (5.65%) (Figure 7.1).

Table 7.1 Digestate characteristics before and after microwave irradiation at various temperatures and heating rates (*)

Heating rate	**1.4°C/min**	**1.4°C/min**	**1.4°C/min**	**7.8 °C/min**	**Control**
Final Temperature	**75°C**	**95°C**	**115°C**	**85°C**	**-**
Parameters/Units					
pH	8.94 (0.02)	8.62 (0.03)	9.06 (0.02)	8.93 (0.03)	8.58 (0.01)
TCOD (g/kg)	44.40 (16.11)	48.06 (15.20)	41.50 (16.85)	43.16 (12.76)	40.71 (12.87)
SCOD (g/kg)	36.80 (6.20)	34.92 (5.75)	38.95 (5.40)	37.75 (6.35)	5.43 (1.50)
Ammonium (g/kg)	14.73 (0.60)	14.43 (0.59)	12.86 (0.80)	15.36 (0.76)	3.15 (0.47)
Alkalinity (mgCaCo$_3$/L)	4650.00 (84.50)	4204.00 (67.10)	3900.00 (55.20)	4540.00 (89.40)	2104.00 (45.10)
TNb (g/kg)	25.58 (0.98)	24.85 (0.49)	22.80 (0.35)	26.46 (0.89)	5.45 (0.90)
TVFAs (mg/L)	379.90 (38.13)	191.05 (47.14)	231.49 (40.10)	246.66 (45.17)	99.76 (29.61)
VS (w.b) (g/kg)	22.06 (1.76)	18.21 (1.01)	21.93 (1.38)	22.50 (1.55)	24.33 (0.90)
VS/TS (%)	58.00 (5.20)	57.40 (3.82)	57.95 (5.56)	58.42 (4.30)	58.93 (0.55)
Phosphate (g/kg)	113.00 (1.99)	112.50 (1.95)	113.70 (2.33)	113.33 (1.85)	182.66 (2.50)

*results expressed as means with standard deviation in brackets.

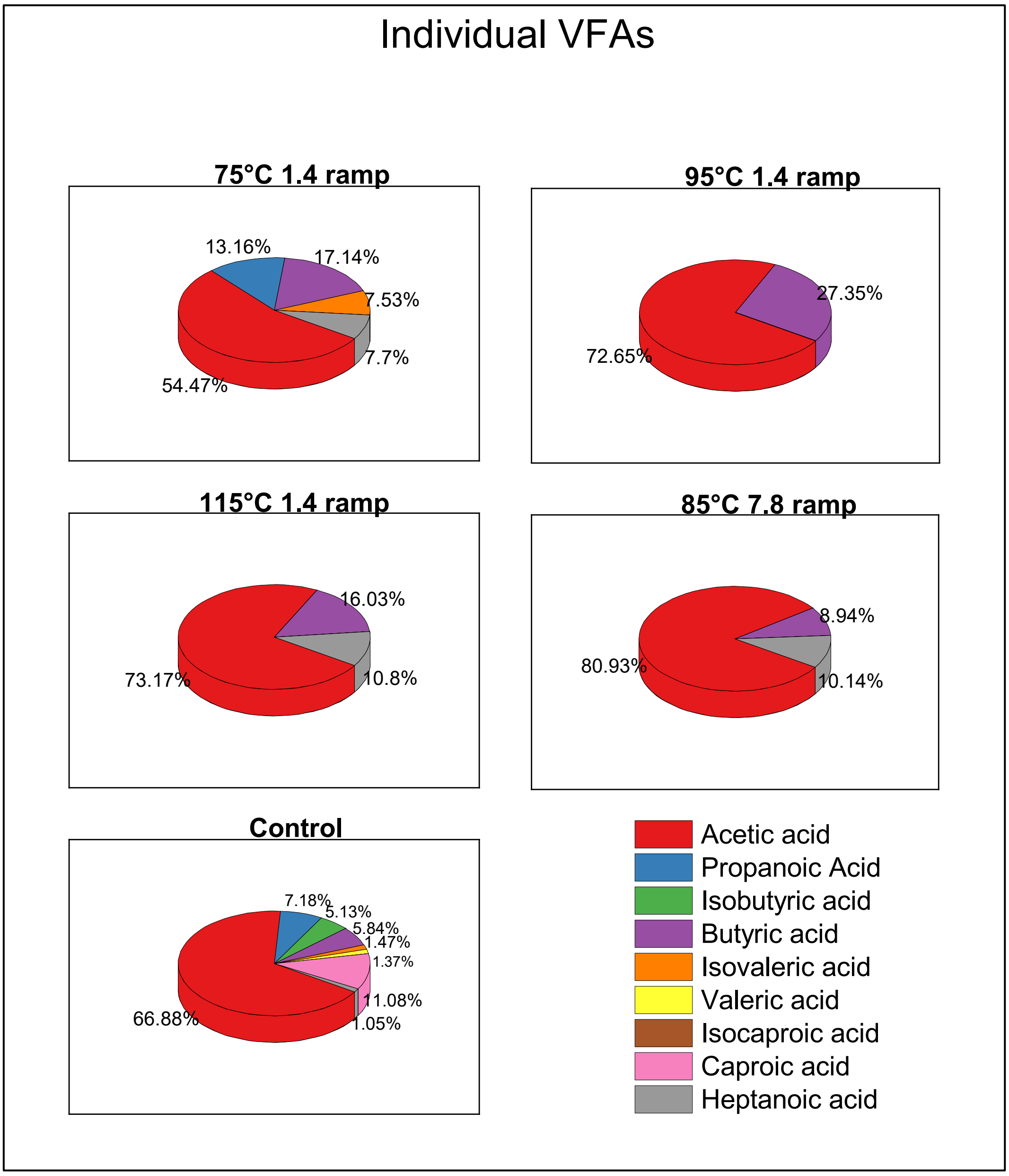


Figure 7.1 Individual VFAs fraction before and after microwave pre-treatment of digestate under different operational conditions.

These results are in agreement with those reported by Park and Ahn (2011), who evaluated the effects of microwave pre-treatment and thermal pre-treatment (magnetic hotplate stirrer), both attaining a final temperature of 80°C, on a mixture of primary and secondary sludge. The authors reported a TVFAs increase in relation to the control as a result of both pre-treatments. This increase was greater for the MW samples with a 12.03 and 18.26% increase in acetic and propanoic acids respectively.

7.2.1.2 Solubilization of organic matter - total and soluble chemical oxygen demand

Thermal pre-treatment of digestate promoted an increase in TCOD for all conditions studied. The most significant increase in relation to the control (18.05%) occurred at 95°C 1.4 ramp and the smallest (1.92%) for the samples treated at 115°C 1.4 ramp. Digestate treated at 85°C 7.8 ramp showed a 6.09% increase in TCOD concentration, representing an improvement with respect to samples exposed to 115°C 1.4 ramp, despite a shorter (65 minutes less) exposure to MW irradiation (Table 7.1). These results suggest that lower temperatures and shorter exposure time are more favourable for TCOD enhancement when thermally pre-treating digestate.

The increase in TCOD after thermal treatment, such as MW irradiation, is a common finding when digesting organic substrates such as sewage sludge. Eskicioglu *et al*. (2008) evaluated the synergetic pre-treatment of sewage sludge by MW irradiation (60, 80, 100 and 120°C) and H_20_2 addition on the characteristics of the waste and AD process. In contrast to the results founds here, the authors reported a greater organic matter disintegration- TCOD increase (5.39%) at higher temperature (120°C) with respect to the controls. The TCOD increase was 3.46% and 4.49% at 100 and 60°C, respectively. An exception occurred for 80°C, for which the treated sludge showed a lower TCOD content (5.84%) in relation to the control. The combination of MW irradiation and chemical addition did not yield a positive result in terms of TCOD disintegration, with lower values than the controls.

Similarly, Park and Ahn (2011) observed a TCOD increase when pre-treating a mixture of primary and secondary sludge with thermal and microwave radiation, both at 80°C. The authors reported a 1.20% and 1.92% increase on the TCOD content for the former and latter condition, respectively. The different degree of TCOD increment presented by Park and Ahn (2011) and those presented here may be due to differences in substrate composition and operational conditions.

Coelho *et al*. (2011) also reported an increase in TCOD after microwaving activated sludge using a ramp of 14.4ºC/min and a final temperature of 96ºC. The increase varied from 8.84 to 28.99%, with an average of 15.00%, similar to the results obtained in the present study.

There was also an increase in SCOD in relation to the control for all tested conditions. Microwave treatment at 75, 95 and 115°C, using a 1.4 ramp, resulted in an increase in SCOD from 5.43g/kg (untreated sample) to 36.80, 34.92 and 38.95g/kg, respectively, representing a 577.71, 543.09 and 617.31% increase in relation to untreated samples. Likewise, samples treated at 85°C 7.8 ramp, showed 595.21% increase in relation to the control. These results suggest that lower temperature and shorter exposure time to MW radiation is preferable over higher temperatures and longer exposure time. This is because the decrease in organic matter solubilization of the latter in relation to the former condition is small (2.49%) but the energy saving associated is large (96kWh less).

The increase in SCOD after thermal treatment is in agreement with results presented by Zhang *et al*. (2016). The authors treated sewage sludge with microwave radiation at a final temperature of 100ºC and maximum power of 1000W. Their results showed 125.91% increase on the soluble organic matter in relation to the control.

Eskicioglu *et al*. (2006) also found an enhancement in SCOD after thermal treatment with conventional heating and microwave irradiation of WAS. Despite being performed at a similar temperature (96ºC), the two pre-treatment methods had different results. The solubilized organic matter increased by 356.17 and 139.40% in relation to the controls, for the former and latter condition, respectively.

Park and Ahn (2011) reported a 225.75 and 175.32% increase in relation to the control after primary and secondary sludge mixture was exposed to MW heating and conventional heating at a final temperature of 80ºC. The solubilization obtained in this study was higher than those reported elsewhere regardless of the type of thermal treatment. The differences in the chemical composition of sludge and digestate could explain this, at least in part. Nevertheless, these results suggest that in terms of solubilization, microwave irradiation is a promising alternative technology for digestate which offers an even better substrate than sewage sludge in pursuit of good soluble organic matter disintegration.

All treated digestate samples exhibited an improvement in the amount of soluble organic matter (SCOD/TCOD) in relation to the control. For a heating rate of 1.4°C/min, the solubilisation varied significantly between the final temperatures, with the greatest SCOD/TOC increase (93.85%) observed at 115°C. In contrast, 95°C 1.4 ramp, showed the lowest (72.65%) SCOD/TOC increment (Figure 7.2).

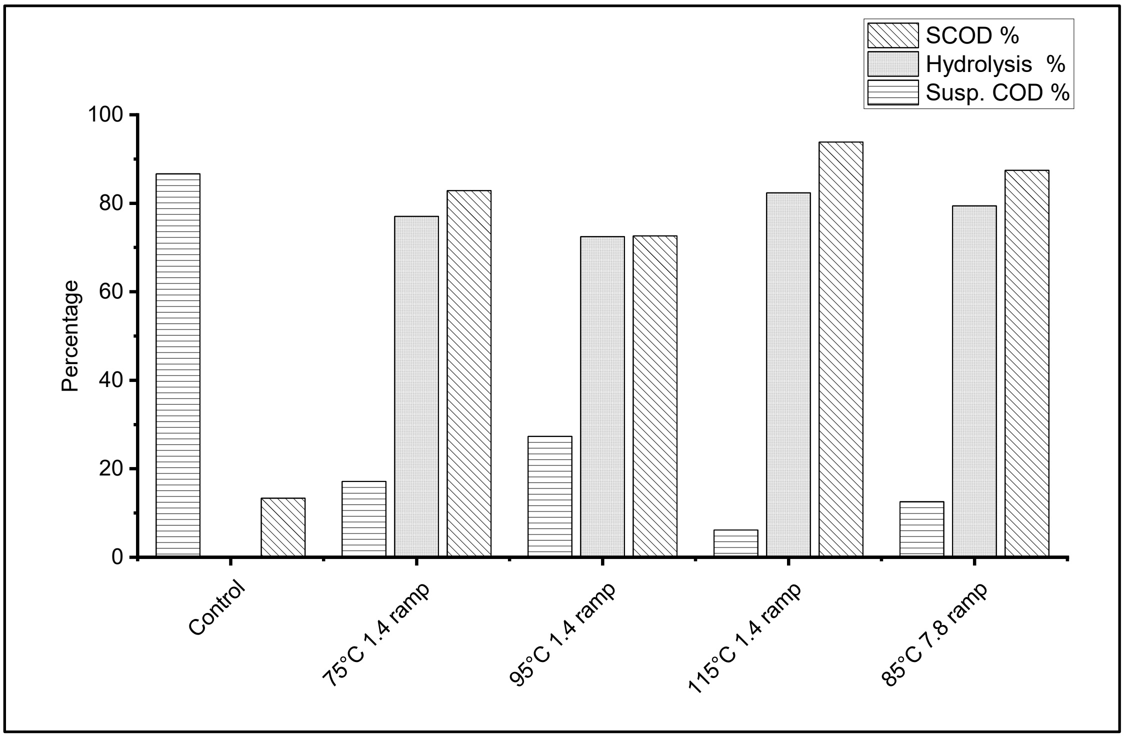


Figure 7.2 SCOD, Suspended COD and hydrolysis percentage of different final temperature and ramp rate of MW digestate.

The solubilisation (%) degree or hydrolysis in general represented an improvement in relation to the control and did not vary significantly amongst the temperatures and ramp teste. Digestate pre-treated at 75ºC 1.4 ramp had a hydrolysis degree of 77.05%, as opposed to 72.43%. 82.33% of 95 and 115°C, respectively. There was no straight co-relation, therefore, between final temperature and the percentage disintegration of organic matter.

Digestate treated at 85ºC 7.8 ramp showed a hydrolysis degree of 79.39%, similar to the highest value for a 1.4 heating rate, again suggesting that this operating condition is preferable. This is because the decrease on the hydrolysis degree is small (3.57% in relation to the 115°C and 1.4ramp) but the energy savings (96kWh, associated with the 65-minute reduction in microwave treatment time) is significative.

The results are in agreement with those presented by Park and Ahn (2011). Before exposing a mixture of primary and secondary sludge to two different thermal treatments, the SCOD/TCOD was 3.9%. After treatment this value was increased by 174 and 217% for the thermal and microwave methods, respectively. The greater increase in SCOD/TCOD (434-617.30%) obtained in relation to Park and Ahn (2011) could be due to differences in the microwave operating conditions and/or differences in the substrate composition.

Due to an improved hydrolysis, digestate treated at 115°C 1.4 ramp showed a reduction in suspended COD concentration in relation to other temperatures tested with the same ramp. This was equivalent to 77.54 and 64.11% in relation to when digestate was treated at 75 and 95ºC respectively. This decrease was even higher when compared to the controls: (92.91%) (Figure 7.2).

The greatest SCOD increase with respect to the control occurred at 115ºC 1.4 ramp (617%) and at 85°C with 7.8 ramp (595%). The difference between these results were statistically significant (Table 7.2). In the former case, the enhanced organic matter was accompanied by an additional 0.12 kWh, compared to 0.34 kWh for the later. The least attractive operational condition for organic matter disintegration is 115˚C 1.4 ramp due to having a yield of 16.76gSCOD/kW, as opposed to 95.05gSCOD/kW obtained when the substrate is treated at a lower temperature and shorter irradiation exposure time.

Table 7.2 ANOVA test for SCOD from microwaved digestate at different temperatures and ramp rates.

Samples	Statistical Value (*p* value)	SCOD increase (%)	Energy Increase (kWh)	kWh Increase per g/SCOD increase	Yield gSCOD/kW
Control vs.75ºC 1.4ramp	$p = 0.0000$	577	0.81	0.0014	22.40
Control vs. 95ºC 1.4ramp	$p = 0.0000$	543	0.85	0.0015	19.60
Control vs. 115C 1.4ramp	$p = 0.0000$	617	0.12	0.0020	16.76
Control vs. 85ºC 7.8ramp	$p = 0.0000$	595	0.34	0.00057	95.05

7.2.1.3 Ammonium and TNb

As previously described, the digestate used in this study originated from food waste. Nevertheless, despite originating from the anaerobic digestion of a nitrogen rich feedstock, the ammonium concentration of the digestate was not considered very high (5.45g/kg) and, in fact, it was similar as concentrations reported in the literature, including the digestate originating from other types of feedstock such as agricultural wastes.

Liu *et al*. (2019), for example, described ammonium values of 3.7g/L for the liquid fraction of the digestate originating from a small-scale biogas plant fed with chicken manure. This is close to the untreated sample/control value (3.15g/kg) in this study. Hu *et al*. (2015) found lower ammonium values (0.653g/L) for the liquid fraction of digestate obtained from continuous reactor fed with corn stovers. Differences in nutrient composition as well as their concentration are expected in the digestate due to interactions of the ingoing substrate and the management of the digestion process (Möller and Müller, 2012; Zirkler *et al*., 2014).

After thermal treatment there was an increase in ammonium concentration in relation to the control for all the conditions tested. For the digestate treated using a 1.4 ramp at different temperatures, the greatest increase occurred at 75ºC, with a final value of 14.73g/kg, representing 367.61 % increase in relation to the control. Digestate samples treated at 115ºC, showed the lowest ammonium increase, with 308.25% increase in relation to the controls.

When the digestate was exposed to a lower temperature and shorter irradiation exposure time (85ºC 7.8 ramp), the increase was the greatest of all conditions tested, with final value of ammonium of 15.36g/kg, representing a 387.61%increase in relation to the untreated samples.

The increase in ammonium after thermal treatment was also observed for sludge and reported elsewhere. Zhang *et al*. (2016) for example, found that microwave irradiation of sewage sludge at 100ºC and 1000W resulted in a 24.0% increase in the ammonium concentration in relation to the control. Similarly, Eskicioglu *et al*. (2008) reported an ammonia (NH_3^+) increase after microwave irradiation of waste activated sludge. The different temperatures tested resulted in different ammonia increases, with an inverse relation between final temperature enhancement and ion concentration. A 325.47, 221.69, 186.79 and 74.52% of ammonia was reported at 60, 80, 100 and 120ºC, respectively. Coelho *et al*. (2011) observed similar ammonia ion behaviour in microwaved activated sludge, with increasing value in relation to the control. The samples microwaved at a final temperature of approximately 96ºC and a heating rate of 14.4ºC/min resulted in 71.36 % increase in relation to the untreated sludge.

A nitrogen-rich digestate is desirable for land application, since N is an essential plant nutrient and NH_4^+ is immediately available to roots, making anaerobic digestate an attractive fertilizer product. Moreover, a high nitrogen content in organic residues reduces the volume needed of this material for spreading on fields (Risberg *et al*., 2017). Nevertheless, high nitrogen substrates can be harmful for bacteria communities, negatively impacting gas production (Angelidaki and Ahring, 1993).

Tampio *et al*. (2015) compared the characteristics of FW digestate and autoclaved FW digestate (160 °C, 6.2 bar) from laboratory-scale reactors. The authors reported a decreased ammonification and low ammonium nitrogen content in the digestate from the autoclaved FW reactor due to thermal treatment of FW, which affected the nitrogen-containing molecules by formation of Maillard compounds. The ammonium value of FW digestate was 4.07 g/kg (similar to the result presented here) as opposed to the autoclaved digestate from FW, which had a value of 1.9g/kg. Notwithstanding, when considering the low NH_4-N/TKN ratio of the autoclave FW digestate (26%) compared to the FW digestate (52%), the former was evaluated to be more suitable for use as soil amendment than as a fertiliser (Nkoa, 2013).

In this study, the ammonium concentration of digestate after microwave irradiation was in most cases similar to that of untreated food waste (13.30g/kg). For this reason, the re-circulation of treated digestate, combined with untreated food waste and inoculum, could exceed the ammonium threshold tolerated by the microbial community and thereby negatively affect the microbial community and methane production during AD. This is especially the case for samples treated at 75°C 1.4 ramp, which exhibited the highest ammonium increase, and for this reason ammonium behaviour was closely monitored during the digestion process.

Overall, there was an increase in TNb concentration in relation to the untreated samples after microwave irradiation. Similar to NH_4^+, there was no simple relationship between final temperature and TNb. The greatest increase (385.50%) occurred for a final temperature of 85ºC 7.8 ramp, and the lowest (318.34%) occurred when digestate was exposed to 115ºC 1.4 ramp. These results suggest that in all cases the TNb increase was primarily associated with the ammonium increase. The TNb value of the control samples in the present study is in agreement with that (4.5g/kg) presented by Liu *et al*. (2019) for the liquid fraction of digestate from a small-scale biogas plant fed on manure.

Similar to the food waste, digestate can undergo Maillard reaction if exposed to high temperatures. Nevertheless, the temperature range on which Maillard inhibitory compounds are formed for the digestate in significantly lower than for food waste. According to Tampio *et al.* (2015), after exposing digestate from food waste to a thermal process (autoclaving at 96ºC), a decrease of the NH_4-N/TKN ratio by 30% in relation to the control was observed. The decrease was later attributed to the formation of Maillard compounds during autoclave treatment.

Similar behaviour of the NH_4/TNb ratio was observed for some of the digestate samples after microwave treatment. A decrease in this ratio in relation to the control was detected for the following operational conditions: 75 and 115ºC 1.4 ramp. This represents >1% and >2.40% decay, respectively, possibly suggesting the formation of small amounts of Maillard compounds.

When food waste was exposed to MW irradiation there was a significant decrease in these values (between 88.76 and 97.88% in relation to the control), specially for longer exposure time (3.9 and 1.9 ramp), and higher final temperatures (175ºC). This suggests that in addition to ammonium volatilization in the microwaving stages of FW, it is possible that inhibitory compounds were being formed (Figure 7.3).

Because amino acids, sugars nor Maillard compounds were not measured here, it is difficult to establish what proportion of ammonium was lost due to volatilization (in the case of FW), and what proportion was lost due to formation of Maillard compounds. Nevertheless, both substrates (FW and digestate) are likely to undergo this process when exposed to microwave radiation. How each particular operational condition affects this is an important question. The formation of Maillard components will be more probable for microwaved food waste due to a greater decrease in ammonium/TNb content.

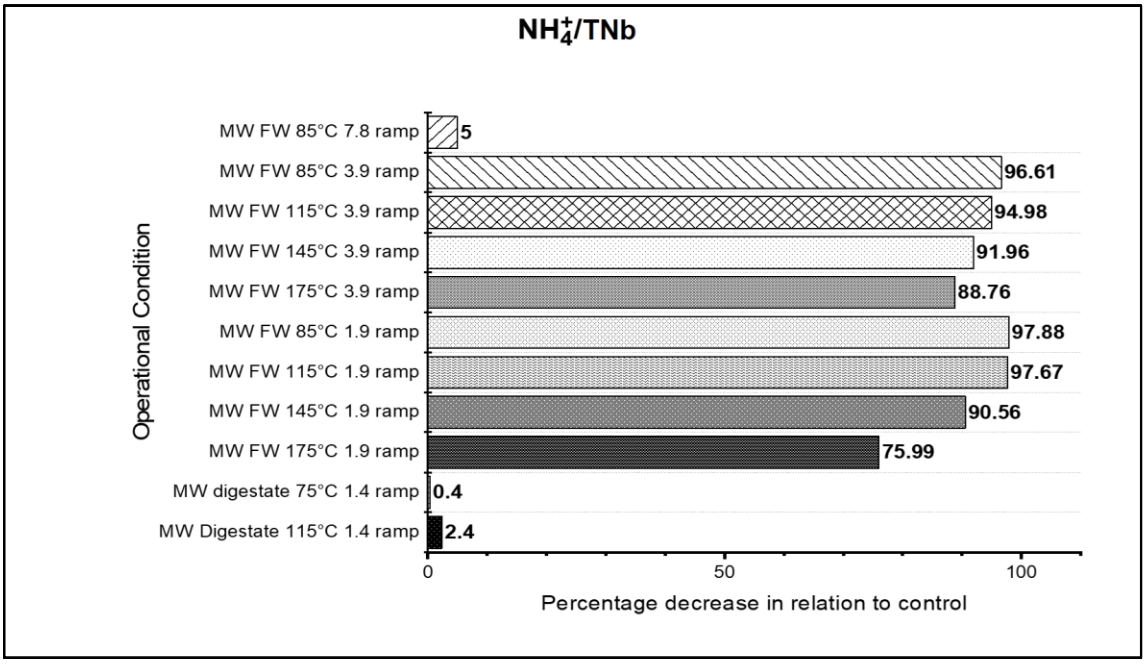


Figure 7.3 Ammonium/TNb ratio decrease in relation to the untreated samples (control FW and control digestate) used as an indicative of Maillard components formation from microwaved food waste and microwaved digestate.

7.2.1.4 Total and volatile solids

Regardless of the final temperature and heating rate applied, a loss of VS in relation to the controls was observed for the microwaved digestate samples. Digestate microwaved at 75°C 1.4 heating rate showed a VS loss of 9.33% in relation to the control, as opposed to 25.15% and 9.86% observed when digestate was treated at 95 and 115°C, respectively. When digestate was exposed to 85°C 7.8 ramp the VS loss was smallest, with a value of 7.52%.

Volatile solids reduction ultimately caused the VS/TS content to drop in relation to the control. At 75ºC 1.4 ramp there was a 1.57% decrease of this value, followed by a 2.59% drop for the digestate treated at 95ºC and 1.66% at 115ºC, 1.4 ramp.

These results are in agreement with those reported by Eskicioglu *et al*. (2008), who found a volatile solid decrease after exposing waste activated sludge to MW radiation at various temperatures. Contrary to the solid’s behaviour here, the decrease of this parameter did not occur for every operational condition. In their case, the VS loss was present at a temperature of 100ºC, corresponding to 2.12%. The remaining operating conditions were characterized by unchanged VS values (80 and 120ºC) or by an increase (60ºC) of this parameter.

Coelho *et al.* (2011) observed a VS decrease after the pre-treatment of sewage sludge with microwave radiation using a 14.4 heating rate and final temperature of 96ºC. The loss was similar to most of those here, with a value of 1.26% on average.

Volatile solids loss occurred despite precautionary measures being taken to avoid them. These included only opening the microwave vessel screw cap once the treated sample had reached room temperature. Nevertheless, the loss was smaller when food waste samples were irradiated and when solid and liquid fraction separation was performed. It was therefore concluded that thermal treatment leads to solids loss regardless of substrate used and operating conditions chosen. Changes in the characteristics of the substrate should be monitored, therefore, before application in an anaerobic digestion process. Moreover, it raises the possibility that each substrate has a specific ideal temperature for minimising VS loss at the moment the microwave tubes are opened.

7.2.1.5 Phosphate

According to Guo *et al.* (2014), digestate is usually rich in phosphate, with concentrations varying from 125-500mg/L, depending on the substrate used. In this study, treated digestate exhibited similar values of phosphate, suggesting that this parameter is not greatly affected by microwave operating conditions. In general, there was a decrease in phosphate in relation to the controls. The greatest decrease (38.41%) occurred at 95ºC and a 1.4 ramp and the smallest decrease (37.75%) was observed at 115ºC 1.4ramp.

Tampio *et al.* (2015) compared the characteristics of FW and autoclaved FW digestate from laboratory-scale reactors and reported a Total P (Total Phosphorous) value of 19.9 and 16.2g/kgTS respectively, corroborating the hypothesis that phosphorous decreases after submitting digestate to thermal treatment.

Although Total P value of digestate was not measure in this study, phosphate values indicate that the former parameter would not possibly be significantly altered due to MW irradiation. Henceforth, the small variation in phosphate concentration (and possibly total P) before and after microwave pre-treatment of food waste digestate could be beneficial to maintaining the adequate levels for application as a soil amendment or fertilizer.

7.3 Effect of re-circulation of microwaved digestate on the MAD of FW

7.3.1 Volatile fatty acids profile and behaviour during the re-circulation of microwaved digestate on the MAD of FW

In all reactors the greatest TVFAs production occurred between days 0 and 2 during which hydrolysis was intensified, with the intermediate products concentration varying significantly amongst the different operating conditions applied (Figure 7.4). For the reactors containing digestate treated at 75ºC and a 1.4 ramp, there was a 2364% increase in the TVFAs concentration between days 0 and 2 and a further increase between days 2 and 4, corresponding to 15.52% in the TVFAs concentration at a volume of 25% treated digestate. The kinetic unbalance between VFAs production (acetolactic bacteria) and consumption (methanogenic bacteria) by the microbial community persisted, with a subsequent accumulation at day 7 of digestion, where the TVFAs reached its maximum value of 1989.06mg/L.

The reactors containing 50% vol. of treated digestate showed a similar behaviour, with a three consecutive points of TVFAs accumulation. The increase in intermediate products between days 0 and 2 was 2075% as opposed to a 16.69% increase between days 2 and 4, followed by 3.30% increase between days 4 and 7. The highest TVFAs concentration occurred at day 7 of digestion, with a maximum value of 2015.10mg/L. Following a similar trend, the reactors containing 75% vol. of treated digestate, showed a Kinect unbalance between days 0 and 7. The increase between days 0 and 2 was 1483% with a further 11. 89% increase between days 2 and 4, and a 7.07% increase between days 4 and 7. Again, the maximum intermediate product concentration was observed at day 7, with a value of 2089.65mg/L.

The reactors with 95ºC 1.4 ramp had a shorter period of TVFAs accumulation, with only two points of kinetic unbalance during the digestion period. For the reactors with 25% vol. of treated digestate there was a 2284%increase in the intermediate products concentration between days 0 and 2, representing the greatest increase and consistent with the intensified hydrolysis step. The production of TVFAs between days 2 and 4 showed a further increase of 13.68%, with a maximum concentration of 1658.49mg/L. Reactors with 50% vol. of treated digestate had a 2084% increase of the intermediate products concentration between days 0 and 2, followed by a 3.41% increase between days 2 and 4 during which it reached the maximum TVFAs concentration of 1692.04mg/L. Lastly, the reactors with 75% vol. of treated digestate had a 1724%increase in the first two days of AD, reaching a maximum concentration of TVFAs of 1819.04mg/L, followed by a 3.24% decrease in concentration between days 2 and 4 of digestion.

As microwave pre-treatment temperature rose to 115°C 1.4 ramp, the reactors containing different volumes of treated digestate showed three points of intermediate products accumulation during the digestion, regardless of the final volume of digestate used. The kinetic unbalance was observed for the first seven days of digestion, with the 25% volume of treated digestate showing an increase 1418% during the first two days of digestion. A further 40.32% increase in the intermediate products concentration was observed between days 2 and 4, the period during which the maximum concentration (1533.02mg/L) of intermediate products was observed, contrasting to the previous conditions, for which the highest TVFAS concentration occurred between days 4 and 7 of digestion. Nevertheless, despite a small decrease of 3.21% in the TVFAs concentration between days 4 and 7, the TVFAs concentration was still elevated, at 1483.73mg/L. Similarly, the reactors with 50% volume of treated digestate showed a 1527.49%increase in TVFAs between days 0 and 2, a 19.09% increase between days 2 and 4, and a 10.38% increase between days 4 and 7, the latter period being that during which the maximum concentration (1819.41mg/L) of intermediate product was reached. For 75% volume of treated digestate the TVFAs increased 1122% during the first two days of digestion, hence representing the lowest increase for this period out of all the conditions tested. A further 29.93% increase was observed between days 2 and 4, followed by a 20.91% increase between days 4 and 7, reaching a maximum value of 2079.37mg/L. during day 7.

The reactors of digestate treated at 85ºC 7.8 ramp also showed a kinetic imbalance between VFAs producers (acetoclastic bacteria) and consumers (methanogenic bacteria) at the start of the digestion process, with three TVFAs accumulation points. The increase in TFVAs between days 0 and 2 was less than for the other conditions regardless of the volume of treated digestate used. Reactors with 25% volume of treated digestate showed a 1121.05%increase in the first two days of digestion, followed by a 37.99% increase between the second and fourth day of digestion and 13.37% increase between days 4 and 7. The highest TVFAs concentration occurred at day 7, with 1993.26mg/L. The reactors containing 50% volume of treated digestate showed an 1084.26% increase in TVFAs between days 0 and 2, followed by 40.32% and 9.05% increases between days 2 and 4 and 4 and 7, respectively. The highest TVFAs concentration was observed on day 7 of digestion equivalent to of 2051.85mg/L. The 75% volume of treated digestate reactors showed a 1167.46%increase between days 0 and 2 and a further 5.05% increase between days 2 and 4. A third point of accumulation was observed between days 4 and 7 with a 42.83%increase in TVFAs. The maximum concentration of 2346.71mg/L was also observed for this period, representing the highest maximum concentration of all the conditions tested.

Comparing the TVFAs behaviour during the AD of the 85ºC 7.8 ramp treated digestate and microwaved food waste under the same conditions, the principal difference is the absence of intermediate product accumulation in the latter. As previously discussed, microwave irradiation proved to be effective in promoting hydrolysis of the organic matter present in the food waste causing faster consumption of this material and, ultimately avoiding intermediate products accumulation.

The untreated/raw digestate also exhibited intermediate products accumulation during the digestion period. Regardless of the volume of untreated digestate applied, two points of TVFAs accumulation during the first week of digestion were observed. High TVFAs concentration occurred between days 2 and 4 of digestion with 25% and 50% of raw digestate volume reactors showing greatest TVFAs maximum values. The former showed a 3463.27% increase between days 0 and 2 of digestion, followed by a further 9.86%increase between days 2 and 4, reaching its maximum value of 1644.56mg/L on day 4 of digestion. The latter control on the other hand, showed a 2059% increase between days 0 and 2 of AD, followed by a 34.27% increase between days 2 and 4. Similar to the 25% volume control, the TVFAs maximum value of 1608.06mg/L was reached on day 4 of AD. These results represent an improvement in terms of process performance compared to the treated digestate at 75 and 115ºC with a 1.4 ramp, and at 85ºC with a 7.8 ramp, which had four and three consecutive days of TVAS accumulation, respectively. The better process performance could have been the result of the absence of inhibitory compounds formed (i.e. phenols, humic acids formed by the Maillard reaction) during the MW irradiation of digestate, known for inhibiting methanogenesis, hence altering the consumption dynamics of these products.

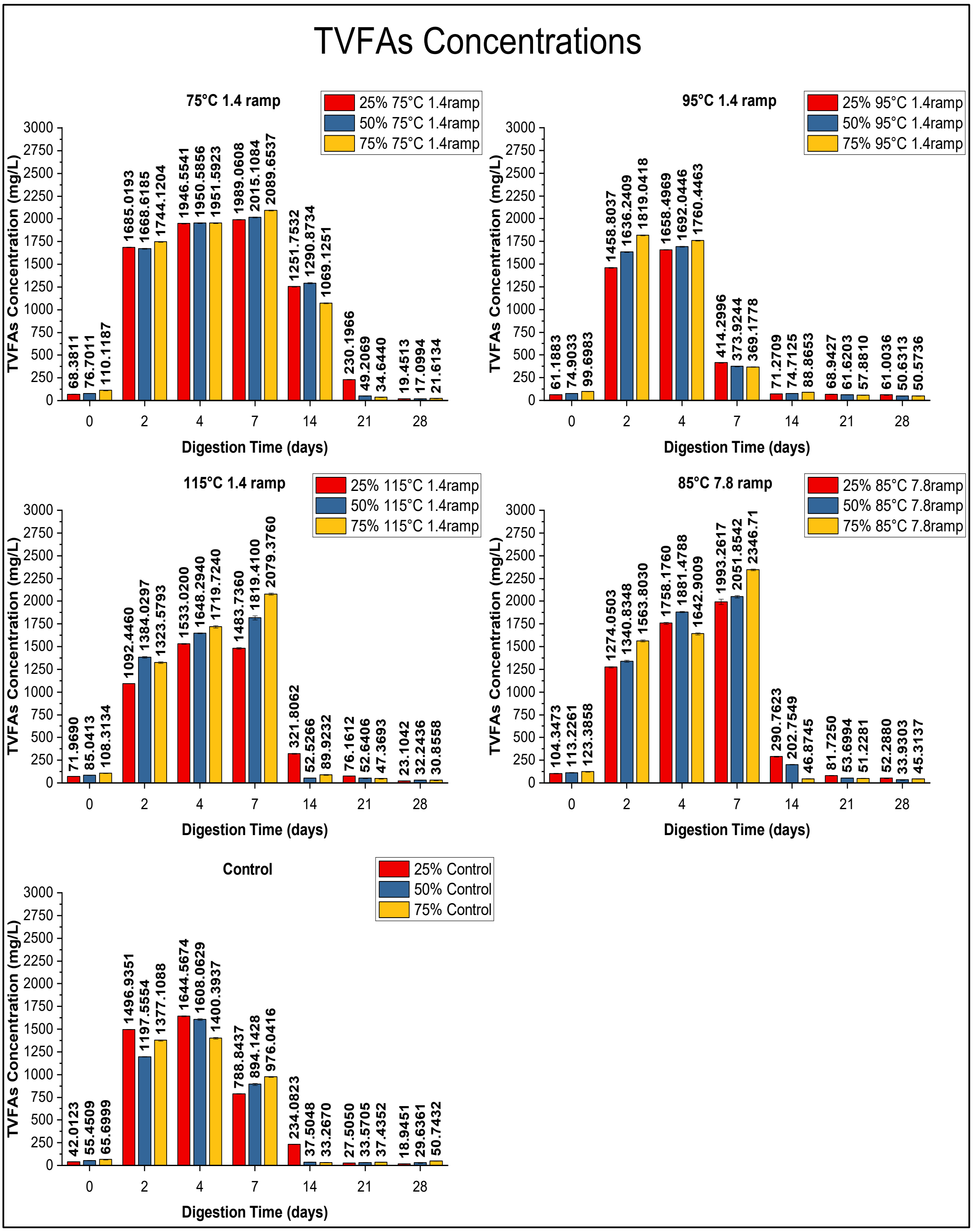


Figure 7.4 TVFAs behaviour during the re-circulation of microwaved digestate (different temperatures and volumes) on the MAD of FW.

The untreated food waste (control) had two consecutive points of TVFAs accumulation between days 0 and 4, due to high hydrolysis and poor kinetics, with a maximum TVFAs of 2100.00mg/L, similar to the maximum values found for several of the treated digestate conditions. The similarities in TVFAs concentration between the two processes are most likely associated with the easily biodegradable characteristics of food waste, together with the rapid transformation of the LOF into VFAs, which is enhanced by the increased TVFAs concentration of the digestate after the pre-treatment, overloading the system if there is insufficient acetoclastic and methanogenic bacteria present. These results suggest that the digestion process of raw food waste together with microwaved digestate is equally challenging.

These results contrast with those reported by Zhang *et al.* (2016) who evaluated the effects of co-digesting microwaved FW and sewage sludge. Different scenarios were analysed including: the AD of raw sewage sludge as a sole substrate, MW FW with untreated sewage sludge, and MW sewage sludge with raw food waste. According to the authors, there was a single TVFAs accumulation point throughout the anaerobic digestion process that happened at day 5. After this, the TVFAs decreased gradually resulting in increased methane production rates. The maximum values of these intermediate products during the kinetic unbalance point were significantly higher than those observed here, especially for the reactors containing raw food waste and microwaved sewage sludge (higher than 20000mg/L). This difference is most likely related to higher volatile solid concentration in the sewage sludge (84.98g/L) than the digestate used here (average of 20.00g/L). As is well known, the volatile solids concentration plays an important role in the metabolization of TVFAs and methane production under adequate microbial concentration and suitable conditions such as pH, ammonium concentration, etc.

High TVFAs digesters performing co-digestion of different waste have been reported to perform without process failure. Dinsdale *et al.* (2000), for example, reported intermediate products concentration between 1330 and 1800 mg/L in the effluent of a successful methanogenic reactor treating fruit and vegetable waste with WAS as a co-substrate. On the other hand, Misi and Forster (2001) showed lower levels of intermediate products (55 – 505mg/L) in functional digesters treating agro-wastes. These results suggest that the successful co-digestion of organic wastes is possible under non-ideal levels of VFAs.

Despite the intermediate product accumulation with a consequent decrease in pH at several points of the treatment of digestate with raw food waste, none of the reactors suffered process failure. The digestate treated at 75ºC, 1.4 ramp showed a decrease in pH from the second to the seventh day of digestion, corresponding to the period of TVFAs accumulation. Such a pH decrease occurred for all proportions of treated digestate, with 25% showing the lowest pH value (6.83) on the fourth day of digestion. This represented the lowest pH value of all tested conditions. Due to a higher concentration of TVFAs in relation to the other operational conditions and more rapid decrease in pH (to a value within the range 6.83 - 6.92) it can be said that there was acidification of the system for all samples treated at 75ºC, 1.4 ramp (Figure 7.5).

After this period there was a pH recovery for all the 75ºC 1.4 ramp reactors. In all cases the pH continued to increase until the end of the digestion period, which coincided with the concomitant, progressive TVFAs removal, and further methane production. The acidification was therefore reversible. On the other hand, digestate treated at 95°C and 115°C 1.4 ramp did not show acidification of the system, suggesting a more stable digestion (Figure 7.5).

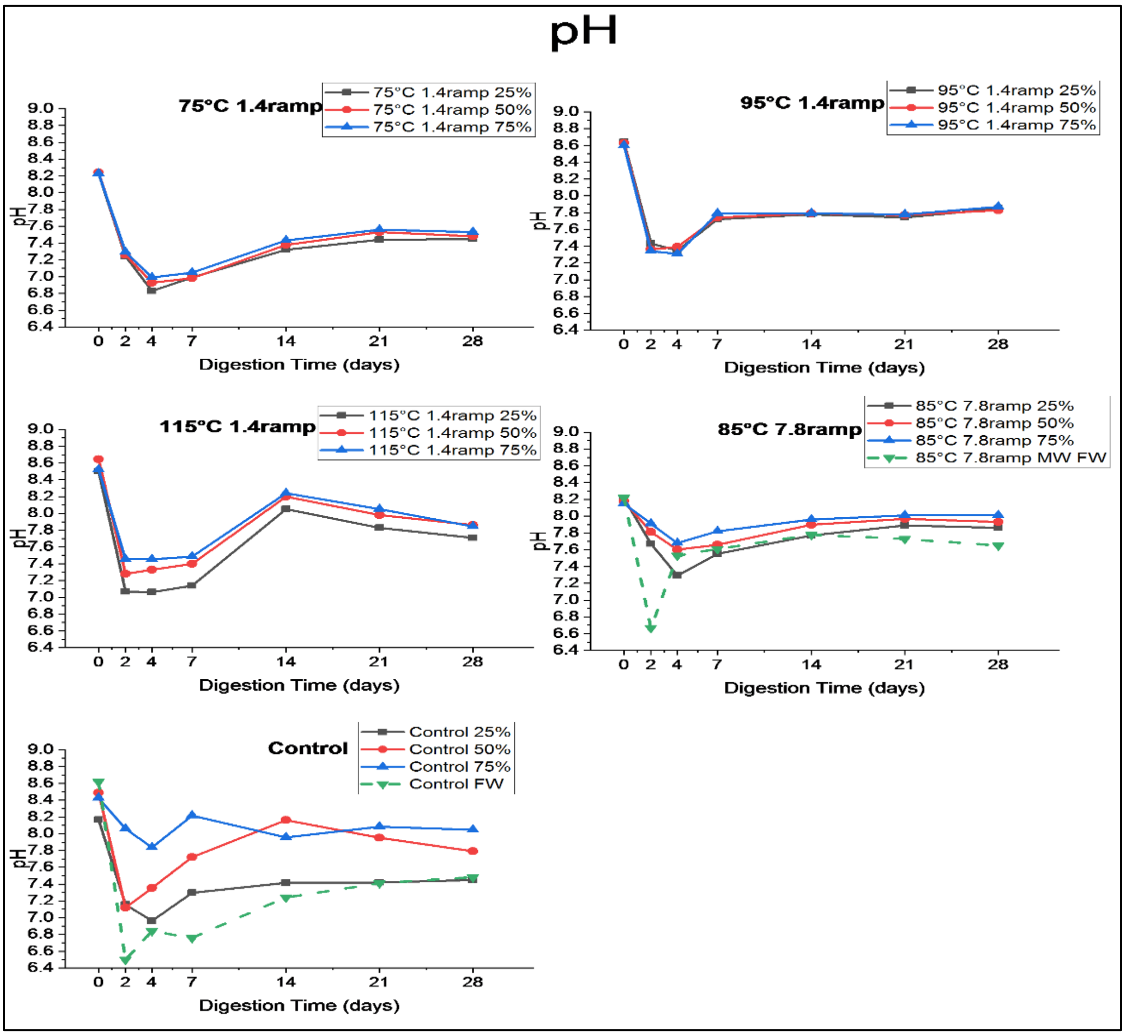


Figure 7.5 pH range during AD of microwaved digestate under different temperatures, ramp rates and phases, as well as controls.

Digestate thermally treated at 85ºC 7.8 ramp had a longer exposure to a lower pH, from the second to the seventh day of digestion. The largest decrease in pH occurred in the 25% volume digestate, changing from 8.21 to 7.29 on the fourth day of digestion. This period corresponded to the greatest TVFAs concentration (1993.26mg/L). These results contradict the relationship between TVFAs concentration and pH decrease, since the 75% volume digestate had the greatest maximum values of TVFAs during the accumulation period (2346.71mg/L), and a pH value of 7.68. A possible explanation for this finding is the higher ammonium concentration (8.85%) of these samples in relation to other temperatures tested. The 75% volume of digestate, would therefore contain proportionally higher amounts of ammonium. Ammonium acts as a buffer agent, hence reducing the decrease in pH drop in cases of elevated intermediate products.

An important observation was made when comparing the pH behaviour during the digestion process of microwaved digestate and microwaved food waste at 85ºC 7.8 ramp. It regards the lower pH of the microwave food waste during the hydrolysis phase (6.67 as opposed to 7.29 for the treated digestate) despite a lower TVFAs concentration (533.27mg/L as opposed to

1758.17mg/L of the treated digestate). In spite of co-digesting the raw food waste with the microwaved digestate, and for this reason having to deal with the common acidification of the system due to the rapid consumption of the labile fraction of the former waste, the re-circulation of digestate seems to have prevented great pH decrease under higher intermediate products concentration.

The acknowledgment of the digestate as a potential buffer agent in the co-digestion of several organic substrates such as food waste and press-waster it is not new. According to Nayono *et al.* (2010), the co-digestion of these substrates together with digestate, offers an increase in the buffering capacity of the system, thus avoiding the need for pH correction and/or a pH control system during the process, thus potentially favouring the process performance, and offering a better route for food waste digestion.

Untreated digestate (controls) on the other hand, showed a brief exposure to low pH, lasting from the second to the fourth day of digestion. The highest pH drop occurred for the 25% volume raw digestate, reaching a value of 6.96 on the fourth day and TVFAs concentration of 1644.56mg/L. In this case, the pH drop was significantly high, in relation to other reactors treating microwaved digestate, which had a higher pH value. It seems that although the untreated digestate is capable of acting as a buffer agent, this effect is not as intense as when treated digestate is present. This can be explained by the significant augmentation of ammonium contents when digestate is exposed to microwave radiation. This reinforces the buffer capacity of the system, avoiding a less sharp pH drop.

Overall, by re-circulating untreated digestate in the MAD of FW it was possible to reduce the acidification period. The shorter duration of a low pH resulted in greater removal of TVFAs and hence a smaller decrease in pH (Figure 7.5). These represent a major benefit of utilizing raw and specially treated digestate as a co-substrate during the AD of FW.

The pH of all reactors recovered after the 4th or 7th day of digestion, with the concomitant progressive TVFAs removal and further methane production. The TVFAs accumulation during the first days of digestion for all reactors, resulted in slow and poor methane production during the hydrolysis step in relation to when food waste was microwaved at different temperatures, and treated solely.

The percentage of the total gas generated for the whole digestion period, obtained between days 0 and 2 for digestate samples treated at 75°C 1.4 ramp varied by small amounts amongst the different volumes of treated digestate applied, and was as follows: 3.32, 3.04 and 2.48% for the 25, 50 and 75% volume of treated digestate reactor, respectively. The volume of gas produced between days 2 and 4 was even lower, as a result of the higher TVFAs concentration for that period. The proportions were 0.19, 0.18 and 0.69%, for the 25, 50 and 75% volumes of treated digestate, respectively. On the 7th day of digestion, during which there was the highest TVFAs accumulation, the proportion of total methane produced was 0.90, 1.83 and 1.52% for the 25, 50 and 75% volume of treated digestate respectively. The slow rate and low volume of methane production during the first week suggests that hydrolysis was negatively affected by the high accumulation of intermediate products (Figure 7.6).

When digestate was exposed to temperature of 95ºC 1.4 heating rate, there was a small improvement on process performance during the first days, with a better production rate and volume of biomethane. Again, there was little variation between the amounts of methane obtained at different proportions of treated digestate. This suggests that proportion of treated digestate is not a critical parameter for gas generation. The percentage of the total gas generated for the whole digestion period, obtained between days 0 and 2 for digestate samples with 25, 50 and 75% of treated digestate, respectively, was 10.62, 10.13 and 10.39%. Between days 2 to 4 the corresponding percentages were 2.43, 3.00 and 2.54%.

Digestate pre-treated at 115°C 1.4 ramp showed a small improvement in the biomethane production between days 0 and 2 in relation to those treated at 75°C, varying by small amounts between the reactors containing different volumes of treated digestate: 5.80, 5.74 and 5.60% for the 25, 50 and 75% volume of treated digestate respectively (Figure 7.6). A further 1.95, 2.83 and 2.38% of methane gas of the total gas generated for the whole digestion period was obtained between days 2 and 4.

When digestate was exposed to 85°C 7.8 ramp, the greatest methane production occurred on the first day of digestion rather than the second day as observed for the other temperatures, hence suggesting a better hydrolysis rate. The percentage of the total gas generated for the whole digestion period, obtained for the first day was: 8.95, 9.02 and 8.71% for the 25, 50 and 75% volume of treated digestate respectively, with the last reactor producing less biomethane due to an average of 17.04% more TVFAs in relation to the other reactors (25 and 50% volume treated digestate). Nevertheless, at the fourth day of digestion the volume of biomethane produced was similar to the other conditions with 2.94, 2.65 and 2.97% of the percentage of the total gas generated for the whole digestion period for the 25, 50 and 75% volume of treated digestate reactors, respectively. These results contrast with those observed for when food waste was exposed to microwaved radiation at 85°C 7.8 ramp. Under this condition, there was a faster and greater consumption of VFAs during the first days of digestion which resulted in enhanced methane production. The percentage of the total gas generated for the whole digestion period obtained between days 0 and 2 was 31.53%, and 64.20% between the days 2 and 4, representing a 250% and 2500% increase in the volume of biomethane generated in relation to when raw food waste was co-digested with microwaved digestate.

The earlier peak in methane production was also observed by Liu *et al.* (2019) when digesting wheat straw that had been previously treated with liquid digestate. The liquid digestate came from a small-scale biogas plant fed on chicken manure, and was applied to the wheat straw during several pre-treatment durations (3, 5 and 7 days). The rationale supporting the benefits of using the liquid fraction of the digestate relies on the fact that it contains abundant lignocellulosic degrading microbes and ammonium, which is expected to hydrolyse the hard-to-digest compounds such as cellulose, hence reducing the costs associated with synthetic chemical agents, whilst increasing gas production. Moreover, by re-circulating the liquid fraction of digestate it is possible to increase the rate of bioconversion of the substrate and reduce the amount of liquid digestate post-treatment. The authors reported two gas production peaks for the pre-treated wheat straw, as opposed to only one for the control straw. The first peak occurred immediately after digestion started, at day 1. There was a total of 197, 208 and 212 ml of gas produced for the 3, 5 and 7 days of pre-treatment, respectively. The second peak occurred on day 13, with 163, 181 and 173ml produced, for the 3-, 5- and 7-days of pre-treatment, respectively. These results suggest that the chosen pre-treatment is effective, offering greater amounts of substrate for biological conversion.

The slower methane production observed herein for the co-digestion process of MW digestate and raw FW at the first days does not necessarily mean that microwave pre-treatment of digestate is not effective, but rather that the presence of raw/untreated food waste slows the hydrolysis down, due to the system being overloaded with TVFAs, which are produced in extremely fast manner in response to the LOF of FW being easily degradable.

Untreated digestate (control samples) had a highest methane production on day 1 of digestion. The total gas generated for the whole digestion period produced by the 25, 50 and 75% volume of treated digestate was higher than that for 75 and 115°C with a 1.4 ramp. This again suggests these conditions result in inhibition. The volume of biomethane obtained for this period was 4.58, 6.92 and 8.72% for the 25, 50 and 75% vol. of untreated digestate, respectively. The subsequent point of TVFAs accumulation occurred at the fourth day, influencing biomethane generation for that period, with a less gas generation: 3.98, 4.52 and 8.49% produced by the 25, 50 and 75% vol. of untreated digestate, respectively (Figure 7.6).

The control digestate reactors, regardless of the final volume showed a better performance in terms of methane production during the first week of digestion than those treating raw FW solely (controls). There was a 2476% improvement in biogas production from the former condition in relation to the latter, which can be explained by the lower TVFAs concentration of the former in relation to the latter. If the application of microwave irradiation were determined solely by economics, therefore, the use of untreated digestate combined with untreated food waste in a co-digestion would offer a better option for the management of these wastes. However, as already discussed, the optimum conditions for an AD plant operation should consider several aspects of process performance.

Methane production was more intense between days 7 and 14 of digestion for most of the conditions tested. For the 75°C, 1.4 ramp reactors, the percentage of the TVFAs removed for this period varied between 35.94 06% and 48.83%. Acetic acid was the principal acid consumed, with its volume being reduced by up to 57.74%, resulting in a maximum of 657.7ml of methane produced for the 75% vol. of treated digestate reactor.

The TVFAs removal behaviour was different for the 95°C 1.4 ramp treated digestate reactors. An intense TVFAs removal occurred earlier in the digestion process, between days 4-7, regardless of the final volume of treated digestate used. This suggests a better kinetic between acidogenic and methanogenic bacteria in the reactor during this period, with a better hydrolysis rate and a lower inhibition rate of methanogenic bacteria due, for example, to a lower concentration of inhibitory compounds formed during the microwaving of the digestate.

The TVFAs consumption during this period varied between 75.00 and 79.09%. In all cases acetic acid was the main intermediate product consumed, reduced by an average of 93.00%. As a result, up to 370.00 ml of gas was obtained (by the 75% vol. of treated digestate reactor).

Nevertheless, the greater methane production occurred between days 7-14. The TVFAs removal for this period varied between 75.92 and 82.79%. Acetic acid was the principal intermediate product removed, with concentrations reduced up to 43.09% (25% vol. of treated digestate reactor), resulting in a maximum of 653.18ml of biomethane produced for the 75% vol. of treated digestate reactor.

Digestate treated at 115°C 1.4 ramp, regardless of the final vol. of treated digestate used showed enhanced TVFAs removal from day 7 of digestion. The consumption of TVFAs from day 7 to 14 varied from 78.31% to 97.11%, hence representing an improvement compared to the digestate treated at 75°C 1.4 ramp. Similar to other conditions, acetic acid was the main intermediate product to be consumed, having its concentration reduced by 97.56%, heralding high methane production for this period for all vol. of treated digestate tested. The greatest vol. of methane produced was for the 75% vol. of treated digestate reactor, which yielded 931.82ml.

It was noticed that the percentage of TVFAs removed for this period was not equivalent to the volume of gas produced. It can be therefore, postulated that there was methanogenic inhibition at the 50% vol. of treated digestate, since it produced 10.86% less methane than the 75% reactors, despite having 1.50% more TVFAs removed. The reduced methanogenic community activity could have resulted from the presence of Maillard reaction products together with the 75°C 1.4 ramp, as previously discussed.

Similar to the other conditions tested, the co-digestion of microwaved digestate exposed to 85°C 7.8 ramp together with raw food waste showed a high rate of intermediate products removal later in the digestion: between days 7-14. For this period, the intermediate product consumption varied between 85.41% and 98.00%, and in all cases acetic acid was the principal intermediate product consumed, reduced up to 98.20%. Consequently, up to 748.93ml of biomethane was obtained (75% vol. of treated digestate reactor).

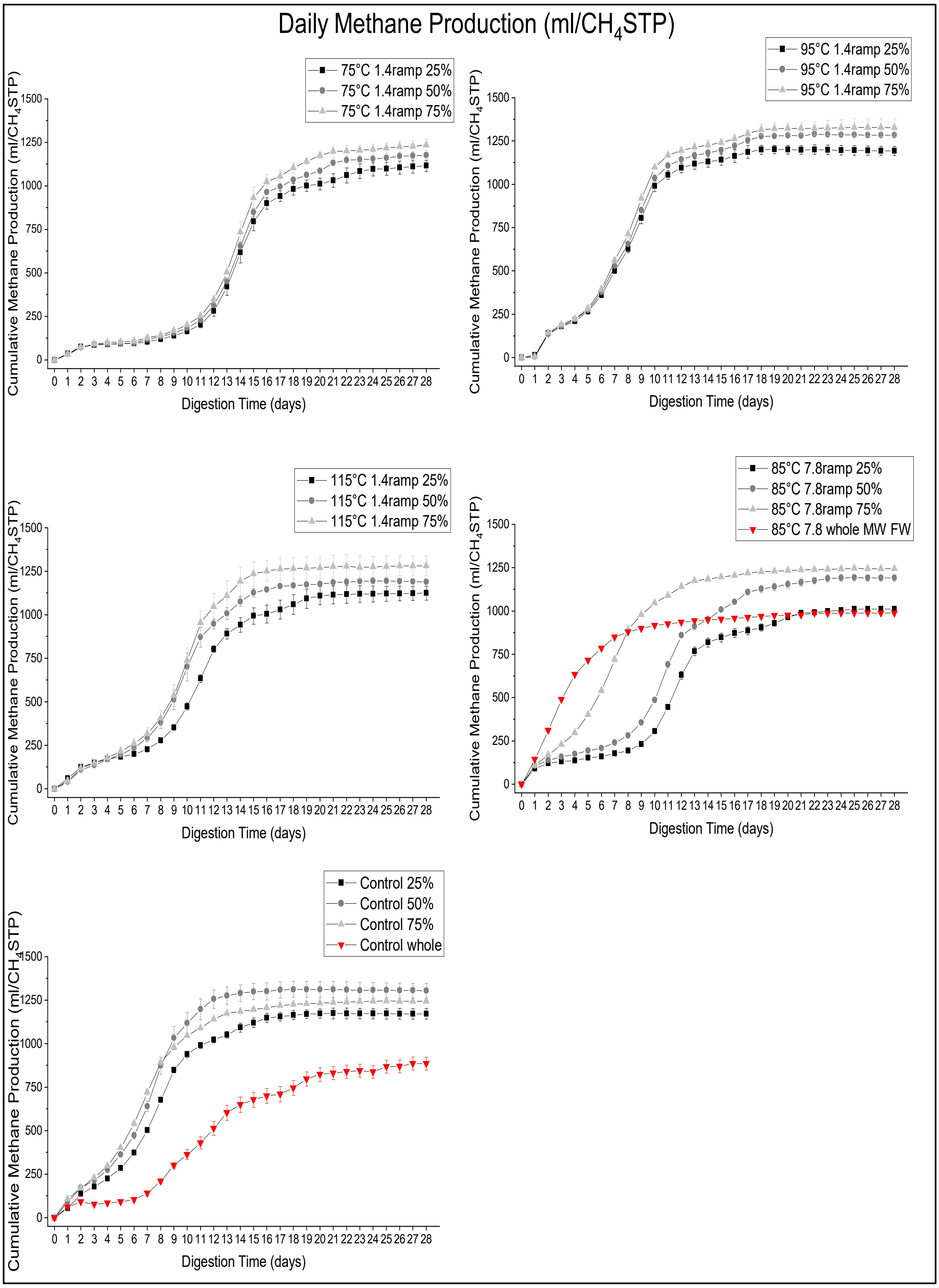


Figure 7.6 Daily methane production of microwaved digestate at different temperatures and heating rates, as well as controls.

Under this operational condition, another cycle of significant TVFAs removal occurred between days 14 and 21 for most of the reactors. Intermediate products reduction of 71.89% and 73.51% was observed for the 25 and 50% treated digestate, with a small increase in TVFAs concentration (9.28%) for the 75% vol. of treated digestate reactor. In contrast to the other conditions, acetic acid was not the principal acid to be consumed in this period, in fact showing an increase in concentration. The main intermediate product consumed in this case was propanoic acid, with reductions varying between 47.64-98.61%. The volume of biomethane produced for this period was equivalent to: 221.2ml, 254.35 and 265.07 for the 25 and 50% treated digestate, respectively. The aforementioned accumulation of acetic acid might have resulted from a combination of factors: a) an inhibition of the acetoclastic methanogens, and b) the shift to a 'range' where the degradation of propanoic acid is more thermodynamically feasible than that of acetic, possibly due to the fact that the propanoic degrading syntrophs are slow growing microorganism only becoming active later in digestion. This route for methanogenesis is discussed ahead.

Although several studies (Banks *et al.*, 2012; Yirong *et al.*, 2014; Capson-Tojo, Trably, *et al.*, 2017) have suggested that syntrophic acetate oxidation (SAO) and hydrogenotrophic methanogenesis (HM) are predominant pathways for methane production during AD of FW, propanoic acid has a good conversion yield rate. According to Stadtman and Barker, (1951), the decomposition of propanoic acid to carbon dioxide and acetic acid is usually performed by *Methanobacterium propanoicum*. Under complete degradation of this acid, three quarters of the CO_2, could be converted into CH_4, and a further volume of methane can be produced by conversion of acetic acid into CH_4 and CO_2, as follows:

$$4CH_3CH_2\,COOOH + 8H_2O \rightarrow 4CH_3\,COOH + 4CO_2 + 24H \tag{19}$$

$$3CO_2 + 24H \rightarrow 3CH_4 + 6H_2O \tag{20}$$

$$4CH_3COOH \rightarrow 4CH_4 + 4CO_2 \tag{21}$$

$$4CH_3\,CH_2\,COOH + 8H_2O \rightarrow 5CO_2 + 7CH_4 \tag{22}$$

When microwaved FW was treated under 85°C 7.8 ramp and further digested, different TVFAs removal behaviour than in the co-digestion process was observed. Methane production was more intense between days 2-4, after significant TVFAs removal (38.05%) with acetic acid reduced by 86.15%. During this period, a total of 489.08ml of biomethane was produced.

Better process performance of the digestion of MW FW treated at 85°C 7.8 ramp in relation to the co-digestion of raw food waste and digestate treated at 85°C 7.8 ramp is evidenced by improved methane production rate at the hydrolysis phase, demonstrating the effectiveness of the pre-treatment in enhancing organic matter solubilization. The same effect was not observed for the co-digestion conditions, possibly due to food waste being used in its raw/untreated form resulting in TVFAs accumulation associated with the rapid consumption of the labile fraction. Taking the results here shown into consideration, it is believed that the digestion process of microwaved digestate could be refined by the application of microwaved food waste instead of the raw form, despite the extra-costs associated with the pre-treatment step.

In contrast to most of the treated digestate reactors in which there was low methane production during the first days of digestion, the controls showed improved methane generation for the same period. Significant TVFAs removal was observed between days 4-7 of digestion with values ranging from 30.30% to 52.03%. Most of the intermediate product consumption referred to acetic acid removal, with values up to 70%, resulting in up to 424.91ml produced (75% vol. of untreated digestate reactor).

A second peak of methane production between days 7-14 was observed for all of the untreated digestate reactors. During this period TFVAs removal varied between 70.32% and 96.59%. Acetic acid was the principal TVFA consumed for the 25% vol. of treated digestate reactor, with its concentration reduced by 89.21%. For the remaining reactors, propanoic acid exhibited a higher removal rate for this period, equivalent to 98.58% and 99.34% for the 50 and 75% vol. of treated digestate reactors, respectively. As a result, 677.35, 817.69 and 643.53ml of methane was produced for the 25, 50 and 75% vol. of untreated digestate reactors, respectively.

Conversely, during the digestion of the raw food waste/control, 73.89% of TVFAs was removed between days 4-7, with acetic acid being reduced by 94.37%. This was higher than for the untreated digestate reactors. In spite of this, methane production was low, i.e. 111.29ml. This may have been due to acidification, which negatively affects methanogenesis. The TVFAs removal rate between day 7 and 14 of digestion was 90.36%, with propanoic acid being the principal intermediate product consumed. Methane production was greatest for this period with 545ml of biomethane produced. This suggests that that propanoic acid accumulation was the main inhibitory factor affecting methanogenic population in the first week of digestion when food waste was digested alone. Moreover, it can be said that overall, methane production rate during the co-digestion of untreated digestate and raw food waste was better than when raw food waste was digested alone (Figure 7.6).

Individual VFAs behaviour during the digestion of raw, microwaved FW, and sewage sludge (digested solely and in a co-digestion form) was briefly discussed in by Zhang *et al.* (2016). The authors reported that the VFAs profile and behaviour change significantly throughout the digestion period for the various conditions tested. During the co-digestion of microwaved food waste and raw sewage sludge, for example, acetic acid dominated the intermediate products from day 0-12, peaking on day 5. When raw food waste was co-digested with microwaved sewage sludge (under 1:1 I/S ratio), and when raw sewage sludge was digested alone, acetic acid did not accumulate, being consumed during the digestion period, with only small residual concentrations at the end of the digestion (241.48 and 149.80mg/L, respectively). Nevertheless, propanoic acid did not accumulate during the co-digestion of raw food waste and microwaved sewage sludge, nor for the raw sewage sludge.

This was not the case, however, when digesting microwaved food waste with raw sludge (1: 1 I/S ratio). In this case the authors observed that propanoic acid accumulated to high levels (6262mg/L) on day 26 of digestion, while acetic acid decreased between days 12-19, rebounding to 2103mg/L at the end of the process. Methanogenesis was negatively affected by the VFAs accumulation in this period, yielding poor process performance compared to microwaved sewage sludge.

The differences in VFAs behaviour during the co-digestion of raw food waste and microwaved digestate, in which there was acetic acid and propanoic acid accumulation for most of the reactors of study reported here (in contrast to that reported by Zhang *et al*. (2016)), could be explained by an inadequate I/S ratio. Despite functioning well for the digestion of raw FW and microwaved FW, it is believed that when performing co-digestion of FW and MW digestate, the I/S ratio of 3:1 used previously needs to be adjusted. This is because the higher amounts of thermally treated substrate used here (up to 75% vol. of digestate) in substitute of the distilled water content of the reactors, could have overloaded the system, and exceeded the capacity of the methanogenic community to process the significant amount of VFAs coming from the food waste and treated digestate. It would be important, therefore, to adjust the I/S ratio prior to the AD process, increasing the inoculum concentration.

As previously mentioned in Chapter 4, section 4.4.4, propanoic/acetic acid ratios (p/a) greater than 1.4 can be used as a tool for stress detection in the system (Hill *et al*., 1987). This ratio was therefore monitored during the co-digestion of food waste and treated digestate.

For the digestate treated at 75°C 1.4 ramp, the only condition that showed a p/a ratio above the recommended threshold was the 25% vol. of treated digestate on day 21 of digestion, with a value of 5.37 (Figure 7.7).

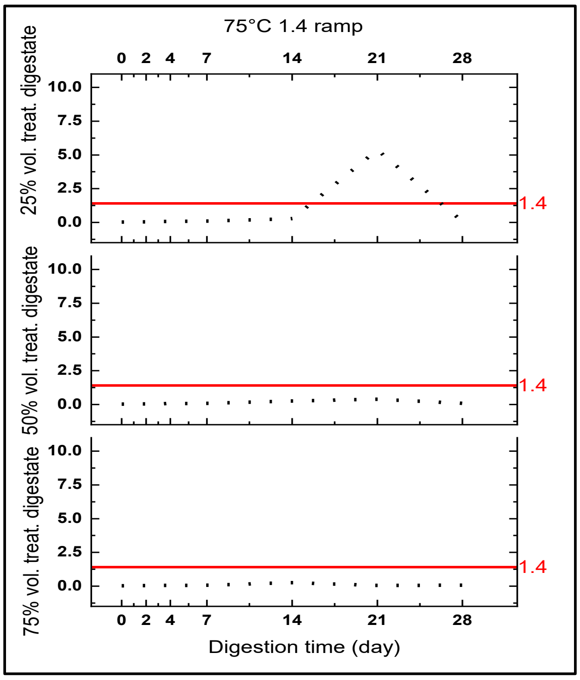


Figure 7.7 p/a ratio of microwaved digestate under 75°C 1.4 ramp and different volumes.

The p/a peak can be explained by the rapid removal of acetic acid (95.80%) coupled with propanoic acid slow metabolization, due to a less pronounced removal of this acid (17.58%) in relation to other reactors (94.94 and 99.20 for the 50 and 75% vol. of treated digestate, respectively). Despite this, methane production for this period was not affected for this period.

When digestate was exposed to 95°C 1.4 ramp, the recommended threshold was exceeded for all the volumes of treated digestate. In general, there was a single p/a peak for all the conditions that occurred on day 7 of digestion. The greatest p/a value (2.29) occurred when 50% vol. of treated digestate was co-digested with raw food waste, and the least prominent peak occurred for the 75% vol. of treated digestate reactor, with a value of 1.99 (Figure 7.8).

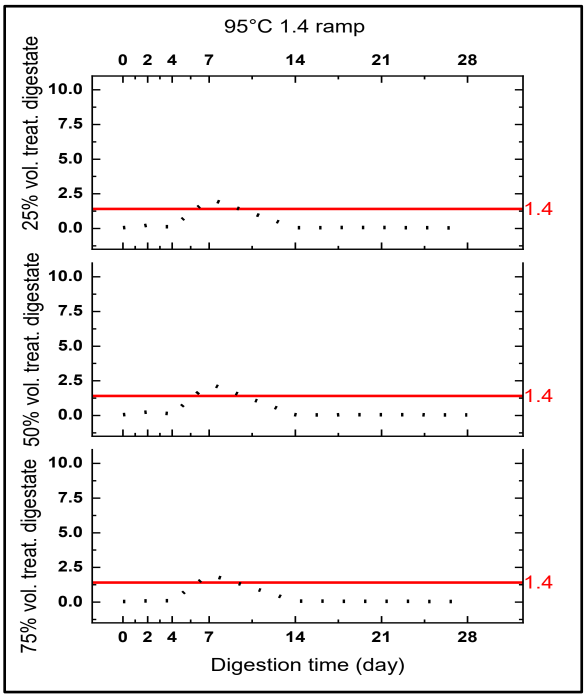


Figure 7.8 p/a ratio of microwaved digestate under 95°C 1.4 ramp and different volumes.

The values above the recommended threshold were all due to propanoic acid accumulation and thus, inhibition of propanoic degrading syntrophs activity. There was an increase of the propanoic acid between days 4-7 for all the treated volumes of digestate, equivalent to 44.59%, 33.01% and 30.69% for the 25 and 75% vol. of treated digestate reactors, respectively. Because the p/a ratio values were similar for all the different volumes of treated digestate, it can be postulated that the propanoic syntrophic inhibition was unlikely to be caused by the presence of inhibitory compounds originating from the thermal treatment, but rather from an unknown intrinsic factor, other than pH, since in all cases, this was within a neutral range. As previously shown, the methane production for this period was not affected.

Similar to the 75°C 1.4 ramp treated digestate, the 115°C 1.4 ramp reactors showed a single point of for which the recommended threshold for p/a was exceeded, also at 25% vol. of treated digestate. The peak was observed on day 14 of digestion, with a value of 8.28 (Figure 7.9). The elevated p/a value observed for this point was caused by rapid acetic acid removal (96.95%) followed by an accumulation of propanoic acid (33.68% increase in concentration between days 7-14 of digestion). This is in contrast to the other vol. of treated digestate reactors exposed to 115°C 1.4 ramp on which there was an absence of propanoic acid accumulation for this period. Despite higher p/a value, the methane production for this period was not affected.

With the exception of 75% volume of treated digestate, all other conditions showed an elevated p/a ratio values when digestate was treated at 85°C 7.8 ramp. The greatest p/a ratio occurred for the 25% vol. of treated digestate, on the day 14 of digestion, with a value of 9.10. This was the largest p/a of all conditions tested, regarding co-digestion process. The least prominent p/a ratio for this condition occurred for the 50% vol. of treated digestate reactors, also on day 14, with a value of 5.0 (Figure 7.10).

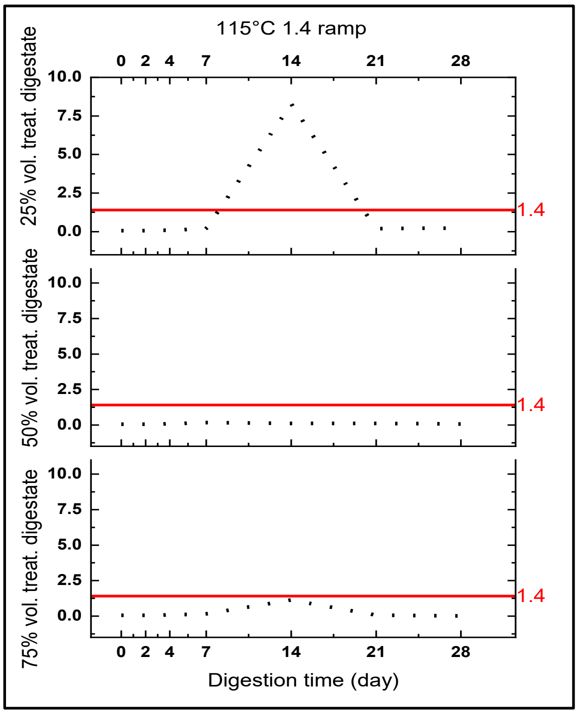


Figure 7.9 p/a ratio of microwaved digestate under 115°C 1.4 ramp and different volumes.

The differences in p/a ratio reflected the propanoic acid metabolization and concentration, which in the 25% vol. of treated digestate reactor showed an increase of 18%, as opposed to the 50% vol. of treated digestate reactor in which there was a reduction of 43% on this acid. Moreover, due to accumulation of the propanoic acid in the former condition, its value was significantly higher than the acetic acid, which was removed at similar rate (98.20%) as the 50% vol. of treated digestate reactors (97.97%), hence leading to a higher p/a ratio of the 25% vol. of treated digestate.

The p/a ratio of the digestate treated at 85°C 7.8 ramp and 25% vol. was significantly higher than that observed at 75°C with a 1.4 ramp, same volume of treated digestate. This is despite the former having a higher pH value (7.77) than the later condition (7.44) at the peak of the p/a ratio. This excludes the possibility of propanoic synthrophs inhibition by a low pH (acidic) range. Another factor, therefore, must have been responsible for the elevated p/a ratio of the 85°C 7.8 ramp and 25% vol. sample. This could be ammonia concentration, for example, or even the hydrogen pressure inside the reactors.

Due to an increase in ammonium after microwave irradiation of digestate, a correlation between its concentration and propanoic acid degraders was taken into consideration. Ammonium inhibition of microbial community including those responsible for propanoic acid degradation has been previously reported by several authors (Li *et al*., 2017; Peng *et al*., 2018; Yang *et al*., 2018).

Bonk *et al*. (2018) evaluated the effects of ammonia increase on the degradation efficiency of three volatile fatty acids (VFAs), namely acetic, propanoic and butyric, by feeding an increased ammonium bicarbonate concentration in the influent with several concentrations, varying from 52 to 277mM. They report that while butyric acid degradation was hardly affected by the increase of ammonia concentration, propanoic acid degradation efficiency was even more inhibited (65% decrease) than acetic acid degradation (31% decrease).

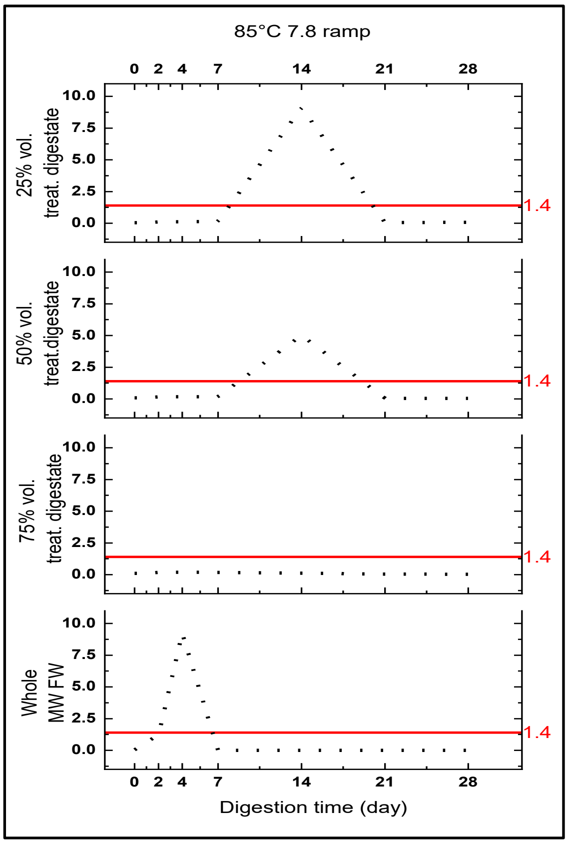


Figure 7.10 p/a ratio of microwaved digestate and food waste under 85°C 7.8 ramp and different volumes.

In this study, ammonium concentration increase was investigated as a basis to justify the discrepancy of p/a results shown for the 25% vol. of treated digestate reactors at 75°C 1.4 ramp and 85°C 7.8 ramp. Under these conditions, ammonium concentration at the peak of the p/a values were similar, with 1511mg/L for the former and 1149 for the latter, hence not sufficiently strong to justify the discrepancy. The remaining explanation would be a high hydrogen partial pressure, unfavourable for propanoic acid degradation. The influence of hydrogen partial pressure on the degradation of this acid is well-known and, according to Dong and Stams (1995), this intermediate product requires a low hydrogen pressure to shift the reaction stoichiometry towards degradation by syntrophic organisms. However, because hydrogen partial pressure was not measure here, this hypothesis cannot be validated. Nevertheless, it raises the need for monitoring of this parameter when future application of the co-digestion process is done.

When food waste was exposed to microwave radiation under 85°C 7.8 ramp, there was also a single point of increased p/a value. It occurred at the beginning of digestion on day 4, with a value of 9.22. This was similar to the 25% vol. of treated digestate reactor. Nevertheless, in contrast to the treated digestate reactor in which the elevated p/a ratio is believed to be caused by unfavourable thermodynamics determined by, for examples, high hydrogen pressure, or acetic acid accumulation, in the microwaved food waste condition, the high p/a ratio was most likely caused by an increased acetoclastic activity, meaning rapid removal of acetic acid form the system, motivated by the higher solubilization of the treated food waste, coupled with propanoic acid accumulation.

The control digestate reactors showed a similar behaviour to most of the microwaved digestate reactors, with a trespassing p/a value for the 25% vol. of treated digestate reactors (5.26) at day 14th of digestion. Similarly, the untreated food waste/control, showed a p/a ratio of 4.61 (also exceeding the recommended threshold) on day 14 of digestion (Figure 7.11).

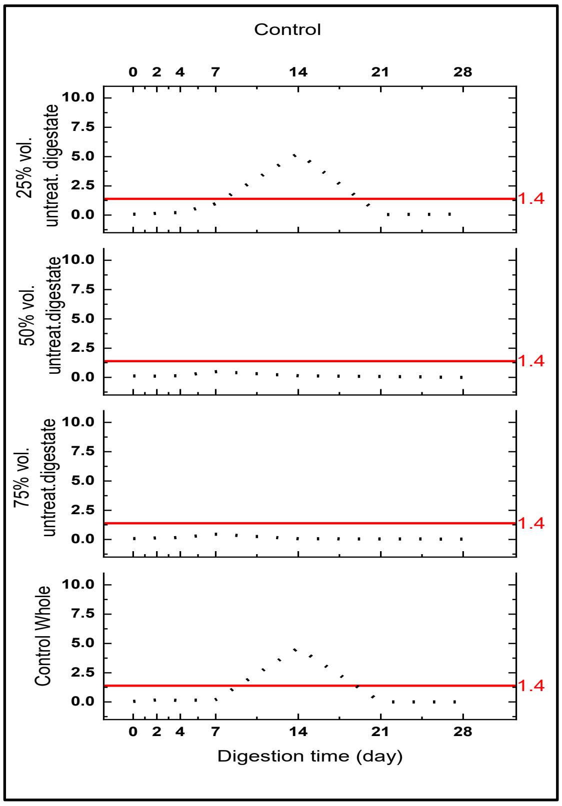


Figure 7.11 p/a ratio of untreated digestate at different volumes and untreated food waste.

Contrary to the control 25% vol. of treated digestate where the p/a ratio became exceeding despite reduction of propanoic acid by 36%, the control FW showed propanoic acid accumulation with an increase in concentration of 30%, suggesting a greater inhibition of the propanoic degrading syntrophs. The inhibition of these organisms in the control food waste could have been a result of a longer period of lower pH, due to system acidification, and acetic acid accumulation.

The accumulation of propanoic acid during the digestion of food waste has been previously shown. Capson-Tojo, Ruiz, *et al.* (2017) reported the accumulation of propanoic acid during the consecutive batch digestion of commercial food waste. According to them, although an initial accumulation of VFAs (mainly acetic acid) is common, during batch AD of FW, there was a transient acetic acid concentration of 17.2g/L during the first days after reactor loading. This suggested that in all the reactors the systems were mostly within a range where the degradation of this acid was more thermodynamically favourable than that of propanoic (region to the right of the vertical red line in Figure 7.12).

Nevertheless, there was a point in the digestion where the authors noticed propanoic acid accumulation. The hypothesis formulated to explain this finding was that Syntrophic Propionate Oxidation (SPO) was not thermodynamically favourable due to the high concentrations of acetic acid and hydrogen or formate in the reactors. Therefore, when acetic acid concentration becomes too high, it jeopardizes the growth of syntrophic propionate, which are slow-growing microorganisms (De Bok *et al*., 2004), further leading to the accumulation of propanoic acid.

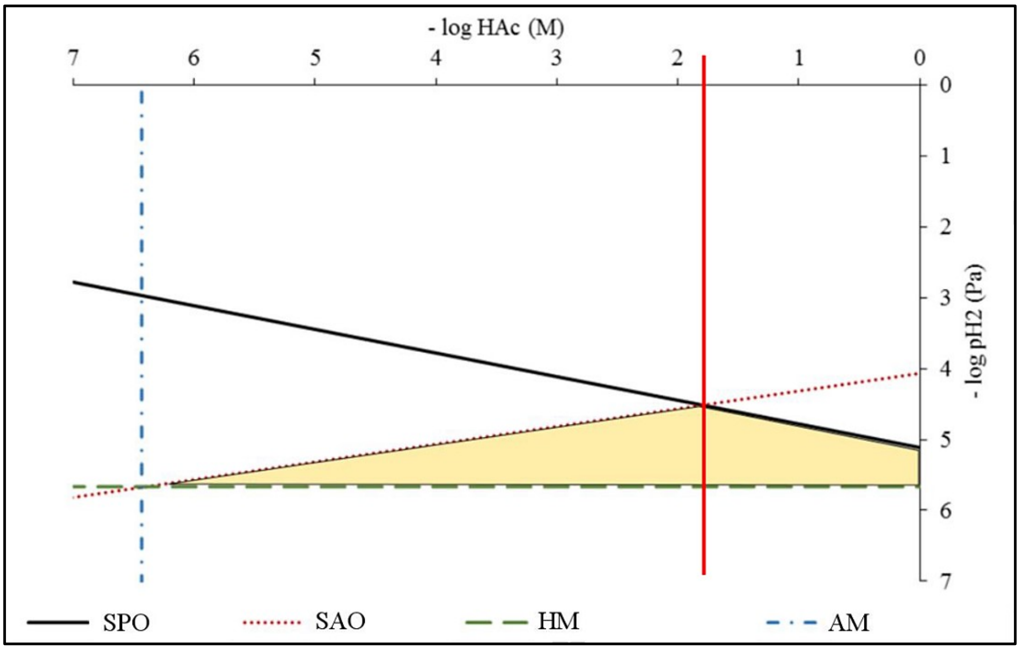


Figure 7.12 Lines of zero ΔG' for the reactions at different acetate concentrations and hydrogen partial pressures. They were calculated assuming 298 K, pH 7, 1 mM HPr (Propanoic) and 0.1 M $HCO3^-$. SPO, SAO, HM, AM and HAc stand for syntrophic propionate oxidation, syntrophic acetate oxidation, hydrogenotrophic methanogenesis, acetoclastic methanogenesis and acetic acid, respectively. Capson-Tojo, Ruiz, *et al*. (2017)

In this study, it is possible that the consecutive high acetic acid concentration (above 800mg/L) when the peaks happened observed for most of the conditions tested influenced the propanoic acid accumulation (Figure 7.13).

These results strongly suggest that despite removing the acidification of the system for most of the co-digestion conditions here tested by combining treated digestate with raw food waste, p/a exceeding peaks can still be observed, especially for a low volume of treated digestate. In none any of the cases, however, does this seem to have had a detrimental effect on methane production.

Because of the degradation of propanoic acid being one of the rate-limiting factors for the whole process, parameters such as pH, hydrogen partial pressure and ammonia concentration should be constantly monitored throughout the process, for a better understanding of these interactions with the propanoic degrading syntrophs.

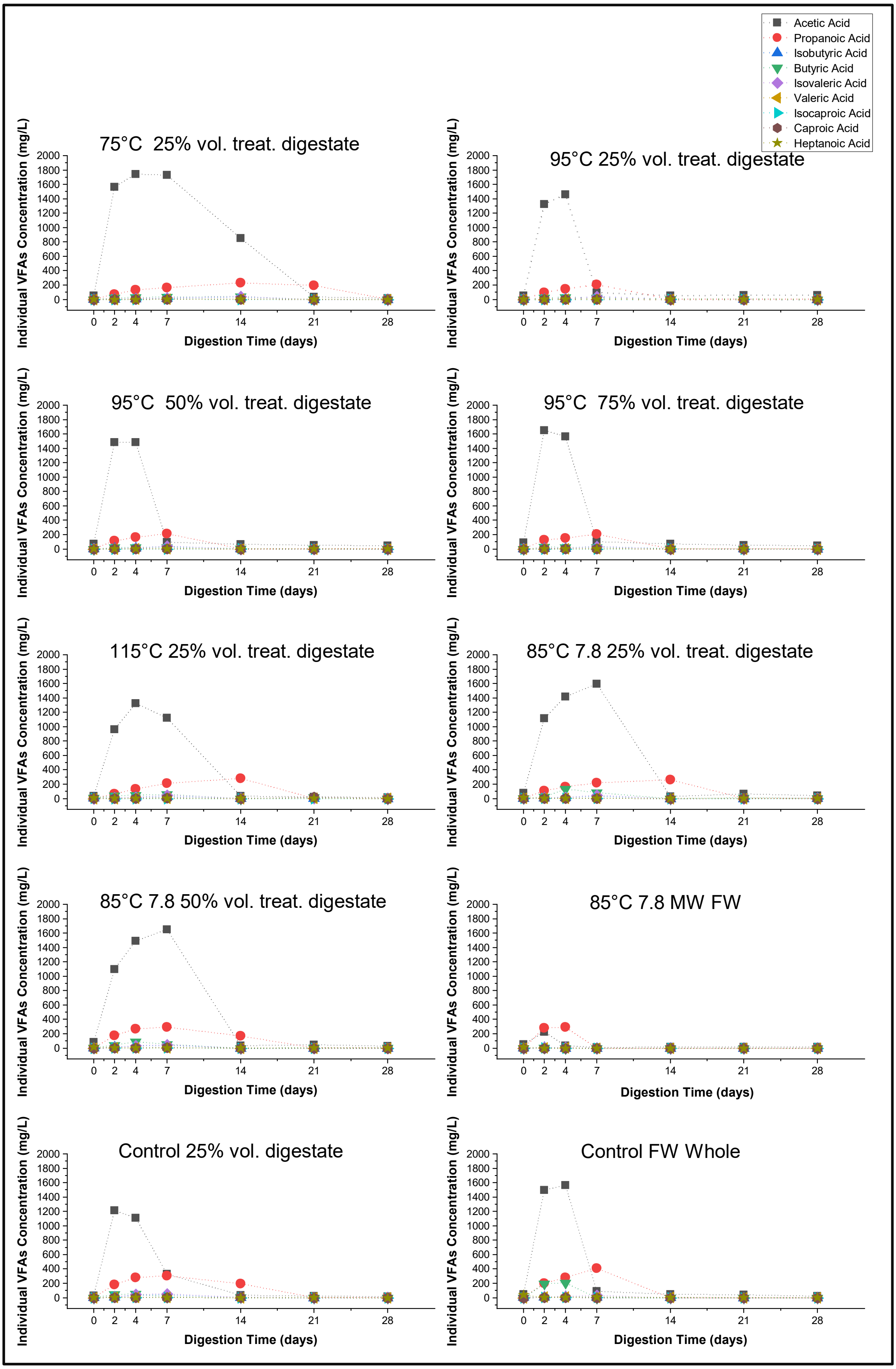


Figure 7.13 Individual VFAs concentration of various conditions tested, including microwaved FW and microwaved digestate, during AD process.

7.4 TVFA to alkalinity ratio during the re-circulation of microwaved digestate on the MAD of FW

When thermally treated digestate and untreated digestate were re-circulated to the anaerobic digestion of raw FW, process stability in terms of the TVFAs/Alkalinity ratio was improved to values lower than the recommended threshold for most of the conditions tested, in relation to when untreated FW was digested alone (Figure 7.14).

For the 75°C 1.4 ramp, the greatest TVFAs/Alkalinity ratio (0.37) occurred for the 25% vol. reactor, during day 4 of digestion. For the 95°C 1.4 treated digestate the greatest TVFAs/Alkalinity ratio (0.28) was observed for the 75% vol. of treated digestate reactors, on day 2 of digestion. In both cases the acidity/alkalinity of the system was within the stable range for the bacteria to perform organic matter conversion representing an improvement in relation to the digestion of food waste alone. The better process performance could have been caused by an increased buffer capacity of the co-digestion due to the addition of thermally treated and untreated digestate which are known to act as a buffer.

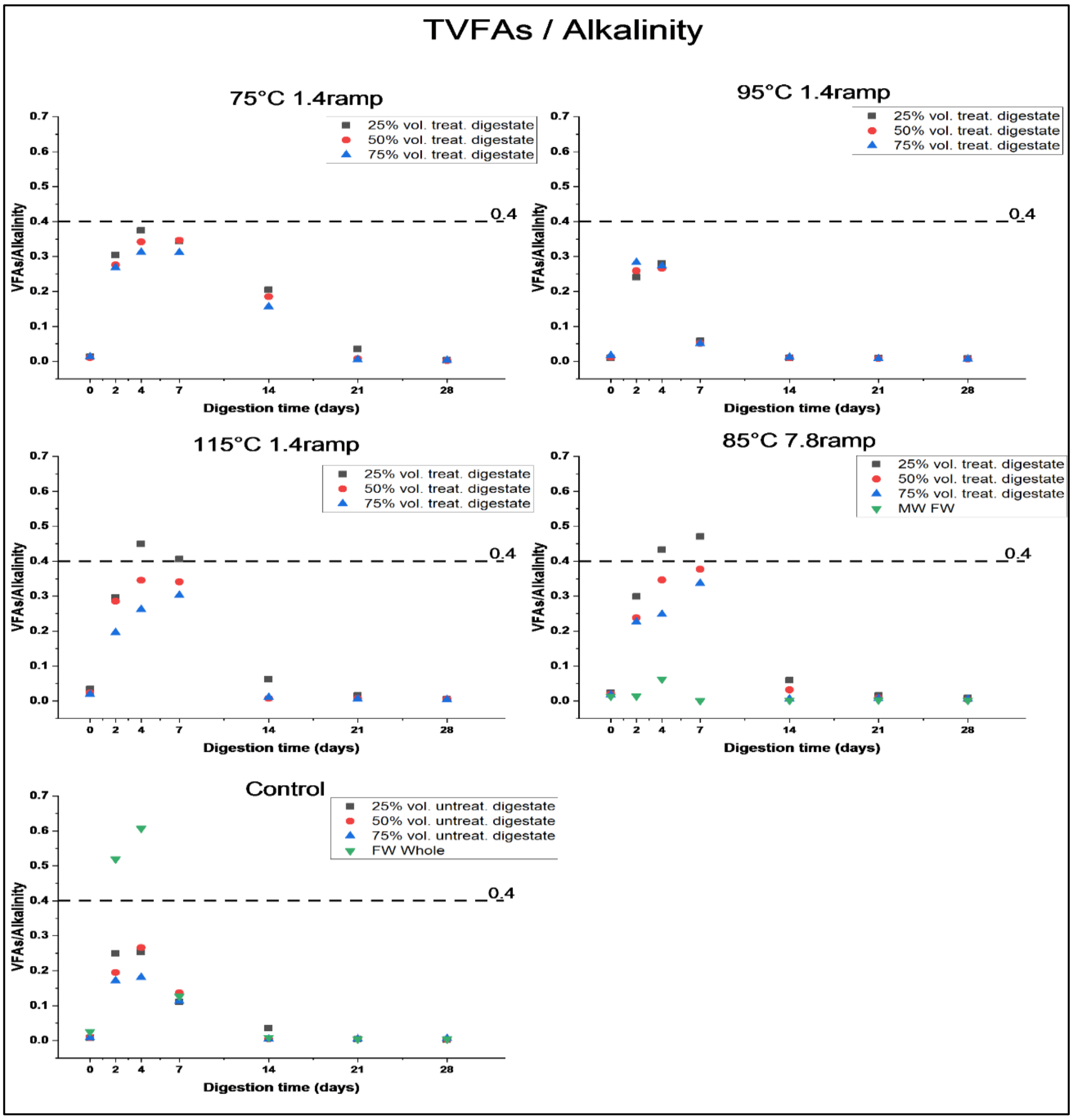


Figure 7.14 VFAs/Alkalinity ratio of microwaved and untreated digestate under various temperatures and volumes including microwaved and control food waste.

Nevertheless, the co-digestion of raw food waste and treated digestate at 115°C 1.4 ramp and 25% vol, showed two consecutive ratios exceeding 0.4 at day 4 and 7 of digestion, with values of 0.44 and 0.41, hence suggesting that attention should be paid to the process in case of possible instabilities.

The increase of the TVFAs/Alkalinity ratio in both cases occurred due to an increase of acidity of the system occasioned by the TVFAs accumulation accompanied by low alkalinity in relation to other temperatures (Figure 7.15). The low alkalinity range (3413.33 – 3975.00ml $CaCO_3$/L) throughout the co-digestion of 25% vol. treated digestate and food waste was not observed for higher volumes of treated digestate reactors. This suggests that a component of the treated substrate was able to increase the alkalinity when present in higher amounts. The component is believed to be ammonium for reasons explained below.

As previously discussed, the ammonium concentration as a result of thermal treatment varied amongst the treated samples with higher concentrations at 85°C 7.8 ramp, and lower at 115°C, 1.4 ramp. The lower concentration of ammonium in the latter condition could explain the low alkalinity levels, and hence buffering capacity, of the system. This is, nevertheless, capable of being corrected once a higher volume of treated digestate is applied (Figure 7.15).

Bouallagui *et al*. (2009) also reported a relation between alkalinity and ammonium during the co-digestion of fruit and vegetable waste (FVW) and different substrates, including: fish waste (fw), abattoir wastewater (AW) and waste activated sludge (WAS) in anaerobic sequencing batch reactors (ASBR). The authors reported that the only co-substrate that yielded an improvement in process stability was fish waste. When mixed with vegetable waste, the average TVFAs/Alkalinity ratio was 0.28, allowing stable anaerobic digestion without the addition of alkaline chemical agents. They report that the increase in alkalinity could be due to generation of NH_4^+ during the digestion of protein in fish waste which resulted in an increased digester buffering capacity and hence, stability of the digesters.

85°C 7.8 ramp at 25% vol. of treated digestate reactors also showed consecutive TVAS/Alkalinity values above the recommended threshold of 0.4. These occurred at day 4 and day 7 of digestion, with values of 0.43 and 0.47, respectively. In this case, the alkalinity levels 4068.33 – 4236.67ml $CaCO_3$ / L) were not as low as in the 115°C 1.4 ramp 25% vol. of treated digestate, however considered significantly lower than the 50 and 75% vol. of treated digestate reactors at the same temperature, which again reflected the ammonium concentration for each condition (Figure 7.15).

These results suggest that although the ammonium concentration showed a more prominent increase for the digestate treated at 85°C 7.8 ramp after thermal treatment, the values were still insufficient to provide an adequate buffer for low volumes of treated digestate (25%). This condition should, therefore, be monitored during AD and corrective measures adopted in case of acidity versus alkali imbalance.

For most of the treated digestate reactors, the alkalinity levels fell within the ideal range reported for properly operating digesters, i.e. 4000 – 7000mg/L, with an average value of 6.500mg/L. (Sharma *et al*., 2000; Chen *et al*., 2008).

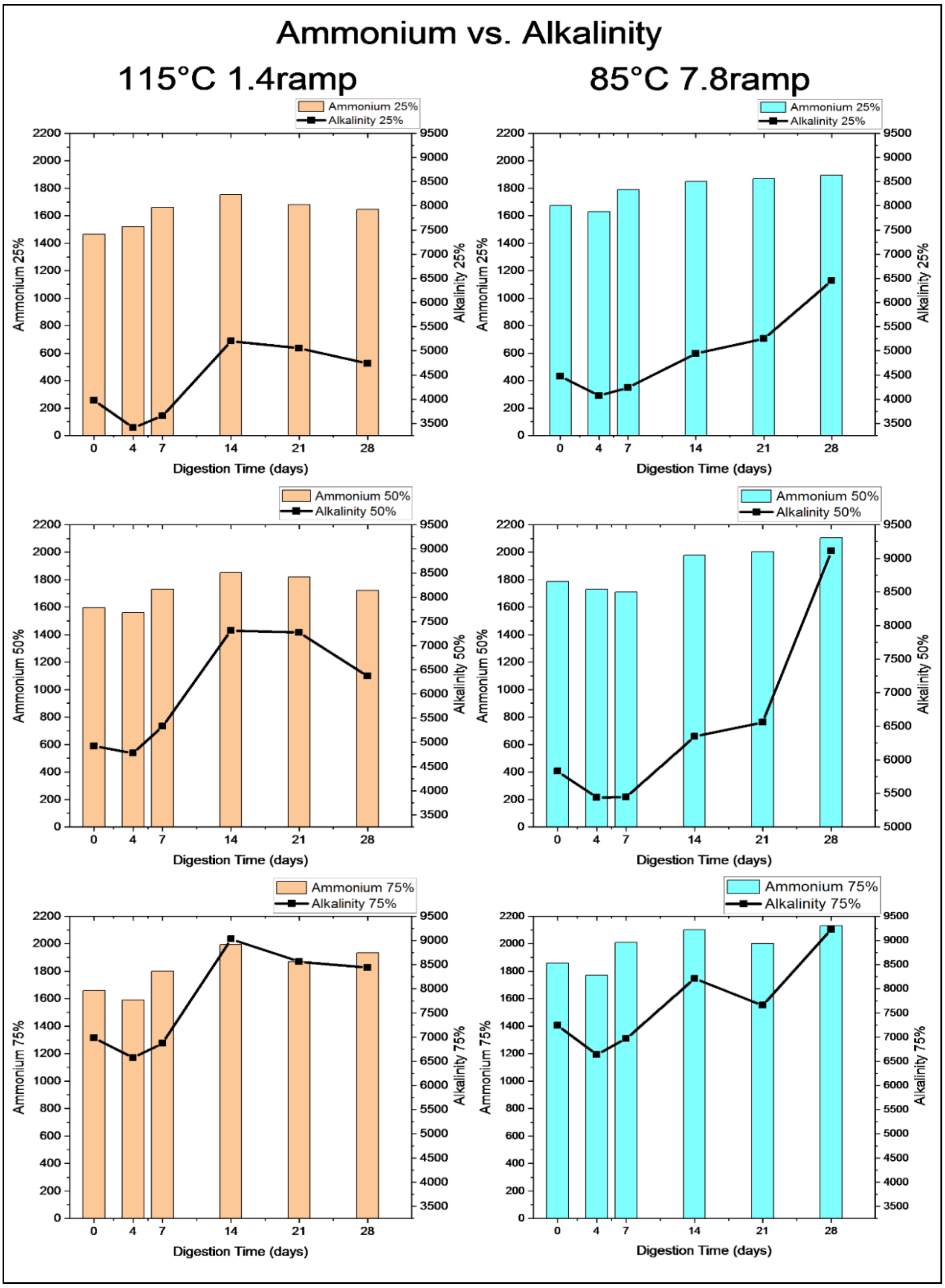


Figure 7.15 Relation between ammonium concentration and alkalinity in the co-digestion of raw food waste and microwaved digestate under 115°C 1.4 ramp and 85°C 7.8 ramp at different volumes.

7.5 Biodegradability of microwaved digestate and controls

Biodegradability was measured to evaluate the effectiveness of the re-circulation of thermally treated digestate on the MAD of FW.

Figure 7.16 shows biodegradability percentage achieved for each set of microwave operational conditions tested. Amongst the samples treated at 75°C 1.4 ramp, the 25% vol. of treated digestate reactor showed the greatest biodegradability percentage (80.18%) of the food waste TMP. This result represents a 132.43% improvement in relation to the raw food waste/control. The 50% vol. of treated digestate reactor showed a biodegradability of 57.03%, corresponding to a 65.32% improvement in relation to the biodegradability of raw food waste. The 75% vol. of treated digestate reactors showed a lower biodegradability (36.31%) and similar to that for the raw food waste (34.50%).

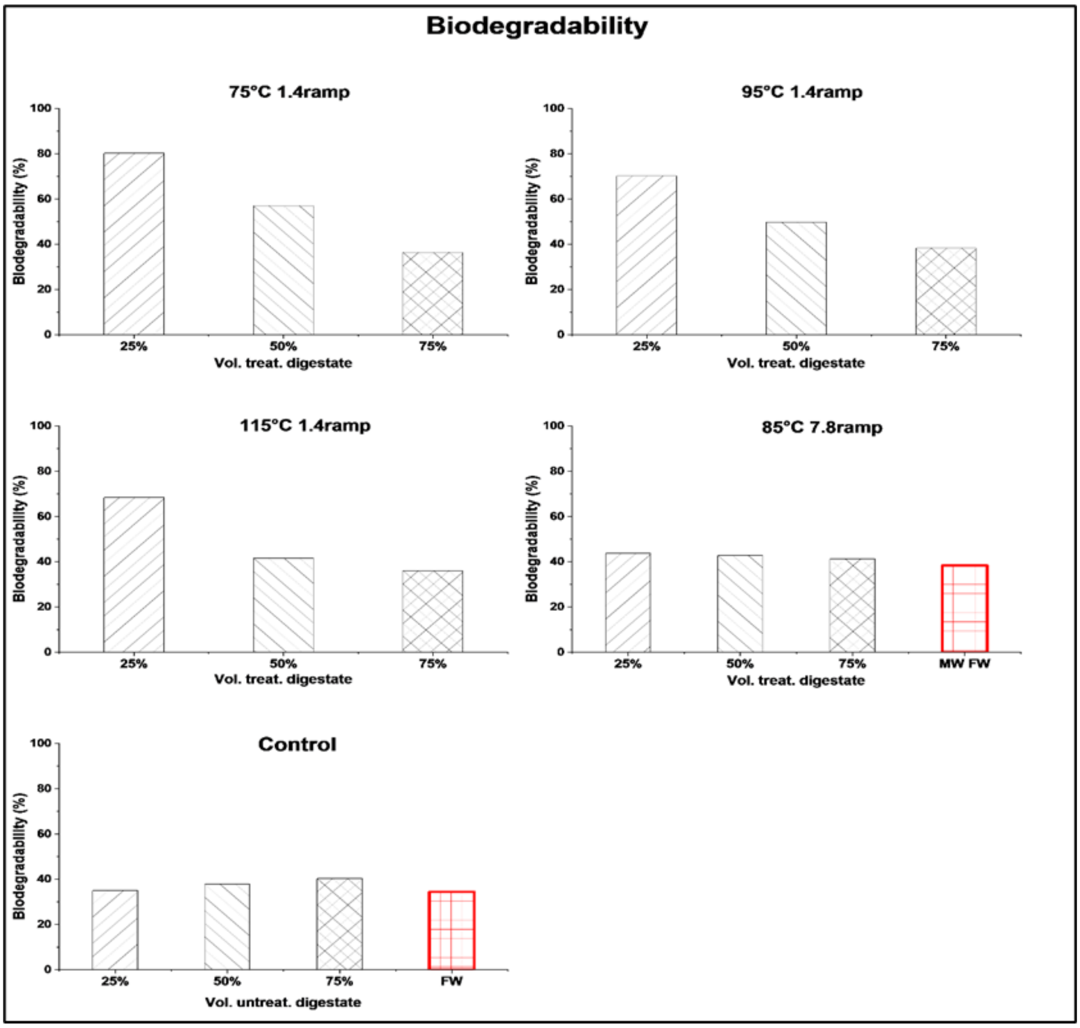


Figure 7.16 Biodegradability of microwaved digestate at various temperatures and ramp rates, as well as MW FW (85°C 7.8 ramp) and raw FW.

Digestate pre-treated at 95°C 1.4 ramp and re-circulated with food waste resulted in a biodegradability of 70.13, 49.79 and 38.31% for the 25, 50 and 75% vol. of treated digestate reactors, respectively. Despite representing a smaller biodegradability percentage than the samples treated at 75°C 1.4 ramp, these results are still better than the biodegradability of raw food waste digested alone. The improvement in relation to raw FW/control is 103.28, 11.05 and 9.95% for the 25, 50 and 75% vol. of treated digestate reactors, respectively.

Similarly, to the other operating conditions, digestate pre-treated at 115°C 1.4 ramp resulted in better biodegradability than the one for raw food waste. The biodegradability was 68.39, 41.57, and 36.02% for the 25, 50 and 75% vol. of treated digestate reactors, respectively, and represented a 98.24, 20.49 and 4.41% improvement in relation to the raw food waste biodegradability.

Overall, the increase of microwave temperature, exposure time and volume of treated digestate caused a detrimental effect on methane yield and consequently, biodegradability. In some cases, the low biodegradability at 75% vol. of treated digestate could have been the result of several factors, including the presence of inhibitory compounds (in the case of 75 and 115°C samples), or low methanogenic activity due, for example, to inadequate I/S ratio, as previously hypothesized.

These results are in agreement with others reported in the literature. Bougrier *et al*. (2008) and Carrère *et al*. (2009) investigated thermal hydrolysis with temperatures ranging from 60 to 210ºC with six different waste-activated sludge samples from municipal and industrial wastewater treatment plants working at high load, medium load, or extended aeration conditions. The authors reported that MAD biodegradability was related to sludge type and that greater organic content was associated with higher AD biodegradability. Moreover, it was found that biodegradability of samples increased with pre-treatment temperature up to 190ºC, but decreased (marginally) after this, suggesting the release, or formation, of recalcitrant or inhibitory compounds at the higher temperatures.

The digestate pre-treated at 85°C 7.8 ramp showed a less prominent increase in biodegradability with respect to the untreated food waste for all vol. of treated digestate tested. The biodegradability was 43.72, 42.81, and 41.21% for the 25, 50 and 75% vol. of treated digestate reactors, respectively. The improvement in relation to the untreated food waste was small, i.e. 24.09, 16.29 and 19.47% for the 25, 50 and 75% vol. of treated digestate reactors, respectively. These results suggest that, in contrast to the other temperatures and ramp rate tested, the volume of digestate used in the reactors did not have a strong impact on methane yield. This was probably due to the low concentration (or even absence of) inhibitory compounds as a result of a shorter exposure time to microwave radiation. The reduced time of the digestate in the microwave, could, in this case, have been beneficial because it offered some improvement in terms of organic matter solubilization without the negative effects caused by producing methanogenic inhibitory compounds (Maillard reaction products).

There was an inverse relation between volume of treated digestate and biodegradability for the digestate pre-treated using a 1.4 ramp (regardless of temperature). In contrast the controls had a direct relationship between these quantities. The 25% vol. of treated digestate reactors showed 34.98% biodegradability, the 50% vol. of treated digestate reactors had 37.79% biodegradability and the 75% vol. of treated digestate reactor had 40.24% biodegradability, representing a 1.39, 9.54 and 16.64% improvement in relation to the raw food waste. Therefore, the controls digestate fulfils the purpose of applying a maximum volume of digestate on the re-circulation process, reducing the volume of the end-product before releasing it to land use.

7.6 Specific hydrolysis during the re-circulation of thermally treated digestate in the MAD of Food waste

Microwaving is a novel method to thermally pre-treat organic substrates such as sludge. In addition to increasing organic matter solubilization, it can control pathogens (Coelho *et al.*, 2011). Moreover, the athermal effect of MW, responsible for breaking of hydrogen bonds leading to denaturation and death of bacteria when the polarized parts of macromolecules align with the electromagnetic field (Loupy, 2002). In this sense, microwave pre-treatment can have a detrimental effect on the various bacterial populations (i.e. fermentative, methanogenic), hence affecting hydrolysis and methane production.

The specific hydrolysis rate considers the suspended/particulate COD (an indirect measure of bacteria volume in a sample) and the volatile solids destroyed. Therefore, it provides information about the bacterial activity during the digestion process. And because in this study it was estimated at various points of the digestion process, including day 0, it helps to understand the origin of the hydrolysis (from the thermal treatment or enzymatic activity of bacteria). The specific hydrolysis rate is generally considered a first-order process and can be calculated using a COD mass balance (Schmit and Ellis, 2001; Puchajda and Oleszkiewicz, 2006) according to the following equations:

$$\text{Specific hydrolysis rate} = \frac{\left(\frac{\text{mass pCOD}}{\text{day}}\right)_{\text{Day 0}} - \left(\frac{\text{mass pCOD}}{\text{day}}\right)_{\text{Day 28}}}{\text{mass of volatile solids within reactor}} \qquad (23)$$

$$\text{pCOD} = \text{particulateCOD} = \text{totalCOD} - \text{solubleCOD} \qquad (24)$$

To complement the bacterial activity information (specific hydrolysis rate), volatile suspended solids (VSS) was measured before and after thermal treatment as well as along the AD process. With this information it is possible to have a better understanding of the effects of the chosen thermal treatment on the microbial community.

Table 7.3 describes the volatile suspended solids before and after thermal pre-treatment. Overall, microwave pre-treatment had a detrimental effect on the volatile suspended solids of the digestate. The greatest reduction occurred at 115°C 1.4 ramp, which could explain the poor process performance (including SCOD removal- results not shown) (Table 7.3).

Table 7.3 Volatile suspended solids of digestate before (control) and after microwave pre-treatment

Condition	Volatile suspended solids (VSS) mg/L
Control	23.05
75°C 1.4 ramp	15.50
95°C 1.4 ramp	14.65
115°C 1.4 ramp	13.60
85°C 7.8 ramp	14.65

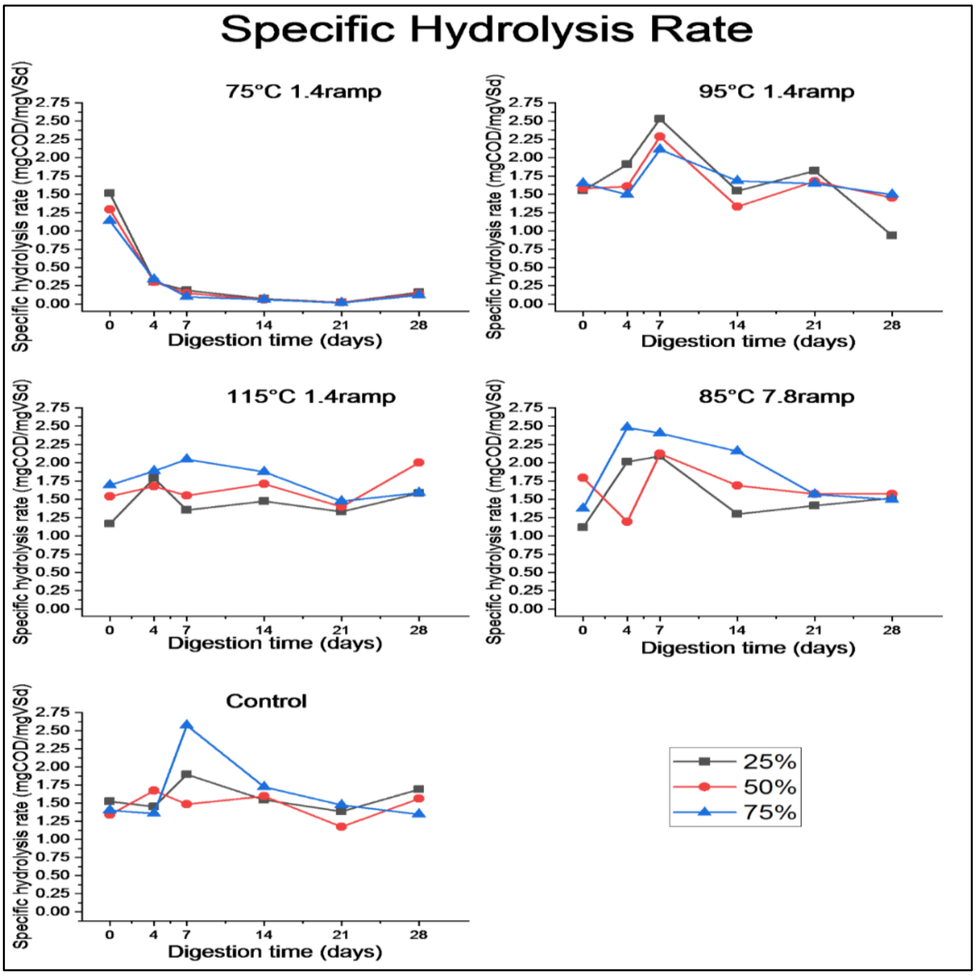


Figure 7.17 Specific hydrolysis rate of microwave digestate at various temperatures and ramp rates.

Regarding the specific hydrolysis rate, the digestate treated at 75°C 1.4 ramp showed an inverse relation with the volume of treated digestate (Figure 7.17). There was a lower specific hydrolysis rate as the final volume of digestate used increased, however it is believed that this did not originate from lower bacterial concentration (measured via particulate COD content and VSS). Inference made from the higher concentration of particulate COD and VSS at the start of digestion (day 0) for the 25% vol. of treated digestate reactors (35.234mg/L) in relation to the 50% vol. of treated digestate (37.622mg/L) and 75% vol. of treated digestate (39.767mg/L), as well as the fact that VSS also increased at day 0 as the volumes of treated digestate increased, support this inference (Figure 7.18). In this case, the reduced hydrolysis rate observed for the higher volumes of treated digestate reactors might have been the result of inhibitory substances arising from the thermal treatment at this specific temperature, as previously postulated. Nevertheless, the higher hydrolysis rate at the beginning of the digestion (day 0) for all the volume of treated digestate reactors, suggests that the organic material originated from the thermal treatment instead of bacterial activity, hence suggesting a more efficient MW result under this operational condition. Digestate treated at 75°C with a 1.4 ramp was the only condition in which there was a decrease in hydrolysis rate as the digestion process progressed. This is indicative of poor process performance, possibly with longer lag phase than the other conditions tested.

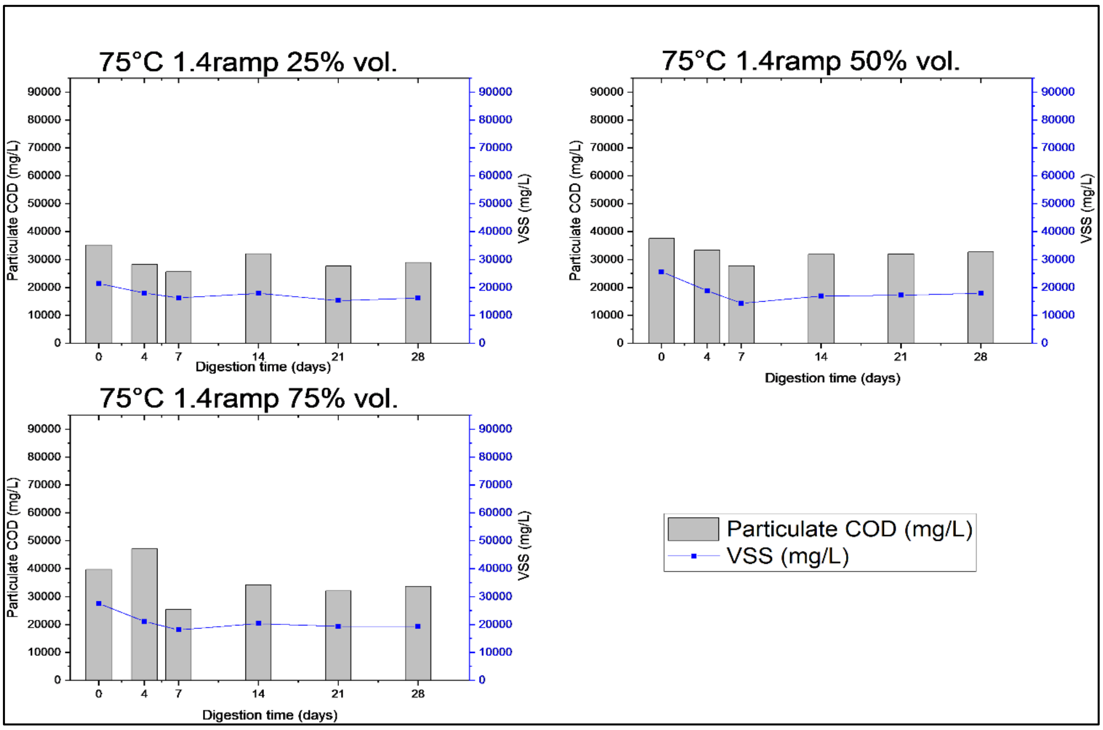


Figure 7.18 Bacterial concentration measured by particulate COD and Volatile suspended solids in the re-circulation of microwaved digestate at 75°C 1.4 ramp in the MAD of food waste.

The 95°C 1.4 ramp thermally treated digestate had different behaviour from the digestate exposed at the former temperature, with a later hydrolysis peak (day 7, instead of 0) for all volumes of treated digestate tested. These results suggest that the organic material increase originated from bacterial activity rather than the thermal treatment. The concentration of particulate COD on the day of the greatest hydrolysis rate (day 7) was equivalent to 37.553mg/L, 38.010mg/L and 36.246mg/L, for the 25, 50 and 75% vol. of treated digestate reactors, respectively (Figure 7.19). The particulate COD values are in most cases lower than those the ones shown for the sample treated at 75°C 1.4 ramp at the greatest hydrolysis rate (day 0), which were equivalent to 35.234mg/L, 37.622mg/L and 39.767mg/L of particulate SCOD. Despite a lower concentration of biomass, there was a better hydrolysis rate/bacterial activity in the former condition, hence corroborating the hypothesis of an inhibition factor at a lower temperature.

The digestate pre-treated at 115°C 1.4 ramp showed large variation in maximum hydrolysis rate and time during digestion in which it occurred, for the different volumes of treated digestate. The most important finding was related to the 50% vol. of treated digestate reactors. In this condition there was an increased hydrolysis rate (2.00mgCOD/mgVSday) at the last day of digestion (day 28), despite not being the digestion period with higher particulate COD concentration, in fact, the highest particulate COD occurred at the start of the digestion period (day 0) corresponding to 45.220mg/L, which resulted in a relatively low hydrolysis rate (compared to other conditions) of 1.53mgCOD/mgVSday, hence suggesting the presence of inhibitory factor(s) (Figure 7.20). Moreover, a greater hydrolysis rate at the end of the digestion process, could suggests that inhibitory compounds are further metabolized by the bacteria in the digestion process. Additionally, for the digestate treated at 115°C with a 1.4 ramp the greatest hydrolysis peak increased with increasing volume of digestate used. This is a consequence of the greater number of bacteria present in the reactor, suggesting that less inhibitory compounds were produced under this condition than for the treatment at 75°C 1.4 ramp.

It has been previously shown that certain strain of bacteria, and fungi are capable of degrading melanoidins from municipal wastewater (MWW). *Coriolus sp.* No.20 (Watanabe *et al*., 1982), in class Basidiomycetes, was the first strain for the application of its ability to remove melanoidins from MWW. Following this, the ability to remove melanoidins from MWW was tested in *Coriolus versicolor* Ps4a (Aoshima *et al*., 1985), *Aspergillus fumigatus* G-2-6 (Ohmomo *et al*., 1987), *Rhizoctonia sp.* D-90 (Sirianuntapiboon *et al*., 1995), *Lactobacillus hilgardii* (Ohmomo *et al*., 1988) and *Bacillus sp.* (Nakajima-Kambe *et al*., 1999). Regardless of the strain used, the decolorization of these compounds are usually accomplished by biological treatments such as aerobic and anaerobic treatment systems (Chuang and Lai, 1978).

Sirianuntapiboon *et al*. (2004) investigated the activity and efficiency of acetogenic bacteria in the process of melanoidins degradation in molasses wastewater. The choice of these microorganisms was justified on the basis of: a) they habit in typical wastewater treatment systems, b) are recognized as oxidative microorganisms and c) are capable of oxidative decomposition of melanoidins. Among 170 strains of acetogenic bacteria tested by the authors, the No.BP103 showed the highest decolorization yield (76.4 ± 3.2%) when cultivated at 30°C for 5 days in molasses pigments medium containing glucose 3.0%, yeast extract 0.5%, KH_2PO_4 0.1% and $MgSO_4$. $7H_2O$ 0.05% and the pH adjusted to 6.0. However, the bacterial activity towards melanoidins degradation is significantly decrease when nutrient supplementation is not applied (9.75 ± 3.0%). Therefore, their study corroborates the hypothesis of inhibitory compounds such as melanoidins degradation of the digestate pre-treated at 115°C 1.4 ramp.

The digestate pre-treated at 85°C 7.8 ramp, showed similar behaviour to that treated at 115°C with a 1.4 ramp in terms of specific hydrolysis rate, with an increase in this parameter as the volume of treated digestate in the reactors increased. Overall, the hydrolysis rate of the reactors treated at 85°C 1.4 ramp was improved in relation to those treated at 75 and 115°C 1.4 ramp. This could be due to the less intense (reduced exposure time and temperature) microwave treatment. Hence having a less negative effect on the bacterial community due to the absence of inhibitory compounds (Figure 7.21).

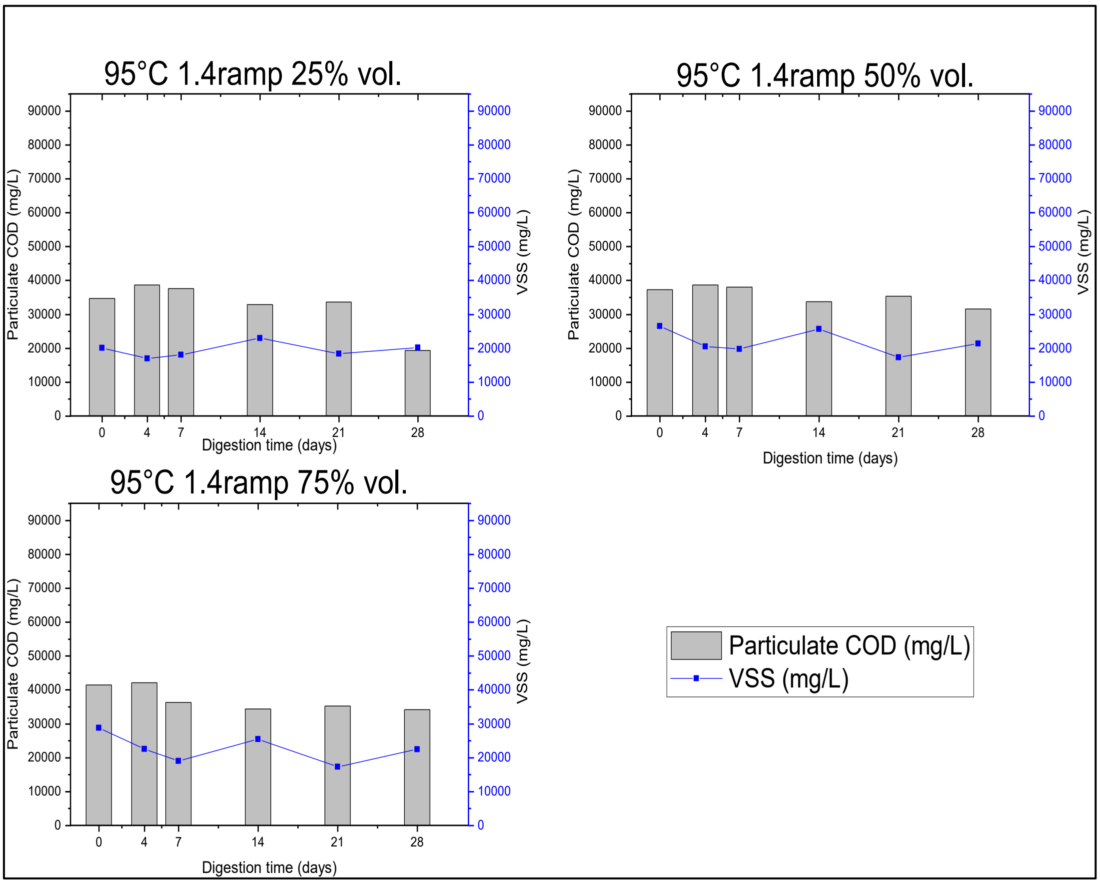


Figure 7.19 Bacterial concentration measured by particulate COD and Volatile suspended solids in the re-circulation of microwaved digestate at 95°C 1.4 ramp in the MAD of food waste.

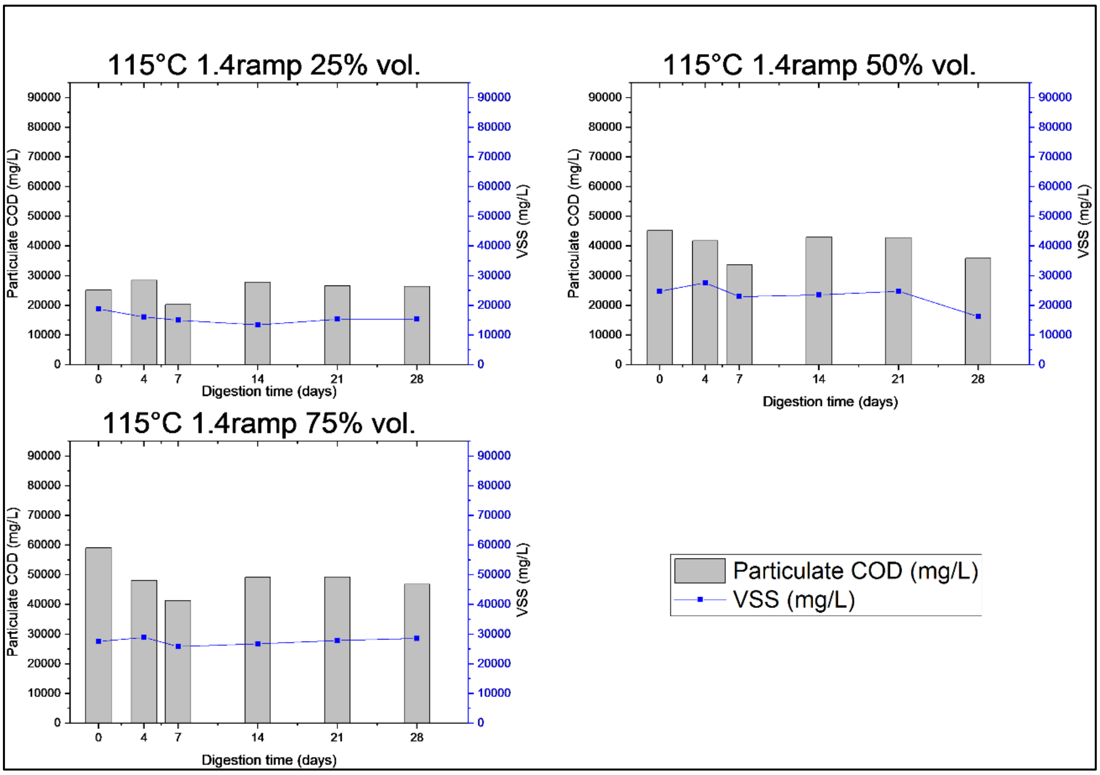


Figure 7.20 Bacterial concentration measured by particulate COD and Volatile Suspended Solids in the re-circulation of microwaved digestate at 115°C 1.4 ramp in the MAD of food waste.

The untreated digestate samples, when re-circulated into the MAD of raw food waste showed similar values of specific hydrolysis rate to most of the treated digestate. The 75% vol. of untreated digestate showed the greatest hydrolysis rate (2.57mgCOD/mgVSday) of all tested conditions tested. This occurred at the at day 7 of digestion, hence suggesting a better process performance when raw digestate is used in under high volumes, possibly due to a combination of larger numbers bacteria, hence activity, associated with the absence of inhibitory compounds (Figure 7.22).

Despite the reduction of volatile suspended solids in relation to the controls (untreated digestate) as a result of microwave irradiation, the BMP tests were successfully performed as this parameter was adjusted positively due to the presence of bacteria coming from the sewage sludge used as a seed.

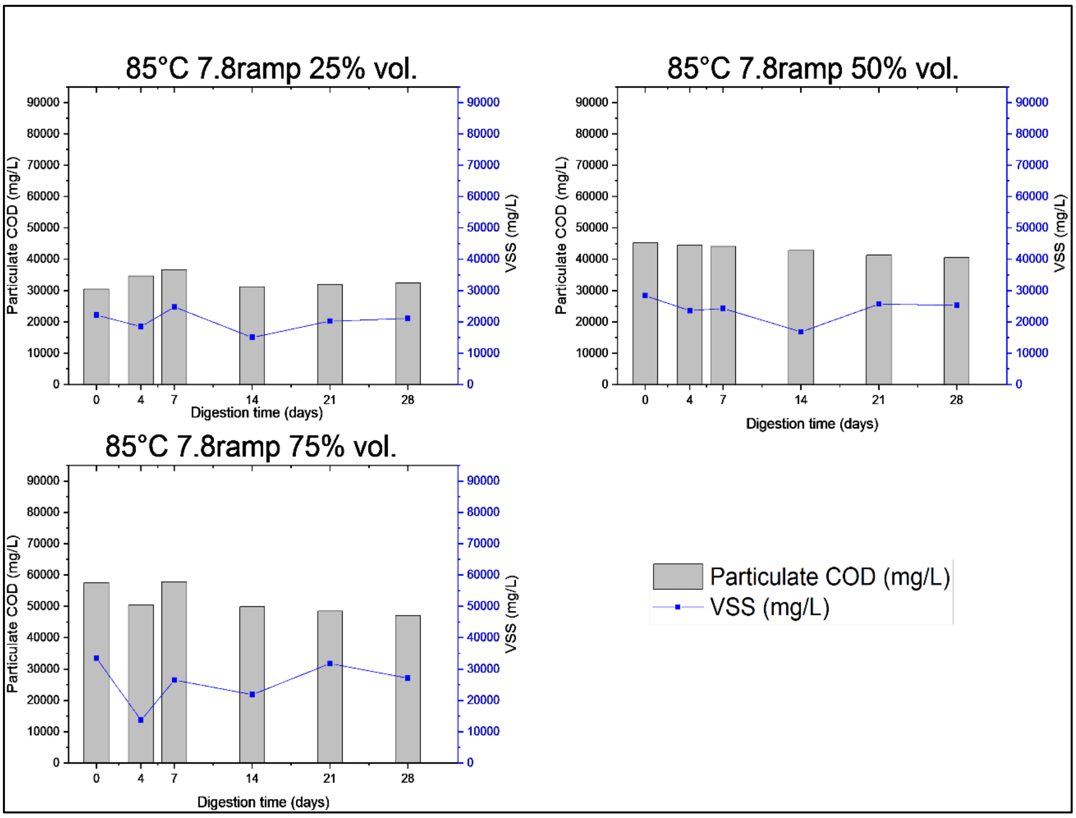


Figure 7.21 Bacterial concentration measured by particulate COD and Volatile Suspended Solids in the re-circulation of microwaved digestate at 85°C 1.4 ramp in the MAD of food waste.

The hydrolysis rate was a multifaceted process affected by several factors, including: volatile suspended solids concentration, suspended COD, and the presence of inhibitory compounds originating from the thermal treatment. The most illustrative example here is the 115°C 1.4 ramp 25% vol. of treated digestate, which had higher hydrolysis rate than the same volume treated at 75°C, despite lower concentration of suspended solids and being the condition with the greatest VSS change/reduction after microwave irradiation.

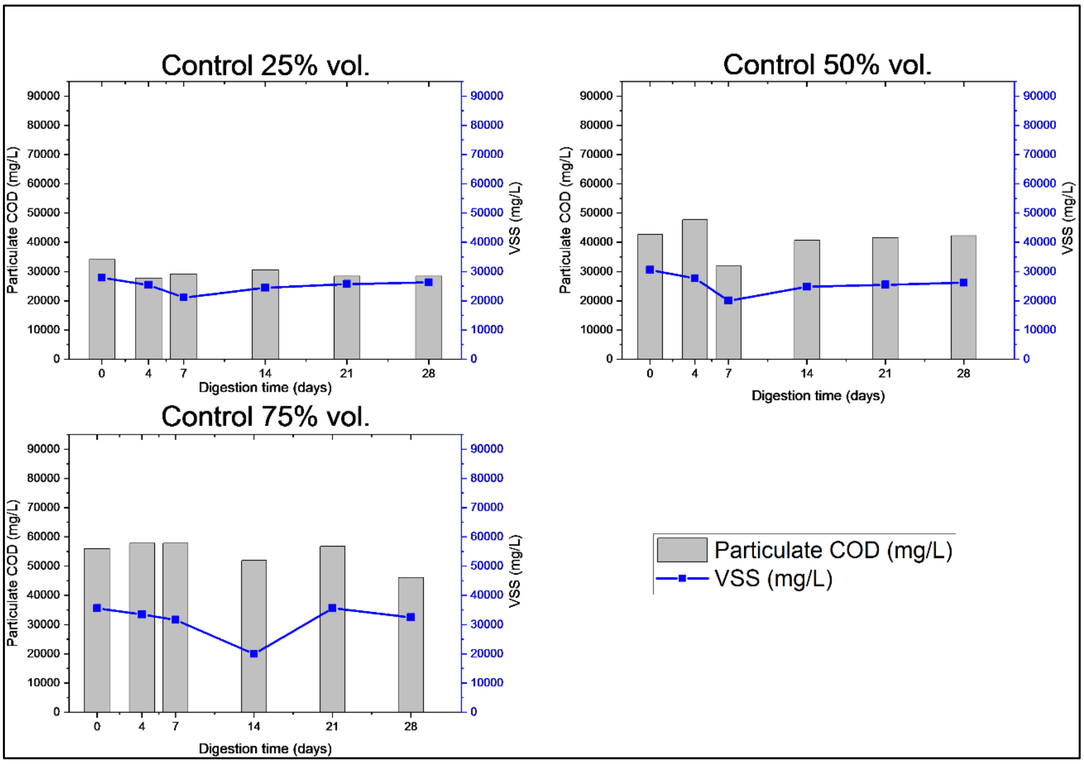


Figure 7.22 Bacterial concentration measured by particulate COD and Volatile Suspended Solids in the re-circulation of untreated digestate in the MAD of food waste.

In general, the specific hydrolysis values of the microwaved digestate in this study are improved in relation to the ones observed in Coelho *et al.* (2011). The authors investigated the effects of MW pre-treatment, staging (single- and dual-stage) and digestion temperature (mesophilic and thermophilic) on the anaerobic digestion of activated sludge.

They report that the reactors with microwaved sludge showed specific hydrolysis rates that were, on average, 25% higher than those with untreated sludge. The highest hydrolysis rates in this case were 0.595 and 0.540 for the single-stage thermophilic reactor. Moreover, they suggest that MW pre-treatment, in addition to solubilizing organic material, could have caused partial hydrolysis. Despite not being able to solubilize the organic matter, partial hydrolysis was apparently capable of modifying the solid substrate to a sufficient extent so as to make its solubilization easier in the following stage. This mostly happened in the two-stage digestion. The authors also reported negative results for the hydrolysis rate of some reactors, such as the thermophilic single-stage treatment of microwaved sludge. They explained these results in terms of total hydrolysis (100% efficacy in solubilizing the organic material) due to an extremely efficient pre-treatment process.

In the present study, negative results for the hydrolysis would be mostly unlikely due to the process being a co-digestion of microwaved sludge and raw food waste. Therefore, as much as the microwave process has proven to be effective to solubilize residual organic matter of the digestate, the enzymatic activity of the bacteria would increase the hydrolysis rate to a positive value when in contact with the raw food waste throughout the digestion period.

7.7 Methane yield and kinetic assessment for the re-circulation of microwaved digestate on the MAD of FW

Digestate treated at a final temperature of 75°C 1.4 ramp showed similar lag phase (λ) irrespective of volume of treated digestate used. The longest lag phase (9.43 days) occurred for the 25% volume of treated digestate and the shortest (9.21 days) occurred for the 75% volume, hence suggesting a detrimental effect of this parameter under lower volumes of treated digestate. In general, these values represent an increase in the lag phase duration in relation to the control, equivalent to 266.92% and 257.52% and 414.52% for the 25%, 50% and 75% vol. of treated digestate respectively (Table 7.4), with the 75% vol. of treated digestate representing the longest lag phase of all the conditions tested.

In contrast, digestate treated at 95°C 1.4 ramp showed a decrease in lag phase, and represented the shortest lag phase of all the thermal treatment conditions tested. The average reduction was 67.31%, 32.44% and 35.04% in relation to digestate treated at 75°C 1.4 ramp, 115°C 1.4 ramp and 85°C 7.8 ramp, respectively. The shortest phase lag (3.01) occurred for 50% vol. of treated digestate and the longest (3.07) for 75% vol. of treated digestates. These represent an average improvement of 32.21% in relation to raw food waste digested alone (control) implying reduced time for hydrolysis.

For the digestate treated at 115°C 1.4 ramp, there was an increase on the lag phase in relation to when untreated digestate was re-circulated in the raw food waste MAD, equivalent to 78.59, 68.33 and 153.07% for the 25%, 50% and 75% vol. of treated digestate in relation to the respective controls. These values were similar to those for raw food waste, and represents less than 1% improvement.

The digestate exposed to 85°C 1.4 ramp showed a positive relation of the lag phase and the volume of treated digestate used. Therefore, the shortest lag phase was observed for the 75% vol. reactor (4.00 days) and the longest was for the 25% vol. reactor (5.55 days). For most of the reactors treated at this temperature a reduction in the lag phase in relation to that for raw food waste was successfully achieved, varying between 0.66 and 10.51% reduction in time.

The improved lag phase of the treated digestate in relation to the raw food waste could be due to an absence of acidification in the first week of digestion. This hypothesis is supported by the prolonged lag phase observed for the 75°C 1.4 ramp 25% vol. of treated digestate sample, which had a pH of 6.83, resulting in a poorest lag phase (9.43) as opposed to the control 75% that showed a pH of 7.8, with no acidification of the system, culminating in a better lag phase (1.79 days). The lower pH of the 75°C 1.4 ramp samples in turn, could have originated from the presence of inhibitory compounds, i.e. Maillard reaction products such as melanoidins. The chemical properties of these refractory organic compounds resemble humic substances, which are acidic, therefore contributing to the acidity of the system (Dwyer *et al.*, 2009). The low pH negatively affects the methanogenic population and their ability to metabolize intermediate products. This delays the hydrolysis step and lag phase.

The control digestate, regardless of the final volume applied, showed the shortest lag phase of all conditions tested. By re-circulating the untreated digestate in the MAD of food waste it was possible to reduce the lag phase/hydrolysis duration by 42.50, 41.38 and 149.72% in relation to when food waste was digested alone.

Overall, the maximum methane yields obtained via the MGompertz model (A) for the treated digestate using different operating conditions were marginally higher than the cumulative methane yield obtained experimentally (B) (Table 7.5). These results for the co-digestion of microwaved digestate and food waste are not in agreement with the model. This is especially the case in the start-up phase were there was a delayed hydrolysis step and lag phase as previously shown.

Nevertheless, the methane yield for the co-digestion of treated digestate and raw food waste was, overall, higher than that for food waste digested alone. This demonstrates the efficacy of microwave treatment in improving anaerobic digestion via the recovery of residual energy from digestate.

The highest methane yield obtained for the co-digestion of thermally treated digestate and raw food waste occurred when the following conditions were applied: 75°C 1.4 ramp and a volume of 25% treated digestate, equivalent to 413.50mlCH_4/gVS added, representing a 132.40% increase in relation to when raw food waste was digested alone. Increasing the volume of treated digestate under a final temperature of 75°C had a detrimental effect on the final methane yield, decreasing it by 28.03 and 49.77% for the 50 and 75% vol. of treated digestate, respectively (Figure 7.23).

When the thermal treatment temperature was increased from 75°C to 95°C (1.4 ramp), the methane yield decreased, as it also did as the volume of treated digestate increased. The highest methane yield (361.64mlCH_4/gVS added) occurred for the 25% vol. of treated digestate, representing an 11.13%, 12.86 and 4.36% decrease in relation to the 75°C 1.4 ramp and 25%, 50% and 75% volume of treated digestate, respectively.

At a higher temperature of 115°C, the effects over the methane yield were also negative, causing a further decrease, the decrease becoming greater as the volume of treated digestate increased. The 25% reactor showed the highest methane yield, 352.68mlCH_4/gVS added, representing a 14.70 and 2.44% decrease in relation to the same volume at 75 and 95°C, 1.4 ramp samples, respectively (Figure 7.23).

Table 7.4 Kinetic assessment of the mesophilic anaerobic digestion of the microwave treated digestate samples at different temperatures and heating rates and controls.

		Predicted methane yield (ml) (A)	sdt	Experimental yield /Specific methane yield (ml) (B)	std	λ (lag phase)	Statistics
MW Operational conditions Temperature (°C) and heating rate (°C/min)	Volume treated digestate	Value	Std Error	Value	Std Error	Value	Adj R-Square
75°C 1.4ramp	25%	414.61	9.80	413.50	8.70	9.43	0.98
	50%	298.37	7.06	294.11	6.89	9.26	0.98
	75%	208.24	4.78	206.00	4.21	9.21	0.98
95°C 1.4ramp	25%	368.46	4.35	361.64	4.12	3.04	0.98
	50%	259.97	2.82	256.74	2.78	3.01	0.99
	75%	199.15	2.18	197.57	1.99	3.07	0.99
115°C 1.4ramp	25%	365.50	8.51	352.68	8.21	4.59	0.97
	50%	219.92	3.47	214.36	3.12	4.36	0.98
	75%	190.15	3.23	185.74	1.87	4.53	0.98
85°C 7.8ramp	25%	232.05	6.79	225.40	6.32	5.55	0.97
	50%	229.11	5.65	220.67	5.21	4.50	0.97
	75%	221.65	5.23	212.41	4.89	4.00	0.98
	Volume untreated digestate	Value	Std Error	Value	Std Error	Value	Adj R-Square
Control	25%	183.07	1.80	180.38	1.67	2.57	0.99
	50%	198.65	2.20	194.88	1.98	2.59	0.98
	75%	208.68	1.54	207.50	1.37	1.79	0.99

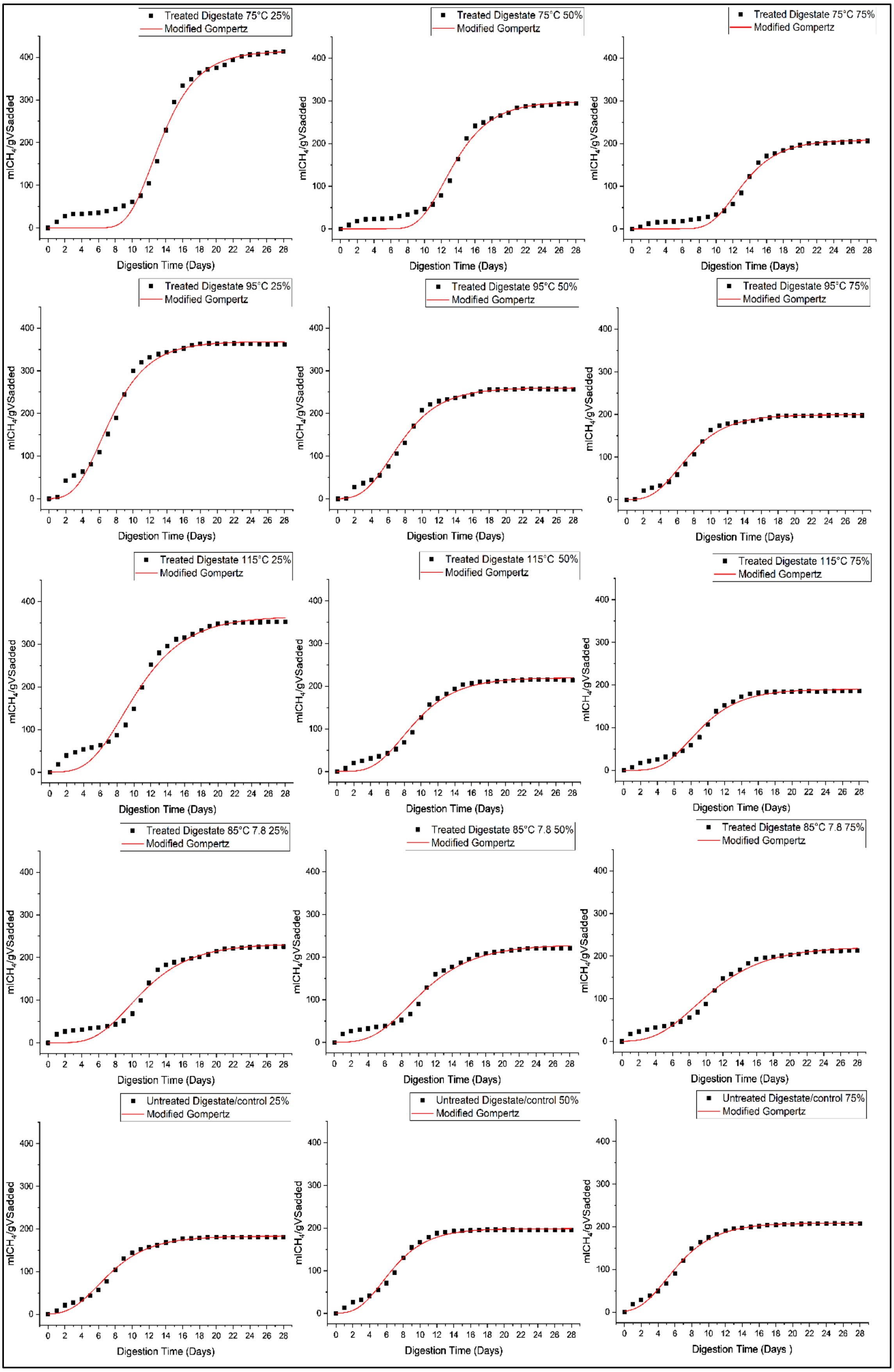


Figure 7.23 Specific methane yield with the MGompertz fitting curves for the digestate samples treated at 75, 95 and 115°C 1.4 ramp, 85°C 7.8 ramp and controls (untreated digestate).

When a lower temperature and shorter microwave irradiation time (85°C 7.8 ramp) was applied, lower values of methane yield were obtained for most of the volumes of treated digestate. The methane yield for 25% vol. of treated digestate was lower than for the other temperatures, representing a 45.48, 37.67 and 36.08% decrease in relation to the same volume at 75, 95 and 115°C 1.4 ramp, respectively. The 75% vol. of treated digestate was an exception but this had a higher concentration of treated digestate. In this case, the methane yield was 3.11, 6.98 and 14.35% greater than the same volume at 75, 95 and 115°C, 1.4 ramp, respectively. An improved methane yield arising from a higher volume of treated digestate suggests the absence of inhibitory compounds. It is possible that a less harsh exposure to microwave irradiation prevented the formation of such compounds.

The controls digestate showed an increased methane yield as the volume of treated digestate increased. Despite the absence of inhibitory substances formed during thermal treatment, the methane yield observed for the untreated digestate was significantly lower than that for microwaved digestate, possibly due lower organic matter solubilization of the digestate in a "raw" form. The final values were: 180.38, 194.88 and 207.50mlCH$_4$/gVS added, for the 25, 50 and 75% vol. of treated digestate, respectively. The highest methane yield for the 75% vol. of untreated digestate reactors represented a 49.81% reduction in relation to the 75°C 1.4 ramp, 25% vol. treated digestate reactors. This suggests that the extra energy applied for the thermal/microwave treatment of the digestate possibly compensates the additional biomethane gas obtained. A more comprehensive analysis of the economic aspects of these scenarios is discussed in Chapter 8 (Figure 7.23).

The methane yields found here are in agreement with those reported in the literature. Zhang *et al.* (2016) investigated the effects of microwave pre-treatment on the co-digestion of food waste (FW) and sewage sludge (SS) as well as the optimum ratio of FW and SS based on MW pre-treatment, microwaves sewage sludge (MWSS). The authors showed that the methane yield (mlCH$_4$/gVSadded) varied greatly for the various conditions tested. The digestion of raw food waste and sludge in the proportion 1:1 (equivalent to the controls of this study), yielded 296.75mlCH$_4$/gVS added, which is marginally greater than observed here. The authors reported a yield improvement by microwaving the sludge and mixing with raw food waste using an MWSS/FW ratio of 1:1, which increased the methane yield by 7% (317.92mlCH$_4$/gVSadded). The maximum methane yield was obtained when the proportion of microwave sewage sludge to the raw food waste was increased to 3:2 in relation. In this case, the methane yield was 389.64mlCH$_4$/gVSadded, representing a 31.30% increase in relation to when untreated sludge was co-digested with raw food waste.

The authors also observed a decrease in the methane yield as the proportion of treated sludge increased in relation to the FW. The decrease was up to 28%, when FW: MWSS concentrations were modified from 2:3 to 1:2. This underlines the importance of the balance between FW and MW sewage sludge. The authors also compared the following conditions: 1) co-digestion of microwaved food waste and raw sewage sludge and 2) co-digestion of raw food waste and microwaved sewage sludge: both with a ratio of 3:2. They report that the thermal pre-treatment of the sewage sludge (389.64mlCH_4/gVSadded) is more advantageous in terms of methane yield than thermally treating food waste (367.62mlCH_4/gVSadded).

Tampio *et al*. (2015) compared the methane yields from co-digestion of FW digestate and autoclaved FW digestate (160 °C, 6.2 bar) with untreated FW (FW) and autoclaved FW (AFW) from laboratory-scale reactors. The authors reported that FW digestate alone yielded 0.132m_3CH_4/kgVS added of methane, as opposed to the autoclaved digestate, which had a lower yield of 0.079m_3CH_4/kgVS added.

These values are lower those reported here for the digestate. Herein, digestate alone yielded an additional 2.46, 16.96 and 29.58mlCH_4/kgVS added in relation to FW control, when re-circulated in an untreated form.

The authors also reported higher values for the co-digestion of treated digestate and food waste than those obtained here. When FW digestate was digested together with untreated FW, a total of 0.452m^3CH_4/kgVS added was obtained. When FW digestate was co-digested with autoclaved FW, 0.411m^3CH_4/kgVS added was obtained. Similar methane yields were obtained when co-digesting AFW digestate with FW (0.451m^3CH_4/kgVS added). For co-digestion of AFW digestate with AFW, the methane yield was significantly lower (by 32%) with 0.307m^3CH_4/kgVS added. Tampio *et al*. (2015) showed that there was a detrimental effect on methane yield when mixing the treated substrate into higher proportions, in this case, represented by the autoclaved FW and digestate. The authors suggest that the methane yield was negatively affected by the formation of hard-to degrade Maillard compounds during the autoclave treatment of FW, leading to reduced biodegradability of the material.

Several conditions previously tested (e.g. the microwaving of food waste at 85°C 7.8 ramp, and utilizing the soluble fraction of untreated food waste (control)) were able to improve methane yield in relation to raw food waste digested alone. The co-digestion of microwaved digestate and raw food waste resulted in the highest methane yield values of all conditions tested. Treated digestate operating conditions with highest methane yield were compared with previously tested conditions (which also had the highest methane yields), to give more comprehensive understanding of the relationship between the operating conditions and methane volume produced (Table 7.5).

It is possible to infer that by pre-treating digestate with microwave irradiation using the operating conditions suggested here and further re-circulating it in the MAD of food waste, a further increase on the yield to between 3.34 and 108.95% in relation to digestion of microwaved food waste at 85°C 7.8 ramp. If microwave pre-treatment is applied to the digestate, with its further application to MAD, it is possible to further increase yield up to 132.40% compared to raw food waste digested alone.

Raw FW in a soluble fraction (control soluble) was considered in the previous chapter to be the optimum condition for methane yield. This implies significant economic advantage since no energy or capital costs would be incurred in microwaving and AD plant. Nevertheless, when compared to the co-digestion process of re-circulating treated digestate into the MAD of raw food waste, methane yield can be further improved by 26.66% if a final temperature of 75°C 1.4 ramp and 25% volume of treated digestate is applied (Table 7.5). Despite the additional economic costs associated with the microwaving of the digestate, this strategy offers the possibility to utilize FW and digestate simultaneously, for energy recovery and digestate volume reduction, hence reducing costs associated with its haulage and disposal to land.

Table 7.5 Comparative of the treated digestate operational conditions with highest methane yield with the process performance of treated and untreated FW.

Samples Names	**75°C 1.4ramp 25%**	**95°C 1.4ramp 25%**	**115°C 1.4ramp 25%**	**85°C 7.8 ramp 75%**	**Control Digestate 75%**
Final Methane yield (mlCH$_4$/gVSadded)	413.50	361.64	352.68	225.40	207.50
Improvement in relation to the microwaved FW 85°C 7.8 ramp (%)	108.95	82.74	78.22	13.90	3.34
Improvement relative to FW Control (whole) (%)	132.40	103.25	98.22	26.68	16.62
Improvement relative to FW Control (soluble) (%)	26.66	9.73	8.03	_	_
Reduction of the lag phase/hydrolysis in relation to FW Control (whole)	-	+	-	-	+

Despite larger volumes of treated digestate reducing methane yield for most of the conditions tested, and for this reason not recommended to be applied in co-digestion with FW, the use of the raw form of this substrate was able to deliver a reduction on the lag phase of the 75% vol. control. Nevertheless, thermal treatment of digestate combined with its recirculation in the MAD of FW yields energy recovery. The greatest volume of gas recovered (235.58ml) was for treatment at 75°C with a 1.4 ramp (25% vol. treated digestate). This was followed by 183.72, 174.77, 47.48 and 29.58ml for the 95°C (25%), 115°C (25%), 85°C with 7.8 ramp (75%) and control (75%), respectively.

A one–way Anova test (Tukey) test for the treated digestate and FW (microwaved and control) was performed. The operational conditions with the highest methane yield shown and discussed previously in other chapters, namely: a) MW FW 85°C 7.8 ramp, b) soluble fraction control, and c) treated digestate at 75°C 1.4 ramp at 25% vol. of treated digestate were compared to each other to better inform the choice of optimum operational conditions for scaling up the process.

The statistical results showed that, with the exception of the FW control vs. treated digestate 75°C 1.4 ramp 25% for which there was a statistical difference, there was no statistical difference between the other conditions compared (Table 7.6). Based on these results, it is possible to assert that the 75°C 1.4 ramp, 25% volume of treated digestate was the optimal condition for methane yield (Appendix H).

Table 7.6 Anova test for methane yield from microwaved digestate and higher methane yield conditions of FW.

Sample's Name	*p* value
FW control vs. MW Digestate 75°C 1.4 ramp 25%	$p = 0.000$
Soluble fraction FW control vs. MW digestate 75°C 1.4 ramp 25%	$p = 0.092$
MW FW 85°C 7.8 ramp vs. MW Digestate 75°C 1.4 ramp 25%	$p = 0.875$

7.9 Technical digestion time of the MAD of FW with re-circulated MW digestate

Overall, the technical digestion time of the co-digestion of thermally treated digestate under and raw FW was shorter than when raw food waste was digested alone (control). The time needed to obtain 80% of the biomethane gas via AD of the microwaved digestate was significantly shorter at 95°C 1.4 ramp, representing a 41.16% reduction in the time needed to reach 80% of the methane production in relation to the FW control (Figure 7.24).

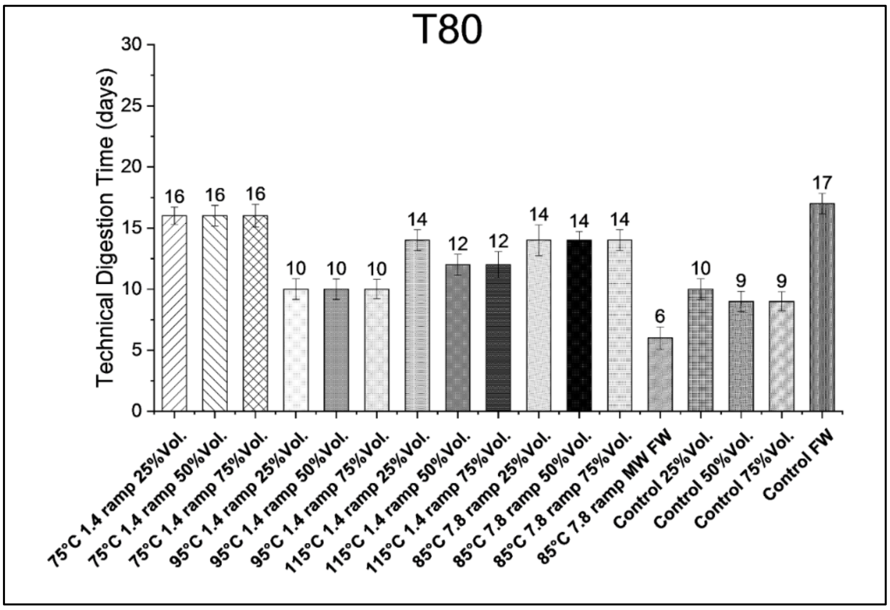


Figure 7.24 Technical digestion time (T80) of microwaved digestate and control food waste.

The untreated digestate/control showed a T80 varying from 9 and 10 days, which represents a 47.05% reduction in relation to when food waste was digestated alone. The digestate controls represent the second-best performance in terms of T80 of all conditions tested in this study, including the soluble fraction of food waste. The best T80 was for the microwaved food waste (whole) treated at 85°C 7.8 ramp, which represented a 64% reduction of the time needed to produce 80% of the biomethane in relation to the FW control.

Although it did not represent the best strategy to reduce the T80 (compared to when microwaving food waste at a lower temperature 85°C 7.8 ramp), it is a promising option since it allows the use of both substrates avoiding their common disposal route and environmental impacts.

7.9 Summary

Microwave treatment of digestate promoted important changes in the characteristics of this substrate, including ammonium and SCOD. Ammonium concentration showed an increase for all the conditions tested. The most significant increase was for treatment at 85ºC 7.8 ramp, yielding a final value of 15.36g/kg, equivalent to 387.61% an increase in relation to the untreated samples. Thermal treatment of the digestate caused an enhancement in SCOD and hydrolysis degree for all the conditions tested. The greatest increase was observed when samples were exposed to 115°C 1.4 ramp, with a hydrolysis degree of 82.33%. Because digestate treated at 85ºC 7.8 ramp showed a hydrolysis degree of 79.39%, similar to that for 115°C, the former operating condition is preferable. It is believed that the decrease in hydrolysis degree (3.57% in relation to the 115°C 1.4 ramp) can be compensated by energy savings associated with reduced microwave treatment duration and consequently, costs (65 minutes less, equivalent to 96kWh less-energy spent on the MW process).

The re-circulation of the thermally treated digestate into the MAD of raw FW shows the typical accumulation of intermediate products in the start-up phase. This is particularly evident at 75ºC 1.4 ramp, which exhibited a monotonic pH drop from the second to the seventh day of digestion, corresponding to the period of TVFAs accumulation. Due to a higher concentration of TVFAs in relation to the other operating conditions and more rapid decrease in pH (falling to between 6.83 and 6.92) it can be said that there was acidification of the system for all samples treated at 75ºC1.4 ramp.

Nevertheless, for most of the conditions tested the re-circulation of digestate into the MAD of FW seems to have worked as a potential buffer, preventing the system of high pH drop under higher intermediate products concentration (TVFAs). This finding is corroborated by comparing thermally treated digestate with untreated digestate. The latter, shows a greater decrease in pH, despite having lower TVFAs concentration than the treated digestate reactors. The explanation could be that, although the untreated digestate is capable of acting as a buffer agent, this effect is not as intense as when treated digestate is present. This is because when digestate is exposed to microwave radiation, a significant augmentation of the ammonium content occurs, thus reinforcing the buffer capacity of the system.

Additionally, when re-circulating the untreated digestate with the raw food waste it was possible to reduce the acidification period. The shorter duration of a low pH offered better removal of TVFAs and hence, a less pronounced decrease in pH.

Despite removing the acidification of the system for most of the co-digestion conditions tested, the combination of treated digestate with raw food waste still resulted in p/a exceeding the recommended value, especially for low volumes of treated digestate. In all of the cases studies this did not have a detrimental effect on methane production.

Amongst the various conditions tested, digestate treated at 75°C 1.4 ramp and 25% vol. showed the greatest biodegradability (80.18%) percentage of the FW TMP. This result represents a 132.43% improvement in relation to when raw food waste (control) is solely digested and demonstrates that the pre-treatment chosen here was effective in recovering residual energy from digestate.

Overall, microwave pre-treatment had a detrimental effect on the volatile suspended solids (VSS) of the digestate, which is an indirect measure of bacteria concentration. The greatest reduction occurred for samples treated at 115°C 1.4 ramp. Bacterial activity (specific hydrolysis rate) was affected by several factors, including volatile suspended solids concentration, suspended COD and the presence of inhibitory compounds originating from the microwave treatment. This finding came after the observation of microwaved digestate treated at 115°C 1.4 ramp and 25% vol. of treated digestate showing a higher hydrolysis rate than the same vol. of digestate treated at 75°C 1.4 ramp, despite lower suspended solids concentration and significant VSS reduction after microwave irradiation. This suggests inhibitory factors acting at a lower temperature.

The specific hydrolysis rate of the 85°C 1.4 ramp reactors were improved in relation to the 75 and 115°C, 1.4 ramp reactors, possibly due to a less intense (reduced exposure time and reduced temperature) microwave treatment, hence avoiding the formation of inhibitory compounds, known for negatively affecting bacteria activity. Despite the reduction of volatile suspended solids (indirect measure of bacteria concentration) as a result of microwave irradiation, the BMP tests were successfully performed for all the conditions tested.

The untreated digestate (controls), showed a 47.05% reduction of the T80 in relation to food waste digested alone. Additionally, by re-circulating the untreated digestate in the MAD of food waste it was possible to reduce the duration of the lag phase/hydrolysis step by 42.50, 41.38 and 149.72%, for the 25, 50 and 75% vol. of untreated digestate reactor, respectively in relation to the when FW was digested alone.

Overall, pre-treating the digestate with microwave irradiation and further re-circulating it in the MAD of FW, results in an increase in methane yield between 3.34 and 108.95% in relation to when FW was microwaved at 85°C 7.8 ramp. Nevertheless, this improvement is even greater (132.40%) when comparing the yields obtained from microwave digestate re-circulation into MAD of FW versus raw food waste digested alone. The co-digestion of MW digestate and raw food waste heralded a 26.66% methane yield improvement in relation to FW soluble fraction (control soluble) discussed in chapter 6 as the optimum condition for methane yield.

Despite the additional economic costs associated with microwaving digestate, this strategy offers the following advantages over the sole digestion of raw FW: a) the possibility to utilize FW and digestate simultaneously for energy recovery and reduction of digestate volume. b) reduction of the costs associated with haulage and disposal of digestate, c) saving in the freshwater requirement for assembling the reactors; the water being replaced by treated digestate.

Despite finding of the non–feasibility of applying the maximum volume of treated digestate (75%) to enhance methane yield, as well as to try to reduce the maximum volume of this end-product before discarding it to the traditional land use, the co-digestion process of treated digestate and raw food waste is able to offer some energy recovery, and improve methane yield in relation to when untreated food waste is solely digested.

CHAPTER 8

8. MASS AND ENERGY INTEGRATION STUDY OF THE CASCADE PROCESS OF MICROWAVE IRRADIATION AND ANAEROBIC DIGESTION OF FOOD WASTE AND DIGESTATE

8.1 Introduction

The application of food waste as a resource for renewable energy has gained much attention in recent decades. The bioenergy industry provides a source of renewable fuel that is sustainable and clean compared to conventional, fossil-based, fuels. Using bioenergy fuel to replace fossil fuels reduces significantly greenhouse gas emissions (Garrett, 1992).

Most bioenergy processes require pre-treatment and/or upgrading to generate a useful feedstock with suitable properties and, hence, more compatible with the specific energy conversion route chosen. Pre-treatment usually involves heating of the feedstock to achieve the desired properties (Beneroso *et al*., 2017). As an example, thermal treatments, which enhance organic matter solubilization, benefit AD by improving mass transfer in the hydrolysis step, reducing its duration (Zhang *et al*., 2016).

Microwave (MW) heating has attracted much attention in recent years since it provides rapid and efficient heating in a controlled environment, increased processing rates and substantially shortened reaction times (by up to 80%). This technology offers numerous other advantages over conventional heating. These include: (i) non-contact heating; (ii) selective heating; (iii) rapid start/stop facility; (iv) high levels of safety and automation; and (v) heating from inside the body of the material (i.e. energy conversion instead of heat transfer) (Menéndez *et al*., 2010). All of these positive aspects of MW heating have been investigated in the context of organic matter (sludge, food waste, biomass wheat-straw, corn stover, etc.) with encouraging results. The further application of pre-treated substrate in AD has resulted in enhanced organic matter solubilization, improved biodegradability and increased methane yield (Marin *et al*., 2010; Shahriari *et al*., 2011).

Although MW heating seems attractive as a pre-treatment for and integrated process of AD there are a number of challenges that need to be addressed and more fully understood. These include: a) assess energy balances, b) determine induced temperature gradients within the heated substrate and c) determination of the dielectric properties of different feedstocks (Kostas *et al*., 2017).

Consequently, despite a wealth of research that has shown unique benefits of MW heating, the growth of industrial-scale MW heating applications is virtually non-existent. The transition from laboratory-scale experimental apparatus to industrial-scale processes is limited not just by poor understanding of the MW heating system but also by incomplete technical information required for commercial design and development (Kostas *et al*., 2017). The latter includes data from demonstration plants with comprehensive evaluation and mitigation strategies for the respective technical risks (Buttress *et al*., 2016; Kostas *et al*., 2017).

In order to couple both treatments (AD and MW) and meet the environmental targets for food waste/digestate minimisation and recovery of valuable products, and also reduce overall treatment costs, several aspects related to the implementation of anaerobic digestion facilities need to be considered. Local circumstances must be examined. These include labour, treatment capacity, transport, collection costs, energy prices, taxation, purchase tariffs, land price, market, price of digested material, residue disposal, additional mixing and pumping. Because these processes involve the use of heat and electricity, their impact upon the environment should be also fully addressed (Beneroso *et al*., 2017). Such information is not always easily available making the process of accurately estimating a full-scale industrial plant difficult (Bolzonella *et al*., 2006; Salsabil *et al*., 2010; Cesaro and Belgiorno, 2014).

Despite these difficulties, a simple analysis of mass and energy balance has been performed and discussed in this chapter based on the experimental results reported in Chapters 5 and 7. Energy and environmental implications are established in order to assess how MW heating can be integrated into AD as a comprehensive food waste and digestate management strategy.

8.2 Material and methods

8.2.1 Overall process description (microwave irradiation coupled with AD)

In order to better understand energy and mass balances discussed in the next sections, a comprehensive schematic representation of the integration of microwave irradiation and anaerobic digestion is now described, including the application of whole FW and the re-circulation of microwaved digestate in the MAD of FW (Figure 8.1).

The integration of microwave pre-treatment with anaerobic digestion comprises three main processes. The first relates to digestion of whole fraction microwaved food waste at different temperatures (85°C - 175°C), and heating rates (7.8, 3.9 and 1.9°C/min) to produce digestate and biomethane under mesophilic anaerobic conditions (37°C).

The second relates to the soluble fraction of food waste pre-treated with microwave irradiation at a final temperature of 175°C and different heating rates (7.8, 3.9 and 1.9°C/min). Once the food waste is thermally treated, the solid fraction is removed by centrifugation and filtration, and a liquid/supernatant is then applied to MAD to produce biomethane and digestate. In this case, the remaining (underutilized) solid fraction is disposed on land. The reason for disposing the solids is to remove inhibitory compounds associated with this fraction, hence improving methane yield.

The third process relates to the further processing and utilisation of the digestate. In whole form the digestate becomes a substrate for microwave irradiation under a range different operational conditions (75, 95, 115°C with a 1.4°C/min heating rate) and 85°C 7.8°C/min. The microwaved digestate is then re-circulated into a digester operating under mesophilic conditions treating raw food waste. The re-circulation of the whole fraction of digestate allows the recovery of residual bioenergy in the form of biomethane. Additionally, avoids the environmental and economic challenges associated with the disposal of the unutilized solid fraction. At the end of the re-circulation process, the digestate produced can be either disposed to land or re-circulated again in the MAD.

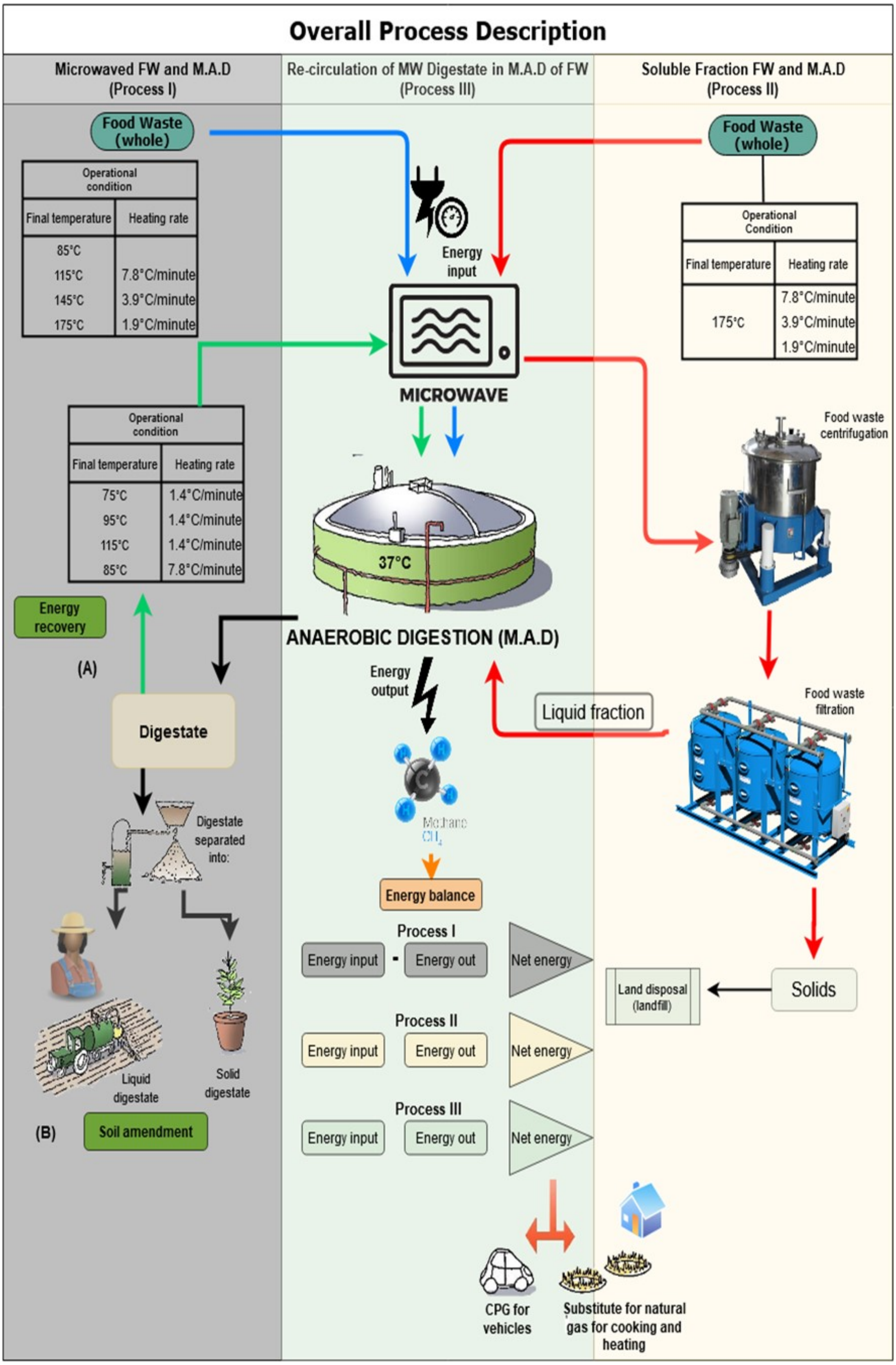
The energy implications associated with the AD system (mixing, feeding, and pumping) has not been taken into consideration. Nor has the centrifuging step for solids removal in process II (Figure 8.1).

The fate of Total and Soluble Chemical Oxygen Demand (TCOD and SCOD, respectively), as well as the Total and Volatile Solids for each condition tested here, composes the mass balance in this study. The option to calculate mass and energy balance for all the conditions here tested arrives from the belief that the choice of optimum condition for scaling-up the combined process should be done by the careful evaluation of its performance (including parameters such as acidity, buffer capacity, methane production and yield) and, most importantly, energy balances values. In this sense, it is possible that a set of microwave operational conditions (temperature and heating rate) which have not previously heralded the best methane yield, show a positive and encouraging energy balance. The rationale behind this is the fact that the energy balance also takes into consideration the power consumption of the microwave ($J\ s^{-1}$), which was very different for each set of conditions, and did not have a straight relation to the final methane yield obtained.

The environmental impact analysis, is based on carbon footprint and expressed as $kgCO_2/t$. In this case, it only takes into consideration the direct CO_2 emissions to the atmosphere and from power generation, and therefore, disregards other aspects such as: land, water, people, etc. Two different scenarios were considered. The first focuses on the amount of CO_2 produced only by the food waste fraction (present in MSW) generated in the UK in the year of 2012 (most recent comprehensive data available) if disposed in landfill. The information utilized to estimate the carbon footprint of scenario I can be found in Jeswani and Azapagic, (2016), and was calculated according to Parra-Orobio *et al*. (2017).

In the second scenario, food waste does not undergo landfilling disposal. Instead, it is applied to energy recovery by anaerobic digestion, and it assumes a loss of 10% of total methane production is emitted directly into the atmosphere, due to small leakage from the pipes that transport the methane during production and distribution in the grid. The methane production obtained experimentally through the combination of microwave irradiation and AD (Chapters 5,6 and 7) is used to establish the carbon footprint of the second scenario, according to Parra-Orobio *et al.* (2017). The environmental impact, mass and energy balance will be used as tools for helping decision making towards scaling up the combined MW and AD process.

All assumptions and methods used as a basis for the mass balance, energy balance and environmental impact calculations of the different scenarios proposed here are presented in Table 8.1.



Operational condition

Final temperature	Heating rate
85°C	
115°C	7.8°C/minute
145°C	3.9°C/minute
175°C	1.9°C/minute

Operational Condition

Final temperature	Heating rate
175°C	7.8°C/minute 3.9°C/minute 1.9°C/minute

Operational condition

Final temperature	Heating rate
75°C	1.4°C/minute
95°C	1.4°C/minute
115°C	1.4°C/minute
85°C	7.8°C/minute

Figure 8.1 Schematic representation of the cascade process: microwave irradiation and anaerobic digestion of FW and digestate.

Table 8.1 Calculation basis considered for environmental impacts, mass and energy balances of the different studied scenarios.

Description/scenario	Equation	Reference	Parameter
	Environmental impact		
Landfill (Scenario I)	$G_1 = BMP \cdot DCO_f / MW_{CH_4} \cdot F \cdot V_{SC}$ $G_2 = BMP \cdot DCO_f / V_{SC} \cdot MW_{CO_2}$ $G_3 = BMP \cdot DCO_f \cdot 0.39 \cdot C / 3.6 \cdot F_E$	Parra-Orobio *et al.* (2017)	G1 and G4: Quantify direct emissions to the atmosphere. G_2: emission from power generation. G_3: GHG offsetting by power generation. $G_{Tlandfill}$: Carbon footprint for landfill. G_{TAD}: Carbon footprint for anaerobic digestion. BMP: Biochemical Methane Potential ($m^3\ t^{-1}$). BMP_d: Biochemical Methane Potential dissolved ($m^3\ t^{-1}$). DCO_f: Fraction of the degradable organic carbon (0.5). MW_{CH4}: Molecular weight methane ($16 g\ mol^{-1}$). F: Value of global warming potential of methane (21). V_{SC}: Volume standard conditions (22.4 L). MW_{CO2}: Molecular weight carbon dioxide ($44 g\ mol^{-1}$). 0.39: Factor of electrical energy production based on internal combustion. C: Heat value of methane (35.9 MJ m^{-3}). F_E: GHG emission factor of power generation with capacity more than 1000 MW (0.8578 kg-CO_2 kWh^{-1}).
Anaerobic Digestion coupled with MW pre-treatment (Scenario II)	$G_4 = BMP_d \cdot DCO_f / MW_{CH_4} \cdot F \cdot V_{SC}$ $G_{Tlandfill} = G_1 + G_2 - G_3$ $G_{TDA} = G_4 + G_2 - G_3$	Parra-Orobio *et al.* (2017)	
	Mass balance		
Volatile Solids Removal Rate	$VSRR\% = \frac{VS_{initial} - VS_{final}}{VS_{initial}} \cdot 100$	Somiya (2001)	VSRR%: Volatile Solids Removal Rate Percentage. SCODRR%: Soluble Chemical Oxygen Demand Removal Rate Percentage. TCODRR%: Total Chemical Oxygen Demand Removal Rate. $VS_{initial}$: Volatile Solids initial amount. VS_{final}: Volatile Solids final amount.
Soluble Chemical Oxygen Demand Removal Rate	$SCODRR\% = \frac{SCODinitial - SCODfinal}{SCODinitial} \cdot 100$	Somiya (2001)	
Total Chemical Oxygen Demand Removal Rate	$TCODRR\% = \frac{TCODinitial - TCODfinal}{TCODinitial} \cdot 100$	Somiya (2001)	

	Energy balance		
Worst Case Scenario	$\mathrm{E_{in}} = \mathrm{s} \, . \, \theta$ $\mathrm{E_{out}} = \mathrm{BMP} \cdot \mathrm{C} \cdot \mathrm{F_r}$ $\mathrm{E_{net}} = \mathrm{E_{in}} - \mathrm{E_{out}}$	Hillel, (1998); Parra-Orobio *et al.* (2017)	$\mathrm{E_{net}}$: energy balance. $\mathrm{E_{in\ BC}}$: energy entering the process (Best case) (J $\mathrm{gVS^{-1}}$). $\mathrm{E_{in\ WC}}$: energy entering the process (Worst case) (J $\mathrm{gVS^{-1}}$). $\mathrm{E_{out}}$: energy generated during the process (J $\mathrm{gVS^{-1}}$). θ: Temperature rise (final temperature- starting temperature (18°C). s: specific heat capacity (water = 4.18kJ/kg°C; FW = 1.92kJ/ kg°C). $\mathrm{P_d}$: power consumption of the microwave (J $\mathrm{s^{-1}}$). t: time of microwaving (s). $\mathrm{C_s}$: concentration of substrate in each experimental unit (gVS $\mathrm{L^{-1}}$). $\mathrm{V_d}$: effective volume of the reactor (L). C: heat value of methane. $\mathrm{F_r}$: percentage recovery of methane (100%).
Best Case Scenario	$\mathrm{E_{in}} = \dfrac{\mathrm{P_d} \cdot \mathrm{t}}{\mathrm{C_s} \cdot \mathrm{V_d}}$ $\mathrm{E_{out}} = \mathrm{BMP} \cdot \mathrm{C} \cdot \mathrm{F_r}$ $\mathrm{E_{net}} = \mathrm{E_{in}} - \mathrm{E_{out}}$	Hillel (1998); Parra-Orobio *et al.* (2017)	

8.3 Results and discussion

8.3.1 Environmental impact of the combined process (AD and MW)

According to Jeswani and Azapagic (2016), one tonne of MSW discarded to UK's landfill in 2012 produced 1102 MJ of biogas. In order to estimate the theoretical methane production (m^3/t) from this amount, a 40% methane content for the biogas was assumed. Additionally, only the organic fraction of MSW related to food waste was taken into account, equivalent to 15% of the methane volume (this percentage represented FW composition on MSW in 2012. Therefore, it was estimated that a total of $80m^{3/}t$ of food waste was produced in 2012. If landfilled this would result in a carbon footprint of 417kg CO_2/t (Scenario I).

If the food waste was anaerobically digested (Scenario II), the carbon footprint is reduced by 98.20% compared to landfilling. The reduction in methane emission can be explained by the controlled environment in which the former occurs. As long as there is little leakage from the pipes that transport the methane in the AD facility during production and distribution in the grid, the emissions to the atmosphere should be small, or even negligible. Therefore, almost virtually all of the methane is utilized for energy or fuel generation, instead of acting primarily as a Green House Gas (GHG).

Regarding scenario II, the lowest carbon footprints were observed for the digestion of microwaved food waste using a medium heating rate (3.9°C/min). The greatest carbon footprint in this case was 8.39kg CO_2/t when the substrate was heated to 115°C, and the lowest was 6.99kg CO_2/t when the substrate was heated to 175°C. These values represent, on average, a 97.00% reduction with respect to the carbon footprint of landfilling FW. Similar results were found by Parra-Orobio *et al*. (2017), with reductions of 90% on the carbon footprint of scenario II in relation to I. The authors compared the landfilling of municipal biowaste (MBW) and the AD of this waste under different particle sizes (<2mm to 12.5mm).

The carbon footprint from the anaerobic digestion of microwaved food waste using a high heating rate (7.8°C/min) was 9.14kg CO_2/t when heated to 145°C, which also represented the highest of all the MW FW digestion conditions. The lowest carbon footprint using a high heating rate was 7.37kg CO_2/t which occurred when the substrate was heated to 175°C.

Despite not being an efficient process in terms of methane yield, microwaved food waste anaerobically digested at higher temperature (175°C) regardless of the heating rate, showed the lowest environmental impact (Figure 8.2).

The carbon footprint obtained from the digestion of raw/control food waste (7.74kg CO_2/t) was similar to that of the food waste treated with microwave irradiation. This suggests that the significant amount of energy recovered from the pre-treated FW could justify the higher environmental impact associated with it.

The environmental impact of the combined anaerobic digestion of raw FW and microwaved digestate resulted in higher carbon footprint than the digestion of microwaved food waste alone. As the volume of treated digestate increased, there was a decrease in the carbon footprint, regardless of the final temperature or ramp rate used. This is because the digestion of higher volumes of microwaved digestate heralded lower methane yields compared to smaller volumes of treated substrate.

The highest value (17.99kg CO_2/t) occurred when treating the latter substrate at 75°C 1.4 ramp using a volume of 25%. This represents an average of a 2-fold increase compared to MW FW digestion footprint. High carbon footprint values (15.73 and 15.34kg CO_2/t, respectively) were also found for the co-digestion of raw food waste and treated digestate heated to 95 and 115°C also using 25% volume of treated digestate.

The co-digestion of raw food waste and microwaved digestate heated to 85°C 7.8 ramp, as well as the co-digestion of raw food waste with control/untreated digestate had a similar carbon footprint to the digestion of microwaved food waste. The co-digestion of raw food waste with control/untreated digestate showed lower values than co-digestion of raw food waste and microwaved digestate heated to 85°C 7.8 ramp, ranging from 7.8kg CO_2/t (25% volume of treated digestate) to 9.02 kgCO_2/t (75% volume of treated digestate).

These results also suggest because of the similarity of carbon footprint of the microwaved FW and co-digestion of FW and untreated digestate, the latter would be more advantageous since it would result in the simultaneous management of FW and digestate.

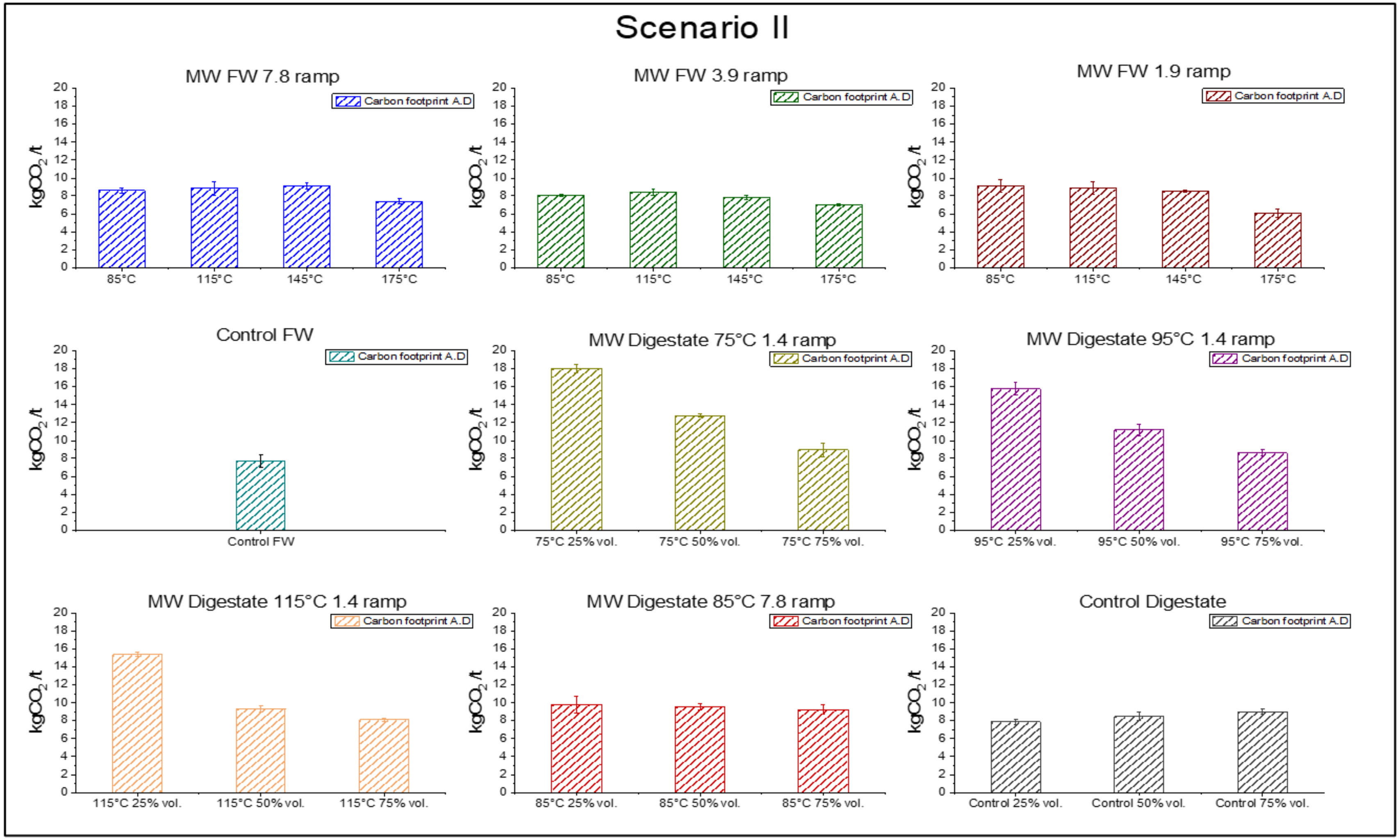


Figure 8.2 Environmental Impact (carbon footprint- kgCO2/t) from the combined process of AD of microwaved food waste and digestate.

8.4 Mass balance

8.4.1 Solids fate for the AD of MW substrates

The experimental data obtained here were used to assess the mass balance for the different microwave operating conditions integrated with AD. Despite the solid's loss observed during the microwave pre-treatment of food waste under certain operating conditions (e.g. heating to175°C), prior adjustments were made before each BMP test to ensure appropriate concentration of this parameter in each reactor. Therefore, for this study, 100% of the SCOD, TCOD, TS and VS was considered as the amount entering the process.

Table 8.3 shows the solids and organic matter fate in the AD of microwaved food waste for the digestion period, and takes into consideration the TS and VS present at day 0 (reactors assembling) and day 28 (last day of digestion). The results point to greater TS removal (an average of 54.45%) for the fast-heating rate (7.8 ramp) than for the medium and slow heating rates (3.9 and 1.9 ramps). Shorter exposure to microwave radiation therefore favoured solids removal for the whole digestion period. For the 7.8 ramp, the TS removal was on average, 9% and 34.24% greater than for the medium and slow heating rates, respectively. As temperature increased to 175°C, regardless of the heating rate, TS destruction for the total digestion period decreased significantly, with final values of 39.58, 32.62 and 22.74% for the 7.8, 3.9 and 1.9°C/min heating rates, respectively (Table 8.2).

The Volatile Solids (VS) removal followed a similar pattern to TS removal for thermally treated food waste digestion, with the fast-heating rate exhibiting the greatest VS removal (an average of 84.87%). This represented a 7.22 and 21.19% increase with respect to food waste pre-treated using medium and slow heating rates, respectively.

The greater removal of solids from MW FW was linked to enhanced methane production (Table 8.3), fast heating rate and lower temperatures resulted in higher methane yields. These results contrast with those reported by Aragon-Briceño (2018), who evaluated the effects of thermal treatment namely Hydrothermal Carbonization (HTC) of sewage digestate coupled with AD. The author showed that, in general, harsher thermal treatment conditions gave rise to greater removal of solids. The hydrothermal treatment at 160°C resulted in a solids reduction of between 47 and 56%, and treatment at 250°C resulted in solids reduction of between 62 and 68%. In this case, higher solids removal was linked to a better process performance, i.e. higher methane yield. The difference between Aragon- Briceño (2018) and the ones seen here could be attributed to the fact that microwave irradiation at high temperatures, leads to the formation of inhibitory compounds for the methanogenic community, hence negatively affecting solids removal and hence, methane production.

Solids removal (TS and VS) for co-digestion of raw FW and microwaved digestate varied significantly between the various scenarios tested (Table 8.4). Regardless of the final temperature, the smaller volume of treated digestate (25%) showed better solids destruction than the higher volumes (50 and 75%). This behaviour was particularly evident for treatment at 95°C, for which TS and VS removal at 25% volume of treated digestate was up to 43.95 and 45% greater than for 50 and 75% volume of treated digestate, respectively. These results suggest that co-digestion of microwaved digestate and raw food waste using a 1.4 ramp is negatively affected by high volumes of microwaved digestate, evidenced by the poorest TS and VS of all co-digestion conditions tested at 115°C with 75% vol. yielding least methane (185.7ml CH_4/g VS_{added}). The lower methane yield is probably due to the presence of inhibitory compounds formed during the thermal treatment of the digestate which undergoes the Mailllard reaction at a lower temperature (>100°C) than food waste (Tampio *et al.*, 2015).

The co-digestion of raw food waste and treated digestate at 85°C with a 7.8 ramp had a more uniform TS and VS removal rate across the various volumes tested, suggesting that the negative effects and/or inhibitory compounds are reduced or absent due to less harsh conditions (Table 8.3).

The solids removal results found here are closer to those reported by Zhang *et al.* (2016), who evaluated the co-digestion of raw and microwaved food waste with raw and microwaved sewage sludge (under various scenarios). The authors reported that TS removal for the co-digestion of MW FW and raw sewage sludge was, on average, 18.85%, with the greatest value (28.10%) observed when MW FW and raw sewage sludge were used in a 2:1 ratio. The VS removal for co-digestion of MW FW and raw sewage sludge was, on average, 47.92% with the greatest value (57.11%) for the MW FW and sewage sludge in a 2:1 ratio. In the Zhang *et al.* (2016) study, the highest methane yield was not associated with the highest VS or TS removal.

According to Zhang *et al.* (2016), the average TS and VS destruction for the co-digestion of microwaved sludge and raw food waste was 17.59 and 48.76%, respectively. Similar to this study, the authors reported an inhibiting effect of higher volumes of treated sludge on the solid's destruction and methane yield. Lower values of these parameters were found when the FW/MW-Sludge ratio was less than 2:3. In this case, TS, VS removal and methane were, on average, 25.24, 16.79 and 14% lower than other conditions.

The organic matter reduction (TCOD and SCOD) during digestion of MW FW overall showed a similar trend to that of solids reduction. The fast-heating rate and lower temperature resulted in greater destruction, with the digestion of MW FW at 115°C 7.8 ramp resulting in the greatest TCOD/SCOD removal. The least removal of organic matter occurred at 175°C 3.9 ramp. These results represent an improvement in relation to when FW was not pre-treated (control). In the former case, this was equivalent to: 160% in the TCOD removal rate and 124.49% in the SCOD removal rate, and 14.32% in the methane yield, hence corroborating the microwave pre-treatment efficiency.

The organic matter reduction (TCOD and SCOD) during the digestion of raw FW and microwaved digestate followed a pattern close to that of the solid's reduction. Smaller volumes of treated digestate resulted in greater organic matter removal (for reasons already explained). The greatest removal occurred for the digestion of raw food waste and microwaved digestate at 95°C using a 1.4 ramp and 25%vol.

Methane production was related to organic matter removal. Co-digestion of raw FW and treated digestate at lower temperatures and lower volumes of treated digestate showed increased methane yield, probably due lower concentration of inhibitory compounds originating from the Maillard reaction.

Table 8.2 Mass balance of the studied processes (pre-treated Food Waste).

Conditions		TCOD removal (%)	SCOD removal (%)	TS removal (%)	VS removal (%)	Methane Production (mLCH$_4$/gVS$_{added}$)
Ramp	temperature					
7.8	85°C	31.30	66.49	55.71	87.50	197.89
	115°C	35.89	64.43	58.72	88.47	203.41
	145°C	31.27	61.39	63.96	92.75	210.21
	175°C	38.37	67.27	39.58	70.77	169.51
3.9	85°C	27.95	52.34	51.74	86.10	185.33
	115°C	25.34	50.29	74.50	98.91	192.95
	145°C	23.16	49.35	49.38	82.01	179.59
	175°C	15.42	32.20	32.62	48.00	160.86
1.9	85°C	21.05	40.12	38.50	69.36	209.12
	115°C	20.29	39.86	36.20	67.56	203.79
	145°C	16.98	35.85	35.90	67.10	196.38
	175°C	25.90	48.69	31.00	63.50	138.99
Control FW		13.79	28.70	19.65	38.70	177.92

Table 8.3 Mass balance of the studied processes (pre-treated digestate).

Conditions	TCOD removal (%)	SCOD removal (%)	TS removal (%)	VS removal (%)	Methane Production (mLCH$_4$/gVS$_{added}$)
75°C 1.4ramp 25% vol.	47.67	67.55	48.32	74.84	413.50
75°C 1.4ramp 50% vol.	45.06	66.60	20.01	35.33	294.11
75°C 1.4ramp 75% vol.	47.03	65.63	21.03	35.54	206.00
95°C 1.4ramp 25% vol.	53.75	83.97	50.85	86.87	361.64
95°C 1.4ramp 50% vol.	41.84	79.45	28.51	47.78	256.74
95°C 1.4ramp 75% vol.	49.52	78.25	37.92	65.25	197.57
115°C 1.4ramp 25% vol.	38.11	57.15	34.10	65.31	352.68
115°C 1.4ramp 50% vol.	30.93	51.63	19.27	32.44	214.36
115°C 1.4ramp75% vol.	36.86	55.35	18.68	30.62	185.74
85°C 7.8ramp 25% vol.	37.20	55.88	25.64	54.85	225.40
85°C 7.8ramp 50% vol.	32.58	53.33	17.32	52.11	220.67
85°C 7.8ramp 75% vol.	38.70	57.64	16.29	42.30	212.41
Control 25% vol.	16.06	20.46	15.39	26.72	180.38
Control 50% vol.	20.76	31.33	13.75	24.39	194.88
Control 75% vol.	23.58	34.20	11.38	21.74	207.51

8.5 Microwave equipment properties and limitations on precise mass balance calculations

8.5.1 Dielectric properties of the substrate

In order to present the energy balance of the various scenarios tested it is important to consider some microwave properties that determine the interaction with the various substrates.

According to Robinson *et al*. (2015), successful MW heating of the substrate, depends on its interaction with the electromagnetic field, and its ability to absorb and convert the field energy into heat. The effectiveness of the substrate in converting field energy to heat energy depend on its dielectric property, as well as the amount of substrate, the shape and distribution of the substrate, amongst others factors (Thostenson and Chou, 1999). The dielectric property can be summarised by two numbers, the permittivity and conductivity of the substrate. Together these numbers define the substrate dielectric loss factor which dictates how efficiently the field energy is converted to heat energy.

A physical explanation of how the alternating electric field is converted to heat can be given as follows. Electrically polarised molecules in the substrate are continually being realigned by the alternating polarity of the electric field. This means that the charged regions of the molecules are moving under the influence of the electric force that the field exerts on these charges. The energy expended (the product of the force on the charge times the distance that the charge moves) is lost to the field and appears as an increased temperature (heat being synonymous with motion at the molecular and/or atomic level) (Thostenson and Chou, 1999).

The dielectric properties (permittivity and conductivity) of simple materials are well known over a wide range of temperatures and frequencies. Materials such as food waste, however, are complicated mixtures of simpler materials (usually in ill-defined proportions). There is a lack of fundamental data about the average (and variation of) the dielectric properties of such substrates. Further information (specifically mean and variance of substrate permittivity and electrical conductivity) along with similar information about the mechanical properties of substrates (e.g. mean and variance of thermal conductivity) is required if heating behaviour and thermal gradients in different substrates are to be modelled and properly understood (Meredith, 1998).

To the best our knowledge, there are currently only a small number of publications which report the dielectric properties of a small selection of substrate types. These include sorghum (Fennell and Boldor, 2014), oil palm (Salema *et al*., 2013; Jie *et al*., 2015; Tripathi *et al*., 2015), Australian wood based substrate (Ramasamy and Moghtaderi, 2010), wood pellets (Robinson *et al*., 2010), Tobacco stems (Zi *et al*., 2013), hay and switchgrass (Motasemi *et al*., 2014), and corn stover (Motasemi *et al*., 2015).

According to Kostas *et al*. (2017), dielectric measurement of substrates at various temperatures is also limited, being most commonly reported up to 50°C. Because these parameters are known for changing (nonlinearly) with temperature, data on the dielectric properties of a substrate at elevated temperatures (within the 100 – 900 °C temperature range) are urgently required.

According to the same authors, within the substrate, there can be distinctly different degrees of MW absorption by the biochemical constituents, with some absorbing more microwave energy than others, and even cases in which there is no absorption at all. Where there is microwave absorption, the degree of polarisation (charge separation in the molecules) depends heavily on the polarity of side groups found on each macromolecule.

Kostas *et al*. (2017) states that the characterisation of the different substrate constituents, including their dielectric property, is extremely complex especially since the biochemical compositions can vary significantly depending on the location and time of the year, as it happens in food waste. By providing this information, the likelihood of specific adjustment of parameters to improve MW heating efficiencies increases. This could further reduce the energy input for the pre-treatment step, whilst still maintaining its efficacy (Wilkinson *et al*., 2015). Developments such as these would allow the successful commercial development of MW based processing (Kostas *et al*., 2017).

8.5.2 Electric field and cavity size

A limitation when using laboratory scale equipment is the relatively low intensity electric field and the absence of reflected power measurements. Consequently, caution should be applied when interpreting energy balances for such equipment, as it is not possible to determine directly, or with precision, the energy absorbed by the sample. As well as the intensity of the electric field, its distribution within the MW cavity is also an important factor. This is because, one of the characteristics of MW heating is the formation of 'hot spots', which usually originates from the inherent heterogeneity of the electric field within a cavity. A consequence is that uneven distributions of the electric field can give rise to large thermal gradients (Jones *et al*., 2002). Regions of higher electromagnetic field inside the cavity are where the hot spots are found (Thostenson and Chou, 1999). Furthermore, the hot spots are not equally distributed leading exacerbating the uneven distribution of heat across the substrate. This means that the degree of substrate pre-treatment is potentially inconsistent.

According to Kostas *et al*. (2017), to date there has been no investigation of the effects of MW frequency, power and substrate position (in the cavity) on field distribution and substrate temperature distribution. They recommend real-time mapping of the power density distribution (which is proportional to the square of the field distribution) on the feedstock that is being pre-treated, as to improve substrate microwave pre-treatment processes.

Robinson *et al*. (2015) reported a concentration of electric field and power density around the edges of internal protruding objects (such as stirrers and temperature probes) within a MW single mode cavity. Nevertheless, there are several techniques available to circumvent such effects, including hot spots. They are: a) changing the geometry of the cavity by modifying its shape and size, b) using multiple MW inputs, c) operating at higher frequencies and ensuring adequate heterogeneity of the material (Thostenson and Chou, 1999).

The uneven distribution of the electric field, and consequently temperature, was observed in the present study when pre-treating food waste with the STARTSYNTH equipment (Figure 8.3).

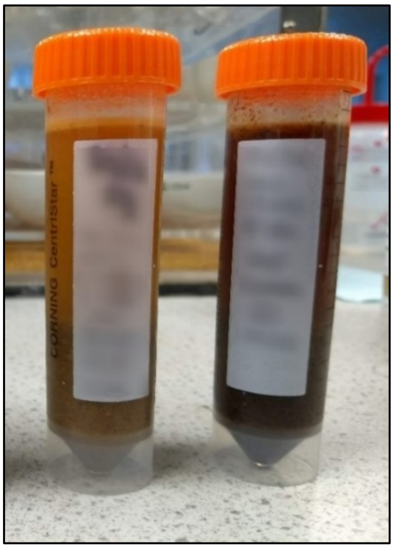


Figure 8.3 Food Waste samples from a same microwave run treated at 175°C 1.9°C/min, showing different colours, possibly due to differences in distribution of the electric field. Picture: Mariana Turnell (2018).

8.5.3 (Precise) temperature measuring device

In this study, uneven temperature distribution was particularly evident for treatment at higher final temperature (i.e. 175°C), where in the same run different samples became differently coloured (e.g. lighter or darker brown), signalling that different regions of the microwave cavity were not being heated equally. Under these circumstances, the sample(s) of 'out-lying colour' were removed and not considered in the characterization process or BMP tests.

Some of the laboratory-scale microwave equipment does not contain temperature probes, and therefore is not able to measure the sample's temperature. In other cases, the microwave device has an optical pyrometer in combination with grounded thermocouple probes to improve the reliability of temperature measurements (Motasemi and Afzal, 2013). Nevertheless, such apparatus gives an indication only of substrate surface temperature, and does not offer a true report of the internal temperature, including the thermal gradients that are commonly present within substrate due to the combination of selective and volumetric heating (Bermúdez *et al*., 2015).

The measurement of high-temperature microwaving processes is even more crucial, since important biochemical changes of the substrate are often observed under these conditions. For biological processes, such as AD, the precise temperatures at which changes occurs need to be reported if the process is to be optimised and unwanted products in the reactors (such as phenols and melanoidins) are to be avoided. (The latter are commonly present at higher temperatures in thermal treatments.) The interpretation of the results in these cases can be controversial because of the uncertainty of the temperature measurement (Kappe, 2013). For these reasons, any reported temperature values should be interpreted with caution (Kostas *et al*. 2017).

As a consequence of the challenges discussed above publications addressing a comprehensive and conclusive energy balance methodology when applying microwave radiation are scarce. According to Bermúdez *et al*. (2015), another reason that restrict a precise mass balance estimate is related to the low sample mass typically used. Lab-scale studies of MW treatments employing only a few grams of sample are very common (Figure 8.4).

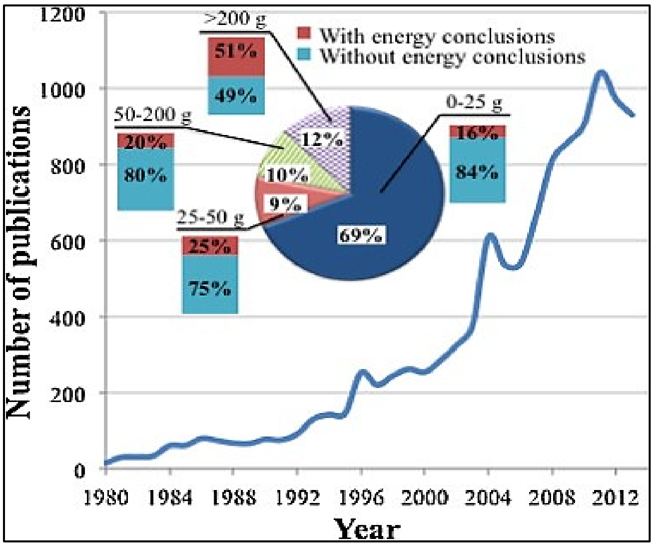


Figure 8.4 Evolution of the number of scientific publications related to microwave heating. Distribution of these publications in terms of the mass used in the study and the energy conclusions drawn (Source: Bermudez *et al*., 2015).

Drawing conclusions about the viability of an industrial-scale process based on results can be risky and, depending on the amounts of sample used in the experiments, can sometimes be meaningless (Bermúdez *et al*., 2015). They suggest that an increase in sample mass from 5 to 100 g reduces the energy requirement (per gram) as well as allowing a more precise mass balance.

8.6 Energy balance

8.6.1 Microwave performance under different operational conditions and MW interaction with the medium

This section briefly describes the behaviour of the STARTSYNTH microwave equipment used in the first step of the research (treatment of food waste) under different operating conditions, namely heating rates and final temperature.

The microwave oven used in this research is not equipped with an integrated internal temperature sensor. The only available mechanism to measure the temperature of the reaction consists of an infrared sensor located inside the microwave cavity, which offers an approximate value of the temperature outside the sample vessels.

The heating rate played an important role on the microwave behaviour towards final temperature and power input as briefly discussed below.

For all the conditions tested, the final external temperature of the vessels exceeded that intended. Temperatures were classified as: a) final temperature, i.e. the temperature goal and b) the final/achieved temperature, i.e. the temperature achieved during the microwave treatment (outside the vessels).

The high heating rate (7.8°C/min) with lower final temperatures (85 and 115°C) was subject to more prominent temperature overshoot. Therefore, final temperatures achieved for 85°C and 115°C were 90ºC and 120°C respectively, compared to 87°C and 117°C for the 3.9°C/min ramp. For the medium heating rate (3.9°C/min) and slow heating rate (1.9°/min) the overshoot was greater for the higher final temperature (145°C and 175°C), with final achieved temperatures of 149 and 183°C/min respectively (Table 8.4).

It is believed that the internal vessel temperatures were even higher than the ones recorded for the outside environment, possibly having a greater impact on the interaction with the substrate than it would have had if the equipment had produced the intended final set of temperatures.

In order to avoid sample overheating, drying and/or burning due to long, continuous, heat exposure, especially at higher temperatures and low heating rates, a discontinuous heating mode was employed. The heating was attained by alternating on-off pulses of microwave power.

The higher the temperature to be reached and the higher the heating rate, the higher the required power input. Treatment at a final temperature of 175°C with a 7.8°C /min heating rate reached the maximum power input recorded (680 W) for the whole set of conditions. Because of the discontinuous heating mode, 1000 W of selected power was not reached on any occasion.

The power pulse mode chosen for the microwave, together with the absence of a device that would automatically record the power fluctuation during each run, limited the power recording to the maximum power registered for each operating condition, thus requiring some assumptions to establish the energy balance for the scenarios tested.

Table 8.4 describes the behaviour of the microwave equipment chosen for this study. E (theoretical) is the chosen power for the microwave; E (max) is the maximum power observed (kW).

Table 8.4 STARTSYNTH microwave performance when treating food waste at a range of heating rates and final temperatures.

Heating Rate	Parameter/Final Temperature	85°C	115°C	145°C	175°C
7.8 ramp (Fast)	E (theoretical) kW	1000	1000	1000	1000
	E (max) kW	287	383	591	680
	Final achieved Temperature (°C)	90	120	148	181
3.9 ramp (Medium)	E (theoretical) kW	1000	1000	1000	1000
	E (max) kW	227	362	433	556
	Final achieved Temperature (°C)	87	117	149	182
1.9 ramp (Slow)	E (theoretical) kW	1000	1000	1000	1000
	E (max) kW	252	289	402	499
	Final achieved Temperature (°C)	88	117	148	183

8.6.2 Specific energy consumption

The use of 2.45GHz equipment in a laboratory-environment is of significant value for the assessment of heating effects and also the opportunities for the technology to be used with different feedstocks. However, as previously discussed, the calculation of an energy balance from lab-scale experiment has limitations and therefore needs to be interpreted with caution. The energy utilization efficiency depends on several factors including: a) the geometries of the resonant cavity and the reactor, b) the design and effectiveness of the impedance matching circuit to minimize reflected power, c) efficiency of magnetron (usually considered to be 60 – 80%, and d) the dielectric properties of the material/substrate. The latter is the degree to which the substrate absorbs the available microwave power (summarised by the impedance match of the load (in this case the substrate) to the power source (i.e. the magnetron) (Sturm *et al*., 2013), previously described in section 8.5.1.

Many studies discuss energy efficiency as an exclusive advantage of microwave heating. However, the energy input required to heat a given quantity of substrate is the same, whether MW or conventional heating methodologies are used (Miura *et al*., 2004).

In a microwave oven, the generation of electromagnetic radiation results from the acceleration of charge. In order to achieve the power and frequency necessary for the device to heat the material, the microwave sources use vacuum tube technology. These devices include magnetrons and travelling wave tubes (TWTs), with the former being more common given its lower market price. Both can be used in domestic and laboratory-scale microwave ovens (Kitagawa *et al*., 1986). According to Thostenson and Chou (1999), magnetrons are only capable of generating a fixed frequency.

A laboratory microwave oven such as those used here, are considered to be generic items of equipment and for this reason are not designed, nor optimised, for a given mass and type of load. It is not, therefore, possible to determine the specific impedance matching efficiency of the materials, which could plausibly be anything in the range of 5 – 80% (Thostenson and Chou, 1999).

For the STARTSYNTH microwave oven used for the thermal treatment of food waste, only the maximum power obtained for each operating condition was used in the energy balance calculations due to the reasons previously explained. The final temperature assumed was the one initially chosen for each set of experiments.

The MARS equipment used to treat the digestate, worked in a continuous mode and the maximum power reached was, therefore, that chosen at the programming step. This was 1200W (section 3.7.1).

Since the load (i.e. substrate) impedance was not designed to match the source (i.e. magnetron) impedance a precise energy balance cannot be calculated with confidence. Nevertheless, some bounds can, and have been, put on the energy balance of the laboratory experiment as described below.

c. Upper bound on energy balance–considering the minimum specific energy (energy per gram of substrate) that could have been supplied to the substrate (E_{in}) is given by the product of the substrate specific heat (s) and the temperature through which it is raised (θ), i.e.:

$$E_{in} = s \, . \, \theta$$

‘s’ for the digestate is assumed be that of water (i.e. s = 4.2 kJ/kg/°C). This is a conservative estimate of the E_{in} required because water will have a higher value of s than the non-water component of the substrate. However, the high specific heat of water and the amount of water present in the substrate mean that water is likely to dominate the overall specific heat of the mixture. In this sense E_{in} represents a realistic, estimate of the minimum E_{in} that must be supplied. A calculation of the energy balance using this figure will thus produce a value close to upper bound on the energy balance.

d. Lower bound –considering the maximum E_{in} that could have been supplied by each microwave oven. For the STARTSYNTH equipment is the maximum power recorded multiplied by the time power was applied divided by the concentration of substrate in each experimental unit (gVS L^{-1}).The maximum E_{in} that could have been supplied by the MARS equipment is the rated power of the oven (chosen at the programming step as 1200W) multiplied by the time this power was applied divided by the concentration of substrate in each experimental unit (gVS L^{-1}). These both represent over estimates of $E_{in.}$ In the case of the first oven it assumes the maximum power observed during the test was delivered to the substrate continuously throughout the test and in the case of the second oven it assumes the maximum possible power capable of being produced by magnetron was delivered the substrate continuously throughout the test. (The degree of over-estimation is greater for the second oven.) It is in this sense that the use of E_{in} so calculated represents a lower bound on the energy balance.

Because microwave of digestate has not yet been tested, there is no reference to its specific heat on the literature. For this reason, its value was assumed to be the same as water, as the moisture content of this material is approximately 95%. A specific heat for the dry component of food waste, however, has been reported to be 1.92 kJ/ $kg^{-1}{}^{\circ}C^{-1}$ (dry FW-solids) in Daniel (1998). A value of s for food waste was therefore determined from a weighted average of the dry solids specific heat and the specific heat of water. The ratios of these components were measured to be solids (32%) and moisture content (68%).

Figure 8.5 and Figure 8.6 show the variation of E_{in} estimated for food waste and digestate, respectively, at various temperatures for the best-case scenario. The energy consumption differs greatly in each condition, and in the case of the microwaved digestate ranges from 0.06 to 0.113Wh/g, whereas in the case of the high temperature heating of food waste it can reach values higher than 0.132 Wh/g.

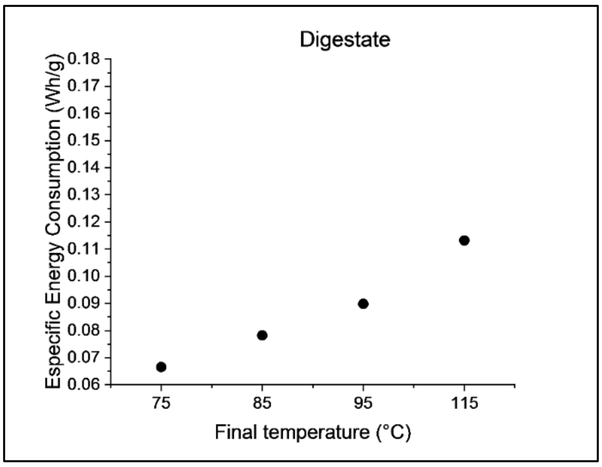


Figure 8.5 Specific energy consumption of digestate microwaved under different temperatures.

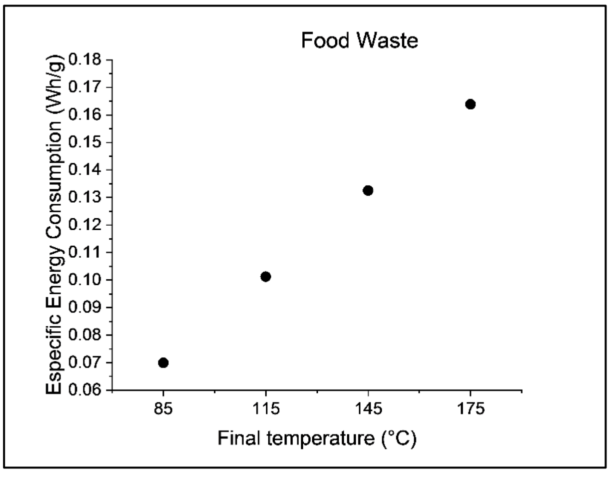


Figure 8.6 Specific energy consumption of food waste microwaved under different temperatures.

Overall, the minimum energy requirement to heat food waste from room temperature (here established as 18°C) to the desired final temperature is greater than that for the digestate. It seems likely, therefore, that the energy required to treat food waste with microwave irradiation at an industrial level would be higher than for digestate, requiring the development of operational strategies as an attempt to compensate this extra cost. On the other hand, the energy required by both substrates can vary depending on the weight of the sample used on the microwave oven, as discussed below.

According to Bermúdez *et al.* (2015), when small samples are conventionally heated, there is a significant additional energy consumption due to fact that the walls and the cavity of the oven first need to be heated to supply the heat to the sample (by radiation, conduction and/or convection). This energy 'overhead' can be improved by utilizing microwave heating. This is because, when a microwave field is applied, the heat is generated inside the material. The sample is thus heated more directly and the energy losses related to the heating of the walls and the cavity of the oven are avoided or, at least, diminished. The sharpest decrease in energy consumption occurs from a few grams to about 50 – 100 g. After this point, the decrease becomes more gradual. This is because the energy used to supply the heating overhead becomes less significant compared to the energy used to heat the larger substrate mass.

An analogous effect is present in microwave ovens. If the impedance match is perfect then all the power is absorbed by the substrate. If the impedance match is sufficiently poor then all the energy is 'reflected' from the substrate and may be absorbed by parts of the oven structure. At least part of the energy is then being used to heat structure of the oven rather than the substrate. This is analogous to the heating overhead in a conventional oven. The impedance match is a complex function of the substrate dielectric parameters (conductivity and permittivity), the amount of the substrate (i.e. the degree to which it fills the cavity), the distribution of the mass of the substrate (one large mass or many smaller masses) and the shape of the substrate masses. The latter may be determined by the shape of the reactor vessels. For generic ovens (ones that are not purpose designed to heat a specific mass of a specific substrate with a specific shape) there is a tendency for matching to become better as the mass of the substrate increases (and more nearly fills the oven cavity) (Thostenson and Chou, 1999; Kostas *et al.*, 2017).

In this study, a fixed amount of sample was used; 2 g of food waste (dissolved in 5 ml of water) and 10 ml of digestate placed in each vessel. The implication of small sample use is that when a microwave system is not used at its maximum sample load capacity (which will depend on the design of its resonant cavity and the precise shape and size of the substrate vessels), there will be a significant waste of energy and loss of efficiency, compared to a system of worse design operating closer to maximum capacity. Consequently, by treating a higher amount of substrate per tube and more tubes per run, less energy per gram is likely to be required to treat the substrate at a given temperature. Such considerations may be an important step in in scaling-up the process.

An industrial microwave heating plant would be designed (or selected) for a given application. The impedance matching of a well-designed industrial oven to a specific load (food waste or digestate,) would probably be between 60 – 90%. According to Meredith (1998), most industrial microwave heating equipment operates at lower frequency than laboratory-scale ovens. The former, usually have a frequency between 896 and 922MHz, with an electrical efficiency of around 85%. Nevertheless, because it is not possible to predict the efficiency of the equipment used, the overall efficiency of an industrial oven assumed here is of 50%.

An energy assessment, known as Net energy balance (NEB), is an important concept when choosing a bioenergy processing platform. This is because only a process having positive (preferably high) NEB is economically and environmentally sustainable (Yuan *et al.*, 2008).

The best-case scenario, for the digestion of microwaved food waste using several final temperatures resulted in positive net energy for all conditions tested. This suggests that the enhanced methane yield (energy out) was greater than the energy required (energy in) to process the samples by microwave. These results show that a full-scale cascade process could be justified in terms of an energy incentive. The greater net energy for the AD of MW FW occurred for the following conditions: 145°C, 7.8 ramp, 115°C 3.9 ramp and 85°C 1.9 ramp, yielding 82.97, 76.16 and 82.57 Wh/g, respectively (Figure 8.7). The net energy of the process is a function of specific heat of the substrate and the consequent energy necessary to heat the material from room temperature to the final temperature. This is a less naive representation of the industrial-scale scenario, since it does not rely on power inputs from laboratory-scale equipment.

The highest net energy was observed for the soluble fraction food waste control. This is unsurprising since the methane produced in this case (E_{out}) was significantly higher than the microwaved FW conditions, and no energy was used (E_{in}) in pre-treating the sample. The surplus energy obtained for the control soluble was 45.98, 52.79, 46.38Wh/g in relation to the 145°C 7.8 ramp, 115°C, 3.9 ramp and 85°C, 1.9 ramp samples, respectively. The process efficiency improvement of the soluble fraction control in relation to the FW control (whole fraction), is an evidence that the solids represent a significant limiting factor for methane production; possibly related to the presence of inhibitory compounds such as melanoidins and phenols. When the solids are removed digestion can be significantly improved, making it desirable for an industrial-scale application. The only disadvantage of solids removal is the disposal of the underutilized fraction of the waste. Nevertheless, because the co-digestion of raw food waste and thermally treated digestate seems promising, the solids removed in the former case could be utilized in the co-digestion process.

The worst-case scenario for anaerobic digestion of microwaved food waste is shown in Figure 8.8. In this case, the net energy is a result of power input and microwave exposure time. This will grossly overestimate the input energy (and therefore represents a harsh, but useful, lower bound on the energy balance).

Overall, the worst-case scenario showed a negative net energy value for most of the conditions tested. These results suggest that that the increased microwave duration, negatively influenced the AD process. Food waste treated using a fast-heating rate (7.8 ramp) exhibited the greatest positive energy balance, with final temperatures of 85 and 115°C yielding 38.97 and 14.68 Wh/g net energy, respectively. Conversely, as the exposure time to MW radiation increased (medium and slow heating rates of 3.9 and 1.9°C/min, respectively) process performance was reduced, synonymous with a decrease in energy efficiency (E_{out}/E_{in}), especially for at a high final temperature (175°C).

Food waste treated using a slow heating rate (1.9°C/min) had a negative net energy balance for all final temperatures, i.e. -13.72, -64.00, -163.39, -318.57 Wh/g for the 85, 115, 145 and 175°C (Figure 8.8). Similar to the best-case scenario, the food waste soluble fraction control showed the greatest positive net energy of all the FW scenarios tested, again indicating that this could be a feasible option when scaling-up the process.

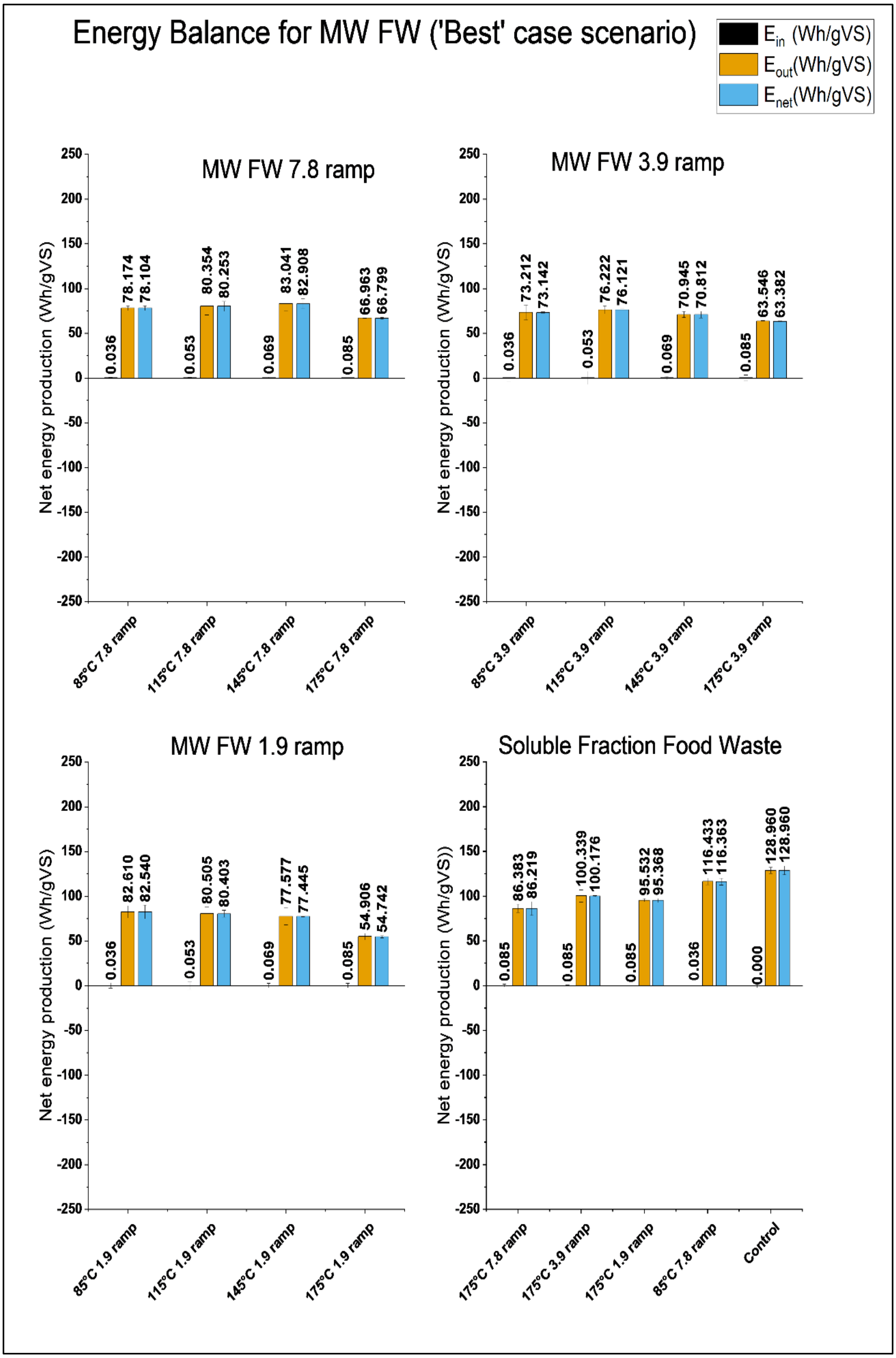


Figure 8.7 Energy Balance – Best case scenario. Net values for microwaved food waste under various temperatures and heating rates.

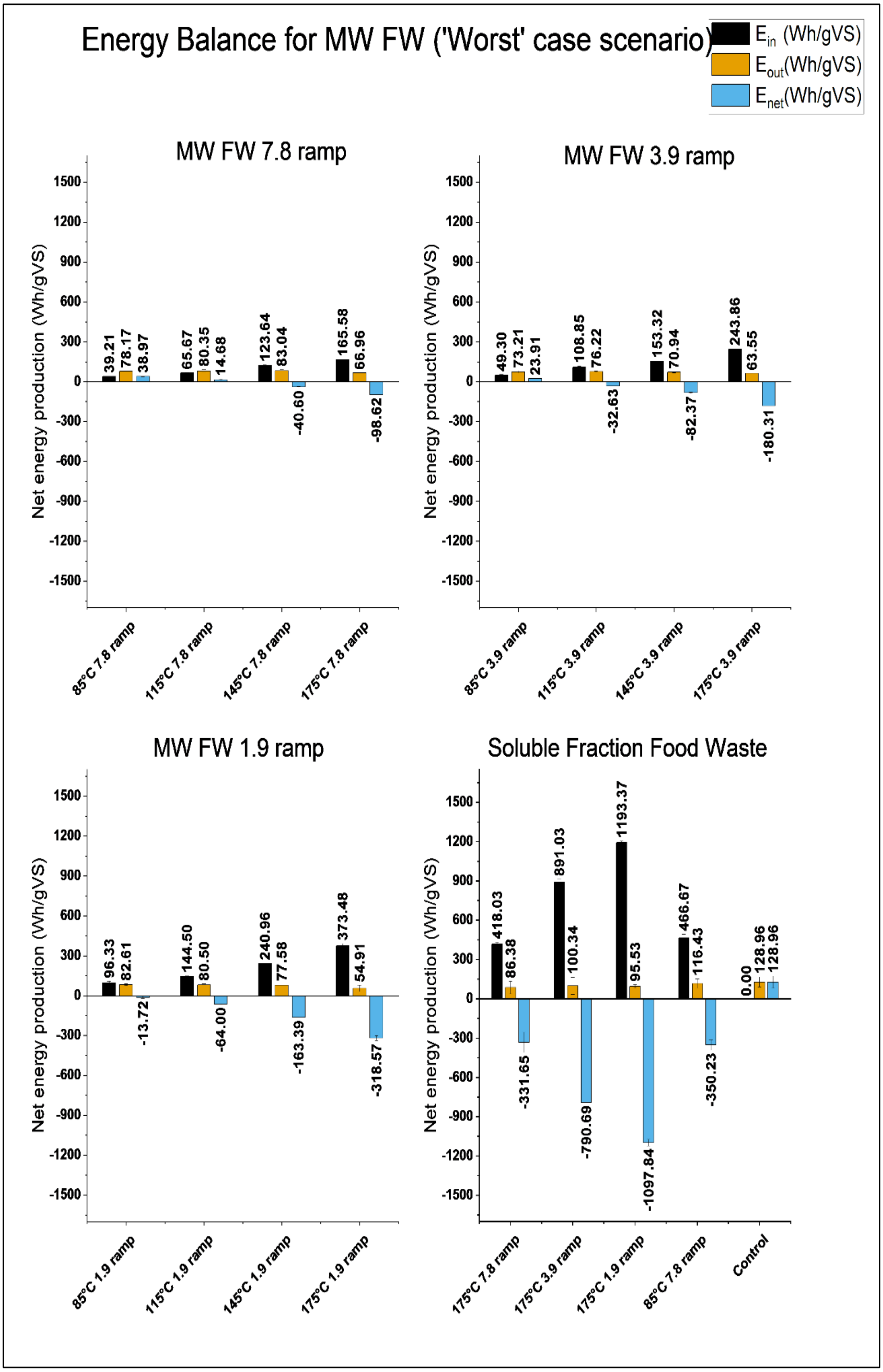


Figure 8.8 Energy Balance – Worst case scenario. Net values for microwaved food waste under various temperatures and heating rates.

The best-case scenario of the microwaved digestate, followed by its re-circulation into the MAD of raw FW also showed positive net energy values for all the tested conditions. The greatest net energy values occurred for the lower volume (25%) of treated digestate, regardless of the final temperature used. The net energy values were 163.13, 142.81 139.26 Wh/g for the 75, 95, and 115°C, respectively, treated at 1.4 ramp and 25% volume of treated digestate, (Figure 8.9). These results point to the negative effect of increasing final temperature on the energy balance, possibly due to the higher energy input required in relation to the poor volume of methane obtained as a result of the possible presence of inhibitory compounds hypothesized for the 115°C samples.

The worst-case scenario for the microwaved digestate, followed by its re-circulation into the MAD of raw FW, had a negative net energy for most of the conditions tested. The greater energy input occurred for the digestate pre-treated at 115°C 1.4 ramp (25% volume of treated digestate), and because the methane produced was not as high as in the other tested conditions, the net energy yield was poor (-458.91Wh/g). In fact, this condition represented the worst net energy balance of all tested scenarios, including microwaved food waste. The only conditions for which significant positive net balance was observed were when digestate was treated using milder operating condition (85°C 7.8 ramp) for all volumes of treated digestate. The net energy balances in these cases were 19.72, 46.63 and 52.94 Wh/g for 25, 50 and 75% volume of treated digestate, respectively (Figure 8.10).

Positive net energies obtained from any of the various processes could be applied to the national gas grid. Based on the energy balance estimates it is possible to infer that the best operating conditions for scaling-up the cascade process for MW FW are a final temperature of 85°C and heating rate of 7.8 °C/min, since it represents the operational condition that offers higher positive net energy for both best- and worst-case scenarios prediction. Control FW soluble fraction is also favourable for large-scale application for the reasons already explained. The best operating conditions for scaling-up co-digestion of microwaved digestate with FW is: final temperature of 85°C 7.8 °C/min using 50 and 75% volume of treated digestate. Nevertheless, the most correct answer would be a value in between the best- and worst-case scenarios, since none of them precisely expresses the energy requirements (E_{in}) of the conditions tested as previously discussed.

Conversely, Mottet *et al*. (2009), reported that the energy balance conducted for both ultrasound and microwave pre-treatment of mixed sludge did not represent an energy incentive in any of the cases they investigated, since the methane yields were not sufficiently large to compensate the required energy for processing.

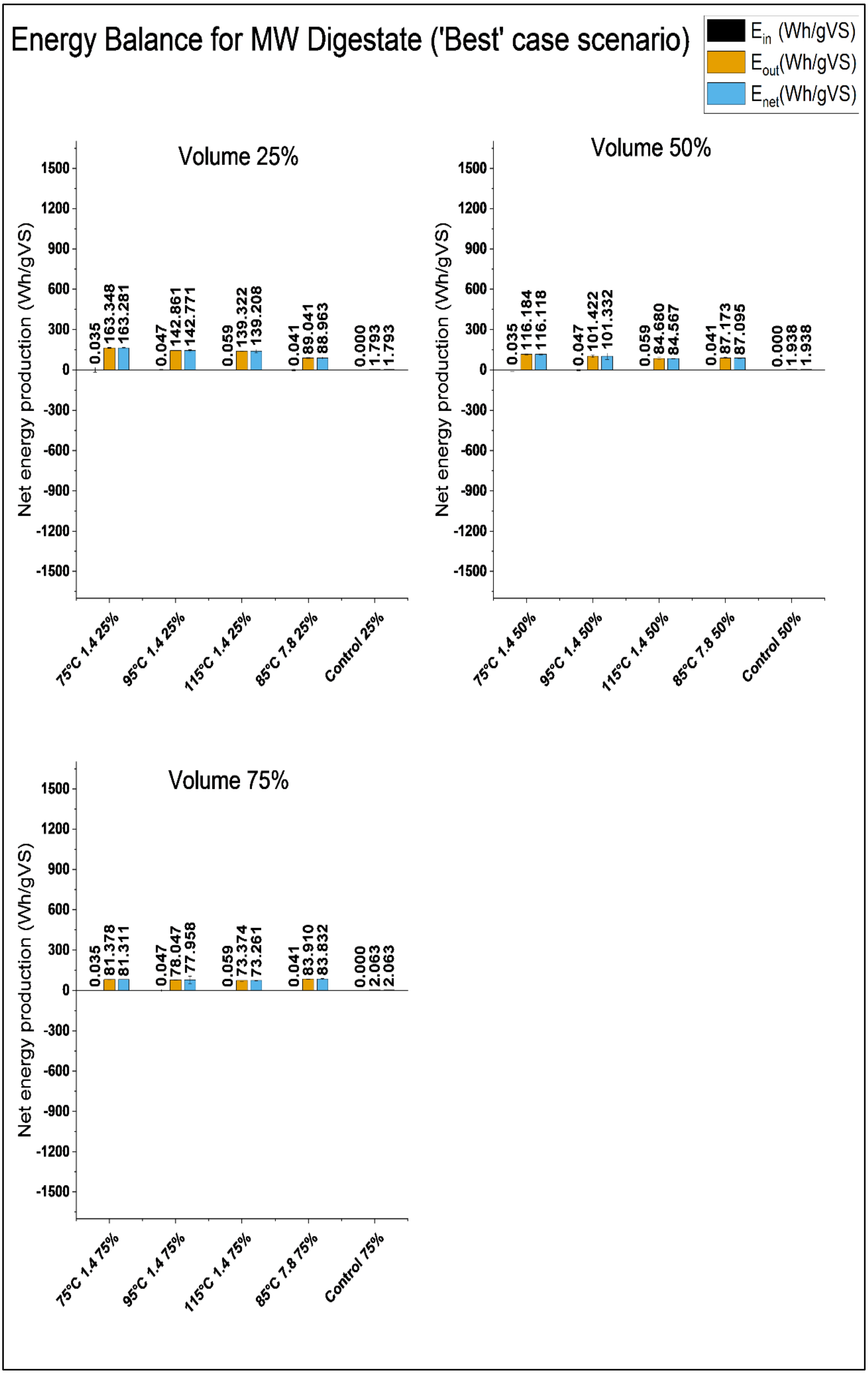


Figure 8.9 Energy Balance – Best case scenario. Net values for microwaved digestate under various temperatures and heating rates.

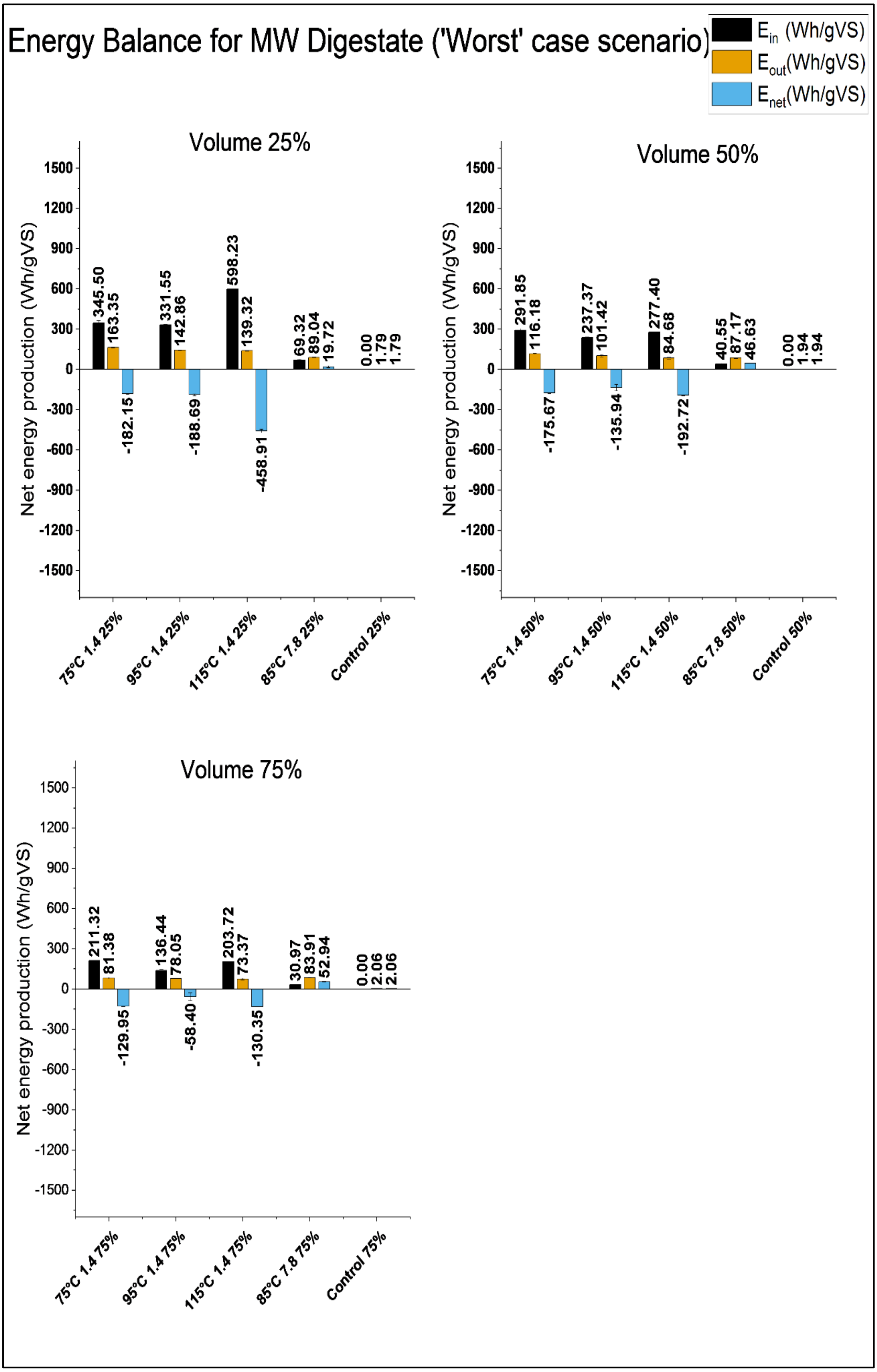


Figure 8.10 Energy Balance – Worst case scenario. Net values for microwaved digestate under various temperatures and heating rates.

On the other hand, Yang *et al.* (2010) concluded that the thermal pre-treatment of sewage sludge using two-phased anaerobic digestion had a positive energy balance. They suggested that the surplus volume of biogas produced could be used to reduce costs of the process via an efficient heat exchanger.

8.7 Summary

In this chapter, the potential to integrate MW irradiation with AD of food waste and digestate was evaluated through environmental impacts (carbon footprint), as well as mass and energy balances.

The results showed that combined treatment has lower environmental impact (reduced up to 98.20%) than food waste disposed of to landfill, as long as there are no leaks in the pipes that conduct the AD recovered methane during production and transportation. The combined MW and AD process therefore, offers a strategy for food waste and digestate management with a low carbon footprint.

Microwave temperature and heating rate had a strong influence on solids destruction during the digestion of MW FW. Higher levels of solids removal for the AD of microwaved food waste are linked to rapid heating and lower final temperatures (85, 115°C and 7.8°C/min), heralding greater methane yields than higher temperature scenarios (175°C), in which the formation of inhibitory compounds is likely. In the latter case, the inhibitory compounds adversely affect process performance including solids removal, and thus, methanogenesis.

The volume of treated digestate applied to the MAD of FW has a strong influence on solids destruction. Greater volumes (50 and 75%) of treated digestate have a detrimental effect on process performance, decreasing TS and VS destruction, and methane yield.

A comprehensive energy balance was not possible here due to: a) the small sample size, b) the pulsed power mode chosen for the STARTSYNTH microwave oven together with the absence of power fluctuation time-series during each run, c) the unknown distribution of the electric field inside the microwave cavity, d) the use of a generic, laboratory-scale, microwave oven that was not impedance matched designed to minimize reflected power, e) unknown magnetron efficiency, f) absence of precise information about the digestate dielectric properties. Moreover, the energy balance calculations do not consider energy inputs related to transportation, particle size reduction of food waste required for the AD.

Nevertheless, a simple energy balance analysis was conducted, showing that the greater net energy for the AD of MW FW in a ‘best case scenario’ occurred for the following conditions: control FW soluble fraction, 145°C 7.8 ramp, 115°C 3.9 ramp and 85°C 1.9 ramp, with a net energy of 128.96, 82.90, 76.12 and 82.54Wh/g, respectively. For the ‘worst case’ scenario, food waste treated using a rapid heating rate (7.8 ramp) and final temperatures of 85 and 115°C showed the highest net energy, equivalent to: 38.97 and 14.68 Wh/g

For the ‘best case scenario’ of microwaved digestate re-circulation into the MAD of raw FW, the greatest net energy values occurred at a lower volume (25%) of treated digestate, regardless of the final temperature used, with net values of 163.13, 142.81 139.6 Wh/g for the 75, 95, and 115°C 1.4 ramp, at 25% volume of treated digestate, respectively. The only conditions in which significant positive net balance in the 'worst case' scenario occurred was for digestate treated using a milder operating condition (85°C7.8 ramp) for all volumes of treated digestate. These net balances were 19.72, 46.63 and 52.94 Wh/g for the 25, 50 and 75% volume of treated digestate, respectively, possibly due to the absence of inhibitory compounds.

Positive net energies obtained from any of the various processes could be applied to the national gas grid. Based on the energy balance estimates it is possible to infer that the best operating conditions for scaling-up the cascade process for MW FW are a final temperature of 85°C and heating rate of 7.8°C/min, since it represents the operational condition that offers higher positive net energy for both best- and worst-case scenarios prediction. Control FW soluble fraction is also favourable for large -scale application. The best operating conditions for scaling-up co-digestion of microwaved digestate with FW is: final temperature of 85°C 7.8°C/min using 50 and 75% volume of treated digestate.

Nevertheless, the most correct answer would be a value in between the best- and worst-case scenarios, since none of them precisely expresses the energy requirements (E_{in}) of the conditions tested.

It is believed that the information presented in this chapter will be useful to decision making in the bioenergy industry in the context of establishing microwave-heating processes as part of a sustainable strategy for food waste and digestate management.

CHAPTER 9
9. GENERAL DISCUSSIONS

9.1 Introduction

This chapters discusses the main findings regarding the use of microwaved FW and microwaved digestate in the mesophilic anaerobic digestion on batch tests and their implications when applied to a large-scale system (e.g. CSTR reactors).

9.1.1 The use of optimum I/S ratio (3:1) on a large-scale system

Batch (BMP) tests conducted in this study showed that FW is best digested in an anaerobic condition using an inoculum to substrate ratio (I/S) of 3:1 and particle size of 1 mm (Section 4.4.1). According to the literature, in batch systems, I/S ratios higher than 4 results in poor biogas production, and decreases the effective volume of the reactors, due to the requirement for higher volumes of bacteria. The limitation to a 3:1 ratio in this study, arose due to the adoption of small working volume reactors (400 ml), which limited the amount of inoculum applied in the AD as well as the fact that lower I/S ratios (2:1 and 1:1) resulted in poor methane yield (Chapter 4).

Batch reactors can efficiently and safely provide important information on the AD of a substrate, including duration of the lag phase, maximum biogas yield, methane production and hydrolysis rate, amongst others. In contrast, CSTRs (continuous stirred-tank reactors) are more appropriate to examine, for example, micro biome's acclimatization for long term operation. Nevertheless, experiments on the latter are laborious and time consuming and therefore can cover relatively few experimental conditions. In contrast, the outcome of lab-scale reactor set-ups, applying appropriate data interpretation, can be extremely useful as input for modelling simulations to expand testing under various conditions, and thereby improve understanding of larger than bench scale AD systems (Tsapekos *et al*., 2018).

Little information is available in the literature concerning the extrapolating to CSTR reactors using data from batch experiments. According to Girault *et al*. (2012), batch experiments can be used to predict methane production in a CSTR, as long as the degradation phenomenon exhibits no inhibition, or when the inhibition is reversible. On the other hand, when inhibitions occur (especially those that are irreversible), it is not possible to directly extrapolate batch observations for the design of full-scale CSTR digesters.

In this study, AD of raw FW in batch systems with a 3:1 I/S ratio exhibited a TVFAs build-up on the 2nd and 4th day of digestion which affected the process performance and resulted in a lower biomethane production in relation to microwaved FW samples. The acidification process, as a result of the TVFAs accumulation, was nevertheless reversible with the system being able to recover itself after the first week of digestion. Therefore, following Girault *et al*. (2012) suggestion on the use of batch results for extrapolating CSTR digesters application, the use of raw FW with I/S of 3:1 I/S could be applied with confidence, to a large-scale system with good expectancy of methane production.

Regarding MW FW, there no TVFAs build-up in the start–up phase, for most of the conditions tested especially for the lower temperatures (85 and 115°C) and fast heating rates (7.8°C/min and 3.9°C/min) (section 5.3.2). This indicates the effectiveness of the chosen pre-treatment and, most importantly, offers the possibility of using these results for further scaling–up of the process under CSTR conditions.

Batch results also provide information on biodegradation kinetics (Girault *et al*. 2012), and hence, commonly used to investigate process inhibition. Overall, MW FW showed a shorter lag phase than untreated FW (section 5.6), with a higher methane production in the first week of digestion. The absence of severe system acidification/inhibition or process failure in the start-up phase of the MW FW batch system experiments reenforces that in this case, it is possible to directly extrapolate batch observations to full-scale systems, with a good methane production prediction.

Nevertheless, MW FW treated at a final temperature of 175°C using different heating rates showed reduced methane production and prolonged lag phase (which negatively influenced methane production kinetics) at the beginning of digestion compared to other MW samples. This behaviour indicates a methanogenic inhibition possibly due to the presence of phenols and melanoidins. Consequently, the use of such results for designing continuous CSTR processes would probably not be simple and, therefore, not advisable.

Overall, a 3:1 I/S ratio for MW FW is advised for CSTR systems. Nevertheless, higher I/S ratios ($\geq$ 3:1) in CSTR systems may also be adequate depending on their specific design. This is because, compared to batch reactors, CSTRs, have higher working volumes, hence benefitting from a higher concentration of inoculum (bacteria), better processing higher amounts of food waste that is constantly feed to the system.

On the other hand, Fitamo *et al*. (2016) showed that the methane yield obtained from the CSTR experiments using a mixture of sludge, food waste, grass clippings and green waste was lower than both the BMPs estimated theoretically and by means of batch assays. According to the authors this result was expected and was justified by the BMP definition which represents maximum practically achievable methane production at an ‘‘indefinite” degradation time. Moreover, the methane yield in CSTR experiments will always permit some degradable organic matter to leave the reactor with the effluent.

Therefore, the type of experiment used here (batch) and its results can be used, with appropriate caution, to predict CSTR full-scale behaviour when treating raw and MW FW.

9.2 Full-scale system buffer capacity and external alkalinity requirements compared to batch experiments

It has been stated that anaerobic digestion of food waste requires the addition of a buffer agent (alkalinity) and/or nutrient supplementation before starting-up and in some cases, during the AD process (Chakraborty *et al*., 2018, Zhang *et al*., 2014). This is because, the usually high OLF (organic labile fraction) of food makes it prone to rapid digestion and TVFAs accumulation, with low pH values (below 6) and system acidification (Kawai *et al*., 2014). The low pH values are not well tolerated by methanogenic bacteria and, for this reason, methane production usually becomes hindered (Zhang *et al*., 2007).

Nevertheless, several studies have shown that is possible to obtain successful digestion without the addition of an external source of alkalinity. The common approach was to use an intrinsic source of alkalinity, such as sewage sludge and manure in co-digestion with FW. The use of these co-substrates is beneficial because: (a) it buffers the system and regulates the pH; and (b) it introduces a wide range of bacteria into the system, including fermentative and methanogenic ones, important for methane generation (Chakraborty *et al*., 2018, Zhang *et al*., 2013, De Vrieze *et al*. 2013).

De Vrieze *et al*. (2013) applied high-rate iron-rich activated sludge as a stabilizing agent for the AD of FW. High-rate activated sludge or A-sludge is obtained during the first stage of the two-phase A/B activated sludge production system, and in the case of De Vrieze et. al, (2013) the sludge was amended with $FeSO_4$. According to the authors, the main benefit of adding this component to the process is to obtain stable methanation and higher methane production, eliminating the likelihood of process failure. The sludge buffers the system, making it tolerant to the production of high levels of TVFAs, especially in the start-up phase (De Vrieze *et al*., 2013).

The authors reported methane production rate values of 1.15 and 1.12 l/day during mesophilic and thermophilic co-digestion, respectively, of a fed mixture consisting of 15% KW (kitchen waste) and 85% A-sludge. These values are higher than those obtained by the digestion of FW supplemented by nutrients, i.e. 0.45 l/day, compared to 0.30 l/day for the control. The authors concluded that the use of sludge, as a co-substrate for KW digestion, is an effective method for process improvement and reduces the costs associated with nutrient supplementation and addition of an external synthetic buffer agent.

Zhang *et al*. (2013) evaluated the main parameters affecting the anaerobic co-digestion of food waste and cattle manure utilizing batch reactors. The authors showed that methane production was enhanced by 41.1%, with a corresponding methane yield of 388 ml/gVSadded. Most importantly, the authors observed that the use of manure as a co-substrate enhanced the buffer capacity of the system (without the need for pH control) avoiding acidification due to TVAS increment during the AD process.

Sewage sludge from ESHOLT WWTP was used primarily as a seed for the experimental work reported here. It was applied as a buffer agent based on previous studies where the efficacy of this material in terms of stabilizing the process was shown. Its pH (8.2) and alkalinity value (average of 4200 $gCaCO_3/L$), suggested its adequate use as a buffer agent. Therefore, when assembling the reactors, sludge was mixed to FW in the proportion of 3:1.

During the AD process, pH values were monitored and never dropped below 6.5 for any of the conditions tested (including untreated FW, microwaved FW at both soluble and whole fractions) indicating that the sewage sludge was effective in stabilizing the system, especially during the first week of digestion were the hydrolysis and acetogenesis were most intense, with a high TVFAs concentration.

It also suggests that the chosen I/S of 3:1 was adequate for batch experiments in terms of offering the system sufficient alkalinity, since system failure did not occur. This is a clear advantage when using batch systems, because, in contrast to CSTR digesters which are under constant adjustment regarding feeding of substrate and removal of digestate, batch reactors are designed to perform using a pre-determined amount of substrate and seed mixed at set-up and then left unadjusted during digestion. The batch system digesting FW and sludge would not therefore be 'disturbed' for alkalinity adjustment.

In the case of CSTR systems, where there is a constant intake of substrate and removal of digestate (together with other components such as bacteria, nutrients and sludge which acts as a buffer agent), the system buffer capacity would at some point become negatively affected, possibly demanding further adjustment and external addition of alkalinity. Therefore, for a large-scale system treating MW FW an additional, external, source of alkalinity would be strongly recommended.

9.3 Inhibitory substances removal efficiency in a large-scale system

In this study it has been hypothesized that phase separation favours melanoidins removal of food waste by centrifugation and filtration. Several parameters indicated possible successful melanoidins and other inhibitory compounds removal, including volume of methane produced in the first day of digestion and peak volume of gas produced. This was particularly evident for FW samples treated at 175°C using a 3.9 ramp which showed a higher biomethane production on the first day of digestion, despite having a lower initial solids concentration in relation to the other samples. This suggested successful removal of inhibitory compounds. In this case, melanoidins was linked to the solids, with centrifugation transferring them to the liquid phase, and later removed from the sample by filtration. Therefore, centrifugation of FW applied under the specific g force chosen here would cause the unwanted (inhibitory) products to stay in the soluble fraction.

Similar to the food waste, digestate can undergo Maillard reaction if exposed to high temperatures. Nevertheless, the temperature range on which Maillard inhibitory compounds are formed for the digestate is significantly lower than for food waste (Tampio *et al.*, 2015). A straight forward method for inferring the formation of melanoidins in a substrate (e.g. digestate) is a decay in the NH_4/TNb ratio after thermal treatment. In the case of digestate, this was observed for the following operational conditions: 75 and 115ºC 1.4 ramp, where it represented >1% and >2.40% decay, respectively, possibly suggesting the formation of small amounts of Maillard compounds.

Digestate was used in a whole form in this study. However, it is believed that centrifugation and filtration could also favour inhibitory compounds removal. The rationale behind this assertion originates from previous findings that showed that mechanical separation of animal manure (similar chemical properties to digestate) can be an effective technique for removing and/or transferring substances from the solid to the liquid phase with the production of a liquid and a nutrient-rich solid fraction. Nevertheless, the efficiency of separators also depends on the physical and chemical composition of substrate and not just on the centrifugation operational conditions (Westerman and Bicudo, 2000).

Møller *et al*. (2002) analysed the efficiency of two different separation methods (centrifuging and screw press) of animal manure. According to them, centrifuging leads to a higher concentration of dry matter (DM) and total phosphorous (TP) (3.1–11.0 and 4.6–12.5 times higher respectively) than untreated manure. This shows that the centrifuge transferred a considerable amount of DM and TP from the liquid to the solid fraction. Moreover, the Total Nitrogen (TN) in the solid fraction was 1.76–3.54 times higher than in the untreated manure. The authors attributed this low TN increase (compared to DM and TP increase) to the mechanical separation merely transferring organic N to the dry-matter-rich fraction, with the dissolved NH_4^+ staying in the liquid fraction.

The screw press separation method produced a solid fraction with increases in the concentrations of DM, TP and TN of 4.77–6.47, 1.69–3.38 and 1.50–2.08 times, respectively, hence not being as effective as the centrifuging method in respect of nutrient transfer between fractions (Møller *et al*., 2000, Pain *et al*., 1978).

Additionally, the authors showed that after centrifugating the manure, there were no particles larger than 0.025 mm in the liquid fraction, whereas in raw manure, more than 30% of the particles were >0.025 mm. This clearly indicates that the centrifuge had transferred all particles > 0.025 mm to the solid fraction.

In contrast with the findings of Møller *et al*. (2002) regarding small particle size substances, melanoidins (molecular weight varying from low to high (65–235 kDa) in this study were transferred from the solid to the liquid phase by centrifugation of FW. This could have been the result of using different g force or centrifuging time applied. Nevertheless, a filtration process would have to be added in order to remove the unwanted substances from the liquid fraction.

In a full-scale plant using microwave digestate as a substrate, additional attention should be paid to ensuring inhibitory compound removal is successfully achieved. This is because, the filtration (responsible for removing unwanted compounds from FW here) is not commonly used in large-scale plants. It is likely, therefore, that melanoidins and phenols would not be effectively removed from the digestate. In case of phase separation of digestate, full scale digestion processes would require a safe and economically feasible method for ensuring unwanted compounds are removed from the liquid fraction of this substrate prior to AD.

Nevertheless, in the case that complete removal of unwanted substances from this substrate is not possible, the whole fraction of digestate could also be used in the AD process, as a co-substrate for FW, with positive methane production results, as show in chapter 6.

9.4 Summary

In this chapter, the potential to apply Microwaved Food Waste and Digestate in a full-scale anaerobic digestion system has been evaluated.

Due to good process performance, with no failure in batch systems, an inoculum to substrate ratio of 3:1 for MW FW is recommended for CSTR systems.

Moreover, the results suggest that an I/S of 3:1 was adequate in terms of offering the batch system sufficient alkalinity, with no pH adjustment during the digestion period when FW and sludge were used as substrates. Nevertheless, in the case of CSTR systems, where there is continuous intake of substrate and removal of digestate (together with other components such as bacteria, nutrients and sludge which acts as a buffer agent), the system buffer capacity would at some point become negatively affected, possibly demanding further adjustments and culminating with the need for an external source of alkalinity. Therefore, for a large-scale systems treating MW FW and/ or digestate, an additional, external source of alkalinity is recommended.

In order to ensure successful removal of inhibitory substances (e.g. melanoidins and phenols) from microwaved digestate and FW in a full-scale plant, an economically feasible alternative method for filtration may be needed, due to this method not being commonly used in a large-scale systems.

Overall, the batch system tests conducted here can be used to directly extrapolate batch observations to full-scale system, with good methane production prediction in most cases.

CHAPTER 10

10. CONCLUSIONS AND RECOMMENDATIONS

10.1 Conclusions

This project investigated different strategies to enhance biomethane production from food waste under MAD conditions, including: optimization of FW particle size and inoculum to substrate ratio (I/S); microwave pre-treatment, and re-circulation of microwave pre-treated digestate.

1. The first part of the research addressed objective I, and focused on finding the best particle size, and I/S ratio for biomethane production from FW under mesophilic anaerobic conditions.
   The results indicate that food waste from Leeds University refectory is a suitable substrate for MAD, with a good potential for biomethane recovery via AD.
   The main outcome was finding optimum values for I/S and particle size for biomethane production from FW without nutrients supplementation. A relatively stable BMP process and increase in biomethane yield was reached at 1 mm food waste particle size and an inoculum-to-substrate ratio of 3:1. Under this condition, the cumulative biomethane yield was increased by 94.59 %, in comparison with the yield at an inoculum-to-substrate ratio of 1:1.
   An inverse relation between I/S ratio and methane yield was found, meaning that the methane yield from the anaerobic digestion of food waste increased as I/S ratio decreased. Therefore, it can be said that food waste requires the addition of a large concentration of bacteria/inoculum compared to other substrates, which highlights the importance of investigating and optimizing operational conditions such as particle size and I/S ratios prior to the digestion process.

2. The second part of the research focused on objective II, testing the effects of different microwave parameters, including final temperature and heating rates, on FW characteristics and MAD performance.
   The most important FW changes after MW irradiation were increased solubilisation (SCOD/TCOD) and TVFAS enhancement, with temperature having a greater influence over heating rate on these modifications.
   This study showed that microwave heating, as a pre-treatment method for enhancing AD of food waste is effective. This is evident from several findings including: a) improved hydrolysis rate, b) reduced T80, c) higher methane yields than untreated samples. These were particularly evident at the lower temperature and fast heating rate (85°C 7.8 ramp), This is a consequence of higher substrate solubilisation (promoted by microwave pre-treatment), which transforms the organic matter into a readily available form, circumventing the intermediate product accumulation observed in the start-up phase of AD of food waste.

Higher temperature (175°C), regardless of the heating rate is unfeasible for MW treatment of FW, since it results in poor process performance. This is a clear indication of bacterial inhibition, possibly related to the formation of Maillard compounds (phenols and melanoidins). Removal of the solid fraction from these samples promoted methane yield enhancement, increased to a maximum of 65% compared to the digestion of raw FW (control), and up to 74% improvement in relation to when microwaved (whole fraction) food waste is anaerobically digested.

It became clear that soluble fraction control (food waste with solid fraction removal and with no microwave pre-treatment) results in the highest methane yield. An improvement of 83.22% in relation to raw FW (control) is achieved. From an economic point of view this scenario seems very promising, since there is no extra cost with microwave pre-treatment, and the centrifugation step for solids removal is usually considered to be low cost. Nonetheless, there would be a significant environmental impact associated with this practice, since the solids removed from the substrate would have to be disposed of, possibly to landfills.

3. The third stage of the research project was associated with objective III, and focused on assessing the effects of final temperatures on the characteristics of digestate, and its effect combined with different volumes of treated digestate when re-circulated in the MAD of food waste. The emphasis was on energy recovery potential.

   The results obtained from digestate characterization suggest that the most relevant changes were: increases in ammonium and SCOD/TCOD. The ammonium enhancement after MW pre-treatment brought advantages to the digestion process such as avoiding the acidification typically observed during start-up phase of raw food waste digested alone. Ammonium acted as a buffer agent. It was also found that digestate pre-treated at 115°C 1.4 ramp showed the greatest enhancement of SCOD (93.85%) and hydrolysis degree (82.33%). Despite being significant, these changes in digestate characteristics did not result in the highest methane yield.

   This study pointed to the effectiveness of MW pre-treated digestate re-circulation in improving overall process performance of MAD of food waste. This was evidenced by: a) avoidance of system acidification, made possible by ammonium enhancement for most of the conditions tested; b) amelioration of TVFAs/alkalinity ratio in relation to the digestion of food waste alone) and, c) increased biodegradability, with the most significant increase observed for co-digestion of raw food waste and treated digestate at 75°C 1.4 ramp and 25% vol. equivalent to 80.18% of the food waste TMP achieved, representing a 132.43% improvement in relation to when raw food waste was digested alone. Energy recovery from digestate was successfully achieved for all of the conditions tested. A 108.95% and 132.40 % increment were obtained in relation to microwaved food waste treated at 85°C 7.8 ramp and raw food waste digested alone, respectively.

Higher volumes of treated digestate re-circulated into MAD of FW has a detrimental effect on the final methane yield. Therefore, the use of the maximum volume of treated digestate (75%) in order to enhance methane yield and reduce its volume before discarding it to land, is unfeasible.

The novel cascading of MW pre-treated digestate followed by AD is thus a feasible option for digestate and food waste management, evidenced by a 26.66% gain in terms of recovered energy in relation to the control FW soluble fraction. It offers a promising option for the simultaneous management of FW and digestate, with greater volumes of methane generated.

4. The fourth and last stage of the project was related to objective 4 and aimed to assess the economic feasibility of integrating MW pre-treatment and AD using mass and energy balances and to evaluate the environmental impact of the cascaded process.

   The results showed that the combined treatment of AD and MW has a lower environmental impact (reducing the carbon footprint by 98.20%) than food waste being disposed to landfill. It is, therefore, an attractive strategy for food waste and digestate management.

   Microwave temperature and heating rate had a strong influence on solids destruction during the digestion of MW FW. Higher levels of solids removal are linked to rapid heating and lower final temperatures, heralding greater methane yields than higher temperature scenarios (175°C), in which the formation of inhibitory compounds is likely. In the latter case, the inhibitory compounds adversely affect process performance including solids removal, and thus, methanogenesis.

   In contrast, the volume of treated digestate had a strong influence on solids destruction in the co-digestion of raw food waste and digestate. It became evident that higher volumes of treated digestate caused a detrimental effect on process performance, lowering TS and VS destruction and, consequently, lowering methane yield.

   A comprehensive Energy balance study was not possible due to: a) the small sample size, b) the pulsed power mode chosen for the STARTSYNTH microwave, coupled with the unavailability of a device that would record automatically the power fluctuation during each run, c) the unknown distribution of the electric field inside the microwave cavity, d) the use of a laboratory- scale microwave oven that lacked the impedance matching (required to avoid reflected power) that would be designed into an industrial-scale microwave applicator, e) unknown magnetron efficiency, f) absence of precise information about the dielectric properties of the digestate. In view of these limitations best-case and worst-case calculations have been made.

   Based on the energy balance estimates it is possible to infer that the best operating conditions for scaling-up the cascade process for MW FW are a final temperature of 85°C and heating rate of 7.8 °C/min, since it represents the operational condition that offers higher positive net energy for both best- and worst-case scenarios prediction. Control FW soluble fraction is also favourable for large -scale application. The best operating conditions for scaling-up co-digestion of microwaved digestate with FW is: final temperature of 85°C 7.8 °C/min using 50 and 75% volume of treated digestate. In practice, the realistic scenarios will lie between the best and worst cases. Overall, the scenarios involving AD of food waste with re-circulated digestate appear to be the most attractive options in terms of energy harvesting. Positive net energies can be applied to the national gas grid.

The positive net balance for these scenarios suggests that residual energy in the form of biomethane can be recovered from digestate with the potential to transform this substrate from a cost to a source of revenue by adopting the cascaded process proposed here. It is believed that the information presented can aid decision-making in the bioenergy industry when considering microwave-heating processes as a sustainable strategy for food waste and digestate management.

10.2 Recommendations

Further work is needed to (a) improve understanding of food waste and digestate treatment in a full-scale industrial processing scenario WWTP, and (b) optimize the use of microwave treatment establishing the proposed cascade process as a viable industrial alternative for substrate management.

The following, more detailed, recommendations for future research have been drawn.

I. The BMP tests in this research were carried out in laboratory batch reactors. Nevertheless, it is recommended that continuous mode system (CSTR) is performed for a better understanding of the impact of microwaving food waste and digestate prior to AD process, hence allowing easy replicability in large scale.

II. In this study, high microwave temperature, low power (slow heating rate) for both FW and digestate (and high volumes of treated digestate) showed poor process performance, possibly due to the influence of inhibitory compounds. These compounds should be qualified and quantified allowing a better correlation with AD process efficiency.

III. The AD of microwaved food waste and microwaved digestate resulted in poor process performance for several microwave set of conditions (temperature and heating rate). Analysis of the microbial population should be undertaken to improve understanding of the relative capacities of each strain to resist the accumulation of inhibitory compounds and effectively process microwaved substrates thus, further allowing the selection of appropriate microorganisms to optimize AD.

IV. It is hypothesised that microwaved digestate may contain inhibitory and toxic compounds (such as phenols). The quality and safety of microwaved digestate should therefore be investigated both to identify those compounds that most effectively limit digestion and to establish the toxicity of compounds that will impact the safe disposal of unused digestate.

V. The benefits of the solid fraction removal, towards improving methane yield of FW pre-treated at harsher conditions (175°C, fast, medium and slow heating rates) has been established. The solids fraction removal strategy at lower temperature range (85-145°C) should be studied to broaden the spectrum of the soluble fraction of microwaved food waste that can be used effectively in AD.

VI. Energy balances of microwaved food waste and digestate have been calculated based on the best available, but incomplete, data relating to power input in two laboratory-scale microwaves ovens. In order to scale-up the process with confidence, measurements need to be repeated with more sophisticated real-time power monitoring equipment. Detail modelling of the heating process also needs to be undertaken including improved characterisation of substrate dielectric and thermal properties, the impedance matching characteristics of microwave applicators designed for specific substrate mixtures and specific substrate throughputs, and the electromagnetic field distribution with the cavities of such purpose-designed applicators. (These studies will probably require the collaboration of engineers and physicists).

VII. A comprehensive industrial-scale energy and economic audit should be conducted to determine whether the economics of the processes investigated here are viable given any particular set of environmental and market circumstances. This audit should include careful consideration of processing capacity, substrate alternatives, capital and operating costs (including operating revenues arising, for example, from bioenergy production). The incentives offered by, and regulatory instruments imposed by governments should also be included in such an audit. This, multidisciplinary work, probably in collaboration with engineers, economists and political scientists, will allow the bioenergy industry to assess the feasibility of specific proposals for commercial-scale microwave processing plants in specific physical, technical and economic environments around the world.

REFERENCES

Abbey, Lord, Udenigwe, C., Mohan, A. and Anom, E. 2017. Microwave irradiation effects on vermicasts potency, and plant growth and antioxidant activity in seedlings of Chinese cabbage (Brassica rapa subsp. pekinensis). *Journal of Radiation Research and Applied Sciences*. **10**(2), pp.110–116.

Adams, A., Borrelli, R.C., Fogliano, V. and De Kimpe, N. 2005. Thermal degradation studies of food melanoidins. *Journal of Agricultural and Food Chemistry*. **53**(10), pp.4136–4142.

Alburquerque, J.A., de la Fuente, C., Ferrer-Costa, A., Carrasco, L., Cegarra, J., Abad, M. and Bernal, M.P. 2012. Assessment of the fertiliser potential of digestates from farm and agroindustrial residues. *Biomass and Bioenergy*. **40**, pp.181–189.

Alice, S. and Janya, S.A. 2012. A Guide for Sustainable Urban Organic Waste Management in Thailand: Combining Food, Energy, and Climate Co-benefits. *Institute for Global Environmental Strategies, Japan*.

Alonso, M.G.V.P. and Themelis, N. 2011. Generation and disposition of municipal solid waste in Mexico and potential for improving waste management in Toluca Municipality. *Waste-to-Energy Research and Technology Council (WTERT)*.

Ames, J.M., Wynne, A., Hofmann, A., Plos, S. and Gibson, G.R. 1999. The effect of a model melanoidin mixture on faecal bacterial populations in vitro. *British Journal of Nutrition*. **82**(6), pp.489–495.

Amlinger, F., Pollack, M. and Favoino, E. 2004. Heavy metals and organic compounds from wastes used as organic fertilisers. Final report for ENV. A. 2./ETU/2001/0024. *Final report for ENV. A. 2./ETU/2001/0024.* (July), p.35.

Amoo, O.M. and Fagbenle, R.L. 2013. Renewable municipal solid waste pathways for energy generation and sustainable development in the Nigerian context. *International Journal of Energy and Environmental Engineering*. **4**(1), pp.1–17.

Angelidaki, I. and Ahring, B.K. 1993. Thermophilic anaerobic digestion of livestock waste: the effect of ammonia. *Applied Microbiology and Biotechnology*. **38**(4), pp.560–564.

Aoshima, I., Tozawa, Y., Ohmomo, S. and Ueda, K. 1985. Production of Decolorizing Activity for Molasses Pigment by Coriolus versicolor Ps4at giant colony method were tested as to decol- for further. *Agric. Biol. Chem.* **49**(7), pp.2041–2045.

Appels, L., Assche, A. Van, Willems, K., Degrève, J., Impe, J. Van and Dewil, R. 2011. Peracetic acid oxidation as an alternative pre-treatment for the anaerobic digestion of waste activated sludge. *Bioresource Technology*. **102**(5), pp.4124–4130.

Appels, L., Lauwers, J., Degrve, J., Helsen, L., Lievens, B., Willems, K., Van Impe, J. and Dewil, R. 2011. Anaerobic digestion in global bio-energy production: Potential and research challenges. *Renewable and Sustainable Energy Reviews*. **15**(9), pp.4295–4301.

Aragon Briceño, C.I. 2018. *Hydrothermal Processes as an Alternative to Conventional Sewage Sludge Management.*[Online] University of Leeds Faculty. Available from: http://etheses.whiterose.ac.uk/22908/.

Arbor 2015. *Nutrient recovery from wastewater.*

Arfaioli, P., Ristori, G.G., Bosetto, M. and Fusi, P. 1997. Humic-like compounds formed from L-tryptophan and D-glucose in the presence of Cu (II). *Chemosphere*. **35**(3), pp.575–584.

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F. and Lens, P.N.L. 2014. Pretreatment methods to enhance anaerobic digestion of organic solid waste. *Applied Energy*. **123**, pp.143–156.

Ariunbaatar, J., Panico, A., Yeh, D.H., Pirozzi, F., Lens, P.N.L. and Esposito, G. 2015. Enhanced mesophilic anaerobic digestion of food waste by thermal pretreatment : Substrate versus digestate heating. *Waste Management*. **46**, pp.176–181.

Baere, L. and Mattheeuws, B. 2010. High rate dry anaerobic digestion experiments on energy-producing plants. , pp.94–97.

Balsari P., Menardo, S., Gioelli F., Dinuccio, E. 2009. Il progetto europeo EU Agrobiogas: finalità, obiettivi e primi risultati ottenuti, Proceedings of IX Convegno Nazionale dell'Associazione Italiana di Ingegneria Agraria – Ricerca e innovazione nell'ingegneria dei biosistemi agro-territoriali, Ischia (NA). [Accessed 11 August 2020]. Available from: http://hdl.handle.net/2318/63666

Banik, S., Bandyopadhyay, S. and Ganguly, S. 2003. Bioeffects of microwave - a brief review. *Bioresource Technology*. **87**(2), pp.155–159.

Banks, C.J., Zhang, Y., Jiang, Y. and Heaven, S. 2012. Trace element requirements for stable food waste digestion at elevated ammonia concentrations. *Bioresource Technology*. **104**, pp.127–135.

Barbanera, M., Pelosi, C., Taddei, A.R. and Cotana, F. 2018. Optimization of bio-oil production from solid digestate by microwave-assisted liquefaction. *Energy Conversion and Management*. **171**(May), pp.1263–1272.

Barbara, B., Claude, F., Ellen, G., John, R., Gabriele, R., Camillo, R., Umberto, V., June 2012. *Food Waste: Cause, Impacts and Proposals*. Barilla Center for Food & Nutrition, Italy.

Battimelli, A., Carrère, H. and Delgenès, J.P. 2009. Saponification of fatty slaughterhouse wastes for enhancing anaerobic biodegradability. *Bioresource Technology*. **100**(15), pp.3695–3700.

Benabdallah El-Hadj, T., Dosta, J., Márquez-Serrano, R. and Mata-Álvarez, J. 2007. Effect of ultrasound pretreatment in mesophilic and thermophilic anaerobic digestion with emphasis on naphthalene and pyrene removal. *Water Research*. **41**(1), pp.87–94.

Beneroso, D., Monti, T., Kostas, E.T. and Robinson, J. 2017. Microwave pyrolysis of biomass for bio-oil production: Scalable processing concepts. *Chemical Engineering Journal*. **316**, pp.481–498.

van den Berg, L. and Lentz, C.P. 1971. Anaerobic Digestion of Pear Waste: Laboratory Equipment Design and Preliminary Results. *Canadian Institute of Food Technology Journal*. **4**(4), pp.159–165.

Bermúdez, J.M., Beneroso, D., Rey-Raap, N., Arenillas, A. and Menéndez, J.A. 2015. Energy consumption estimation in the scaling-up of microwave heating processes. *Chemical Engineering and Processing: Process Intensification*. **95**, pp.1–8.

Bernstad, A., Malmquist, L., Truedsson, C. and la Cour Jansen, J. 2013. Need for improvements in physical pretreatment of source-separated household food waste. *Waste Management*. **33**(3), pp.746–754.

BERR and DEFRA, 2003. Our Energy Future—Creating a Low Carbon Economy. *Energy White Paper.*TSO.

Beszédes, S., László, Z., Horváth, Z.H., Szabó, G. and Hodúr, C. 2011. Comparison of the effects of microwave irradiation with different intensities on the biodegradability of sludge from the dairy- and meat-industry. *Bioresource Technology*. **102**(2), pp.814–821.

Biswas, R., Ahring, B.K. and Uellendahl, H. 2012. Improving biogas yields using an innovative concept for conversion of the fiber fraction of manure. *Water Science and Technology*. **66**(8), pp.1751–1758.

De Bok, F.A.M., Plugge, C.M. and Stams, A.J.M. 2004. Interspecies electron transfer in methanogenic propionate degrading consortia. *Water Research*. **38**(6), pp.1368–1375.

Bolzonella, D., Battistoni, P., Susini, C. and Cecchi, F. 2006. Anaerobic codigestion of waste activated sludge and OFMSW: The experiences of Viareggio and Treviso plants (Italy). *Water Science and Technology*. **53**(8), pp.203–211.

Bonk, F., Popp, D., Weinrich, S., Sträuber, H., Kleinsteuber, S., Harms, H. and Centler, F. 2018. Ammonia inhibition of anaerobic volatile fatty acid degrading microbial communities. *Frontiers in Microbiology*. **9**(NOV), pp.1–13.

Borges, E.A., Loss, A., Silva, E.E., Souza, S.R. and Fernandes, M.S. 2009. Cinética de absorção de amônio e efluxo de prótons em variedades de milho. *Semina: Ciências Agrárias*. **30**(3), p.513.

Bouallagui, H., Lahdheb, H., Ben Romdan, E., Rachdi, B. and Hamdi, M. 2009. Improvement of fruit and vegetable waste anaerobic digestion performance and stability with co-substrates addition. *Journal of Environmental Management*. **90**(5), pp.1844–1849.

Bougrier, C., Delgenès, J.-P. and Carrère, H. 2006. Combination of Thermal Treatments and Anaerobic Digestion to Reduce Sewage Sludge Quantity and Improve Biogas Yield. *Process Safety and Environmental Protection*. **84**(4), pp.280–284.

Bougrier, C., Delgenès, J.P. and Carrère, H. 2008. Effects of thermal treatments on five different waste activated sludge samples solubilisation, physical properties and anaerobic digestion. *Chemical Engineering Journal*. **139**(2), pp.236–244.

Bougrier, C., Delgenès, J.P. and Carrère, H. 2007. Impacts of thermal pre-treatments on the semi-continuous anaerobic digestion of waste activated sludge. *Biochemical Engineering Journal*. **34**(1), pp.20–27.

Boulanger, A., Pinet, E., Bouix, M., Bouchez, T. and Mansour, A.A. 2012. Effect of inoculum to substrate ratio (I/S) on municipal solid waste anaerobic degradation kinetics and potential. *Waste Management*. **32**(12), pp.2258–2265.

Braguglia, C.M., Mininni, G., Tomei, M.C. and Rolle, E. 2006. Effect of feed/inoculum ratio on anaerobic digestion of sonicated sludge. *Water Science and Technology*. **54**(5), pp.77–84.

British Standards Institution, BSI. PAS 110:2010. 2010. Specification for whole digestate, separated liquor and separated fibre derived from the anaerobic digestion of source-segregated biodegradable materials. UK: British Standards Institution Publications. 60p.

Browne, J.D. and Murphy, J.D. 2013. Assessment of the resource associated with biomethane from food waste. *Applied Energy*. **104**, pp.170–177.

Buswell, A.M. and Mueller, H.F. 1952. Mechanism of Methane Fermentation. *Industrial & Engineering Chemistry*. **44**(3), pp.550–552.

Buttress, A.J., Binner, E., Yi, C., Palade, P., Robinson, J.P. and Kingman, S.W. 2016. Development and evaluation of a continuous microwave processing system for hydrocarbon removal from solids. *Chemical Engineering Journal*. **283**, pp.215–222.

Buzby, J.C. and Hyman, J. 2012. Total and per capita value of food loss in the United States. *Food Policy*. **37**(5), pp.561–570.

Callaghan, F.J., Wase, D.A.J., Thayanithy, K. and Forster, C.F. 2002. Continuous co-digestion of cattle slurry with fruit and vegetable wastes and chicken manure. *Biomass and Bioenergy*. **22**(1), pp.71–77.

Cao, X.Q., Chen, J., Cao, Y.L., Zhu, J.Y. and Hao, X.D. 2006. Experimental study on sludge reduction by ultrasound. *Water Science and Technology*. **54**(9), pp.87–93.

Capson-Tojo, G., Ruiz, D., Rouez, M., Crest, M., Steyer, J.-P., Bernet, N., Delgenès, J.-P. and Escudié, R. 2017. Accumulation of propionic acid during consecutive batch anaerobic digestion of commercial food waste. *Bioresource Technology*. **245**, pp.724–733.

Capson-Tojo, G., Trably, E., Rouez, M., Crest, M., Steyer, J.P., Delgenès, J.P. and Escudié, R. 2017. Dry anaerobic digestion of food waste and cardboard at different substrate loads, solid contents and co-digestion proportions. *Bioresource Technology*. **233**, pp.166–175.

Carballa, M., Duran, C. and Hospido, A. 2011. Should we pretreat solid waste prior to anaerobic digestion? An assessment of its environmental cost. *Environmental Science and Technology*. **45**(24), pp.10306–10314.

Carlsson, M., Lagerkvist, A. and Morgan-Sagastume, F. 2012. The effects of substrate pre-treatment on anaerobic digestion systems: A review. *Waste Management*. **32**(9), pp.1634–1650.

Carrère, H., Dumas, C., Battimelli, A., Batstone, D.J., Delgenès, J.P., Steyer, J.P. and Ferrer, I. 2010. Pretreatment methods to improve sludge anaerobic degradability: A review. *Journal of Hazardous Materials*. **183**(1–3), pp.1–15.

Carrère, H., Sialve, B. and Bernet, N. 2009. Improving pig manure conversion into biogas by thermal and thermo-chemical pretreatments. *Bioresource Technology*. **100**(15), pp.3690–3694.

Cesaro, A. and Belgiorno, V. 2014. Pretreatment methods to improve anaerobic biodegradability of organic municipal solid waste fractions. *Chemical Engineering Journal*. **240**, pp.24–37.

Chakraborty, D., Karthikeyan, O.P., Selvam, A. and Wong, J.W.C. 2018. Co-digestion of food waste and chemically enhanced primary treated sludge in a continuous stirred tank reactor. *Biomass and Bioenergy*., pp.232–240.

Chamchoi, N., Garcia, H. and Angelidaki, I. 2011. Methane Potential of Household Waste; Batch Assays Determination. *Applied Environmental Research*. **33**(1), pp.13–26.

Chen, Y., Cheng, J.J. and Creamer, K.S. 2008. Inhibition of anaerobic digestion process: A review. *Bioresource Technology*. **99**(10), pp.4044–4064.

Cho, J.K., Park, S.C. and Chang, H.N. 1995. Biochemical methane potential and solid state anaerobic digestion of Korean food wastes. *Bioresource Technology*. **52**(3), pp.245–253.

Chu, C.P., Chang, B.V., Liao, G.S., Jean, D.S. and Lee, D.J. 2001. Observations on changes in ultrasonically treated waste-activated sludge. *Water Research*. **35**(4), pp.1038–1046.

Chuang, T.C., Lai, C.L., 1978. Study on treatment and utilization of molasses alcohol slop. In: Proceedings of the International Conference on Water Pollution Control in Developing Countries. Asia Institute of Technology, pp. 513–524

Coca, M., García, M.T., González, G., Peña, M. and García, J.A. 2004. Study of coloured components formed in sugar beet processing. *Food Chemistry*. **86**(3), pp.421–433.

Coelho, N.M.G., Droste, R.L. and Kennedy, K.J. 2011. Evaluation of continuous mesophilic, thermophilic and temperature phased anaerobic digestion of microwaved activated sludge. *Water Research*. **45**(9), pp.2822–2834.

Commission of the European Communities 2002. European Union Regulation EC1772/2002 of 3 October 2002. *Official Journal of the European Union*. **2002**(October 2002), p.21.

Corsten, M.A.M., Worrell, E. and Van, D. 2012. The potential for Waste Management in Brazil to Minimize GHG emissions and Maximize Re-use of Materials. *Utrecht University, Brazil*., p.46.

Ćosović, B., Vojvodić, V., Bošković, N., Plavšić, M. and Lee, C. 2010. Characterization of natural and synthetic humic substances (melanoidins) by chemical composition and adsorption measurements. *Organic Geochemistry*. **41**(2), pp.200–205.

Cossu 2016. Back to Earth Sites: From “nasty and unsightly” landfilling to final sink and geological repository. *Waste Management*. **55**, pp.1–2.

Crombie, K., Mašek, O., Sohi, S.P., Brownsort, P. and Cross, A. 2013. The effect of pyrolysis conditions on biochar stability as determined by three methods. *GCB Bioenergy*. **5**(2), pp.122–131.

Cuetos, M.J., Gómez, X., Otero, M. and Morán, A. 2010. Anaerobic digestion and co-digestion of slaughterhouse waste (SHW): Influence of heat and pressure pre-treatment in biogas yield. *Waste Management*. **30**(10), pp.1780–1789.

Curry, N. and Pillay, P. 2012. Biogas prediction and design of a food waste to energy system for the urban environment. *Renewable Energy*. **41**, pp.200–209.

Dahlin, A.S., Eriksson, J., Campbell, C.D. and Öborn, I. 2016. Soil amendment affects Cd uptake by wheat - are we underestimating the risks from chloride inputs? *Science of the Total Environment*. **554–555**, pp.349–357.

Danso-Boateng, E., Shama, G., Wheatley, A.D., Martin, S.J. and Holdich, R.G. 2015. Hydrothermal carbonisation of sewage sludge: Effect of process conditions on product characteristics and methane production. *Bioresource Technology*. **177**, pp.318–327.

Davidek, T., Clety, N., Devaud, S., Robert, F. and Blank, I. 2003. Simultaneous Quantitative Analysis of Maillard Reaction Precursors and Products by High-Performance Anion Exchange Chromatography. *Journal of Agricultural and Food Chemistry*. **51**(25), pp.7259–7265.

Davidsson, Å., Gruvberger, C., Christensen, T.H., Hansen, T.L. and Jansen, J. la C. 2007. Methane yield in source-sorted organic fraction of municipal solid waste. *Waste Management*. **27**(3), pp.406–414.

DEFRA, 2007. Department of Environment Food Rural affair and Agriculture. UK Waste Strategy.

Delft, C. 2016. Biomass and waste pyrolysis-evolution to high-quality marketable fuels. [Accessed 11 August 2020]. Available from: https://www.ce.nl/assets/upload/file/Presentaties/2016/CE_Delft_3I96_presentationDJ.pdf

Department for Environment Food and Rural Affairs (DEFRA) 2015. UK statistics on waste. . (December), pp.1–17.

Dhia, B.H., Alyce, B., Alexander, O. and George, S. 2011. Managing Costa Rica's Waste–Recommendations for a Municipal Solid Waste Management Plan.

Dinsdale, R.M., Premier, G.C., Hawkes, F.R. and Hawkes, D.L. 2000. Two-stage anaerobic co-digestion of waste activated sludge and fruit/vegetable waste using inclined tubular digesters. *Bioresource Technology*. **72**(2), pp.159–168.

Divya, D., Gopinath, L.R. and Merlin Christy, P. 2015. A review on current aspects and diverse prospects for enhancing biogas production in sustainable means. *Renewable and Sustainable Energy Reviews*. **42**, pp.690–699.

Dong, X. and Stams, A.J.M. 1995. Evidence for H2 and formate formation during syntrophic butyrate and propionate degradation. *Anaerobe*. **1**(1), pp.35–39.

Dwyer, J., Griffiths, P. and Lant, P. 2009. Simultaneous colour and DON removal from sewage treatment plant effluent: Alum coagulation of melanoidin. *Water Research*. **43**(2), pp.553–561.

Dwyer, J., Starrenburg, D., Tait, S., Barr, K., Batstone, D.J. and Lant, P. 2008. Decreasing activated sludge thermal hydrolysis temperature reduces product colour, without decreasing degradability. *Water Research*. **42**(18), pp.4699–4709.

Echavarría, A.P., Pagán, J. and Ibarz, A. 2012. Melanoidins Formed by Maillard Reaction in Food and Their Biological Activity. *Food Engineering Reviews*. **4**(4), pp.203–223.

Eggeman, T. and Elander, R.T. 2005. Process and economic analysis of pretreatment technologies. *Bioresource Technology*. **96**(18 SPEC. ISS.), pp.2019–2025.

Elliott, A. and Mahmood, T. 2012. Comparison of Mechanical Pretreatment Methods for the Enhancement of Anaerobic Digestion of Pulp and Paper Waste Activated Sludge. *Water Environment Research*. **84**(6), pp.497–505.

Elsayed, M., Abomohra, A.E.F., Ai, P., Jin, K., Fan, Q. and Zhang, Y. 2019. Acetogenesis and methanogenesis liquid digestates for pretreatment of rice straw: A holistic approach for efficient biomethane production and nutrient recycling. *Energy Conversion and Management*. **195**(May), pp.447–456.

Eskicioglu, C., Kennedy, K.J. and Droste, R.L. 2006. Characterization of soluble organic matter of waste activated sludge before and after thermal pretreatment. *Water Research*. **40**(20), pp.3725–3736.

Eskicioglu, C., Prorot, A., Marin, J., Droste, R.L. and Kennedy, K.J. 2008. Synergetic pretreatment of sewage sludge by microwave irradiation in presence of H2O2 for enhanced anaerobic digestion. *Water Research*. **42**(18), pp.4674–4682.

Eskicioglu, C., Terzian, N., Kennedy, K.J., Droste, R.L. and Hamoda, M. 2007. Athermal microwave effects for enhancing digestibility of waste activated sludge. *Water Research*. **41**(11), pp.2457–2466.

European Parliament and Council 2018a. Directive (EU) 2018/850 of the European Parliament and of the Council of 30 May 2018 amending Directive 1999/31/EC on the landfill of waste. *Official Journal of the European Union*. **2018**(April), pp.100–108.

European Parliament and Council 2018b. Directive (EU) 2018/851 of the European Parliament and of the Council of 30 May 2018 amending Directive 2008/98/EC on waste. *Official Journal of the European Union*. (1907), L-150/109–140.

European Parliament and Council 2018c. Directive (EU) 2018/852 of the European Parliament and of the Council of 30 May 2018 amending Directive 94/62/EC on packaging and packaging waste. *Official Journal of the European Union*. **2018**(November 2008), pp.141–154.

European Parliament and Council 2000. DIRECTIVE 2000/60/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL. *Official Journal of the European Communities*. **October 20**(1), pp.1–72.

European Parliament and Council 2008. Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste and repealing certain directives. *Official Journal of the European Union*., pp.3–30.

European Union 1999. Council Directive 1999/31/EC on the landfill. *Official Journal of the European Communities*. (10), L182/1-19.

Evans, D. 2012. Beyond the Throwaway Society: Ordinary Domestic Practice and a Sociological Approach to Household Food Waste. *Sociology*. **46**(1), pp.41–56.

Fdez.-Güelfo, L.A., Álvarez-Gallego, C., Sales Márquez, D. and Romero García, L.I. 2011. Dry-thermophilic anaerobic digestion of simulated organic fraction of Municipal Solid Waste: Process modeling. *Bioresource Technology*. **102**(2), pp.606–611.

Feng, L., Li, Y., Chen, C., Liu, X., Xiao, X., Ma, X., Zhang, R., He, Y. and Liu, G. 2013. Biochemical Methane Potential (BMP) of Vinegar Residue and the Influence of Feed to Inoculum Ratios on Biogas Production. *BioResources*. **8**(2), pp.2487–2498.

Fennell, L.P. and Boldor, D. 2014. Dielectric and thermal properties of sweet sorghum biomass. *Journal of Microwave Power and Electromagnetic Energy*. **48**(4), pp.244–260.

Fini, A. and Breccia, A. 1999. Chemistry by microwaves. *Pure and Applied Chemistry*. **71**(4), pp.573–579.

Fischer, J.R., Iannotti, E.L. and Sievers, D.M. 1981. Anaerobic digestion of manure from swine fed on various diets. *Agricultural Wastes*. **3**(3), pp.201–214.

Fisgativa, H., Tremier, A., Le Roux, S., Bureau, C. and Dabert, P. 2017. Understanding the anaerobic biodegradability of food waste: Relationship between the typological, biochemical and microbial characteristics. *Journal of Environmental Management*. **188**, pp.95–107.

Fitamo, T., Boldrin, A., Boe, K., Angelidaki, I. and Scheutz, C. 2016. Co-digestion of food and garden waste with mixed sludge from wastewater treatment in continuously stirred tank reactors. *Bioresource Technology*. **206**, pp.245–254.

Fixen, P.E. and Johnston, A.M. 2012. World fertilizer nutrient reserves: A view to the future. *Journal of the Science of Food and Agriculture*. **92**(5), pp.1001–1005.

Fuchs, W. and Drosg, B. 2013. Assessment of the state of the art of technologies for the processing of digestate residue from anaerobic digesters. *Water Science and Technology*. **67**(9), pp.1984–1993.

Fung, D.Y.C., Cunningham, F.E., 1980. Effect of microwaves on microorganisms in foods. *J. Food Prot*. **43**, pp. 641–650.

Garfí, M., Gelman, P., Comas, J., Carrasco, W. and Ferrer, I. 2011. Agricultural reuse of the digestate from low-cost tubular digesters in rural Andean communities. *Waste Management*. **31**(12), pp.2584–2589.

Garrett, C.W. 1992. On global climate change, carbon dioxide, and fossil fuel combustion. *Progress in Energy and Combustion Science*. **18**(5), pp.369–407.

Gioelli, F., Dinuccio, E. and Balsari, P. 2011. Residual biogas potential from the storage tanks of non-separated digestate and digested liquid fraction. *Bioresource Technology*. **102**(22), pp.10248–10251.

Girault, R., Bridoux, G., Nauleau, F., Poullain, C., Buffet, J., Peu, P., Sadowski, A.G. and Béline, F. 2012. Anaerobic co-digestion of waste activated sludge and greasy sludge from flotation process: Batch versus CSTR experiments to investigate optimal design. *Bioresource Technology*. **105**, pp.1–8.

Goberna, M., Podmirseg, S.M., Waldhuber, S., Knapp, B.A., García, C. and Insam, H. 2011. Pathogenic bacteria and mineral N in soils following the land spreading of biogas digestates and fresh manure. *Applied Soil Ecology*. **49**(1), pp.18–25.

Griffin, M.E., McMahon, K.D., Mackie, R.I. and Raskin, L. 1998. Methanogenic population dynamics during start-up of anaerobic digesters treating municipal solid waste and biosolids. *Biotechnology and Bioengineering*. **57**(3), pp.342–355.

Grigatti, M., Di Girolamo, G., Chincarini, R., Ciavatta, C. and Barbanti, L. 2011. Potential nitrogen mineralization, plant utilization efficiency and soil CO2 emissions following the addition of anaerobic digested slurries. *Biomass and Bioenergy*. **35**(11), pp.4619–4629.

Grocery Manufacturers Association, 2012. Food Waste: Tier 1 Assessment (the United States).

Grosso, M., Motta, A. and Rigamonti, L. 2010. Efficiency of energy recovery from waste incineration, in the light of the new Waste Framework Directive. *Waste Management*. **30**(7), pp.1238–1243.

Guo, X.M., Trably, E., Latrille, E., Carrere, H. and Steyer, J.P. 2014. Predictive and explicative models of fermentative hydrogen production from solid organic waste: Role of butyrate and lactate pathways. *International Journal of Hydrogen Energy*. **39**(14), pp.7476–7485.

Gusiatin, Z.M., Kurkowski, R., Brym, S. and Wiśniewski, D. 2016. Properties of biochars from conventional and alternative feedstocks and their suitability for metal immobilization in industrial soil. *Environmental Science and Pollution Research*. **23**(21), pp.21249–21261.

Hansen, K.H., Angelidaki, I. and Ahring, B.K. 1998. Anaerobic digestion of swine manure: Inhibition by ammonia. *Water Research*. **32**(1), pp.5–12.

Hansen, T.L., Jansen, J. la C., Davidsson, Å. and Christensen, T.H. 2007. Effects of pre-treatment technologies on quantity and quality of source-sorted municipal organic waste for biogas recovery. *Waste Management*. **27**(3), pp.398–405.

Hao, H.T.N., Karthikeyan, O.P. and Heimann, K. 2015. Bio-refining of carbohydrate-rich food waste for biofuels. *Energies*. **8**(7), pp.6350–6364.

Hartmann, H., Angelidaki, I. and Ahring, B.K. 2000. Increase of anaerobic degradation of particulate organic matter in full- scale biogas plants by mechanical maceration. *Water Science and Technology*. **41**(3), pp.145–153.

Hassan, M., Ding, W., Umar, M. and Rasool, G. 2017. Batch and semi-continuous anaerobic co-digestion of goose manure with alkali solubilized wheat straw: A case of carbon to nitrogen ratio and organic loading rate regression optimization. *Bioresource Technology*. **230**, pp.24–32.

He, C., Giannis, A. and Wang, J.-Y. 2013. Conversion of sewage sludge to clean solid fuel using hydrothermal carbonization: Hydrochar fuel characteristics and combustion behavior. *Applied Energy*. **111**, pp.257–266.

Hejnfelt, A. and Angelidaki, I. 2009. Anaerobic digestion of slaughterhouse by-products. *Biomass and Bioenergy*. **33**(8), pp.1046–1054.

Hendriks, A.T.W.M. and Zeeman, G. 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology*. **100**(1), pp.10–18.

Hill, D.T., Cobb, S.A. and Bolte, J.P. 1987. Using Volatile Fatty Acid Relationships To Predict Anaerobic Digester Failure. *Transactions of the American Society of Agricultural Engineers*. **30**(2), pp.496–501.

Hillel, D. 1998. *Introduction to Environmental Soil Physics* [Online] 1st ed. San Diego. Available from: https://www.elsevier.com/books/environmental-soil-physics/hillel/978-0-12-348525-0.

Hiramoto, S., Itoh, K., Shizuuchi, S., Kawachi, Y., Morishita, Y., Nagase, M., Suzuki, Y., Nobuta, Y., Sudou, Y., Nakamura, O., Kagaya, I., Goshima, H., Kodama, Y., Icatro, F.C., Koizumi, W., Saigenji, K., Miura, S., Sugiyama, T. and Kimura, N. 2004. Melanoidin, a food protein-derived advanced Maillard reaction product, suppresses Helicobacter pylori in vitro and in vivo. *Helicobacter*. **9**(5), pp.429–435.

Hodge, J. E. 1953. Chemistry of browning reactions in model systems. *Journal of Agricultural and Food Chemistry*, **1**(15), 928–943.

Hogg, D., Barth, J., Favoino, E., Centemero, M., Caimi, V., Amlinger, F., Devliegher, W., Brinton, W. and Antler, S. 2002. *Comparison of compost standards within the EU, North America and Australasia* [Online]. Available from: http://www.endseurope.com/docs/compost1.doc.

Holm-Nielsen, J.B., Al Seadi, T. and Oleskowicz-Popiel, P. 2009. The future of anaerobic digestion and biogas utilization. *Bioresource technology*. **100**(22), pp.5478–84.

Hong, S.M., Park, J.K., Teeradej, N., Lee, Y.O., Cho, Y.K. and Park, C.H. 2006. Pretreatment of sludge with microwaves for pathogen destruction and improved anaerobic digestion performance. *Water Environment Research*. **78**(1), pp.76–83.

Horan, N. 2012. Celebrating the revolution. *Water Environment Magazine*. **17**, p.14.

Horan, N., Smyth, M., Cessford, I. 2015. Optimising the value of digestate and digestion systems. Zero Waste. Scotland: WRAP.

Hou, L. 2013 South Korea's food waste solution: you waste, you pay. Common Wealth Magazine **519** [Accessed 06 August 2020]. Available from: https://english.cw.com.tw/article/article.action?id=573

Hu, Y., Pang, Y., Yuan, H., Zou, D., Liu, Y., Zhu, B., Chufo, W.A., Jaffar, M. and Li, X. 2015. Promoting anaerobic biogasification of corn stover through biological pretreatment by liquid fraction of digestate (LFD). *Bioresource Technology*. **175**, pp.167–173.

IEA. 2006. World Energy Outlook *2006*. International Energy Agency, Paris.

IEA. 2009. World Energy Outlook 2009. p.696.

IFC, International Finance Corporation-World Bank Group. 2012 Municipal Solid Waste in Ukraine: DEVELOPMENT POTENTIAL [online] [Accessed 06 August 2020]. Available from: https://www.ifc.org/wps/wcm/connect/24f11a48-d7a0-4970-9bd1-37ff9244f60e/21.+Municipal+Solid+Waste+in+Ukraine+DEVELOPMENT+POTENTIAL+Scenarios+for+developing+the+municipal+solid+waste+management+sector+.pdf?MOD=AJPERES&CVID=lNpD-tO

Ike, M., Inoue, D., Miyano, T., Liu, T.T., Sei, K., Soda, S. and Kadoshin, S. 2010. Microbial population dynamics during startup of a full-scale anaerobic digester treating industrial food waste in Kyoto eco-energy project. *Bioresource Technology*. **101**(11), pp.3952–3957.

Inoue, S., Hanaoka, T. and Minowa, T. 2002. Hot compressed water treatment for production of charcoal from wood. *Journal of Chemical Engineering of Japan*. **35**(10), pp.1020–1023.

Ioannis, B., Leonidas, M., Almut, R., 2013. *Municipal Waste Management in Turkey*. European Environment Agency (EEA).

IPCC 2006. 2006 IPCC Guidelines for National Greenhouse Inventories – A primer, Prepared by the National Greenhouse Gas Inventories Programme, Eggleston H.S., Miwa K., Srivastava N. and Tanabe K. *Iges*., p.20.

Izumi, K., Okishio, Y., Nagao, N., Niwa, C., Yamamoto, S. and Toda, T. 2010. Effects of particle size on anaerobic digestion of food waste. *International Biodeterioration & Biodegradation*. **64**(7), pp.601–608.

Jagadabhi, P.S., Kaparaju, P. and Rintala, J. 2010. Effect of micro-aeration and leachate replacement on COD solubilization and VFA production during mono-digestion of grass-silage in one-stage leach-bed reactors. *Bioresource Technology*. **101**(8), pp.2818–2824.

Jagadabhi, P.S., Lehtomäki, A. and Rintala, J. 2008. CO-digestion of grass silage and cow manure in a CSTR by re-circulation of alkali treated solids of the digestate. *Environmental Technology*. **29**(10), pp.1085–1093.

Jain, S., Lala, A.K., Bhatia, S.K. and Kudchadker, A.P. 1992. Modelling of hydrolysis controlled anaerobic digestion. *Journal of Chemical Technology & Biotechnology*. **53**(4), pp.337–344.

Jena, M. 2013. India's Food Security Rots in Storage [online] [Accessed 06 August 2020]. Available from: http://www.ipsnews.net/2013/06/indias-food-security-rots-in-storage.

Jeswani, H.K. and Azapagic, A. 2016. Assessing the environmental sustainability of energy recovery from municipal solid waste in the UK. *Waste Management*. **50**, pp.346–363.

Jie, W.S., Abdullah, H., Yusof, N. and Abbas, Z. 2015. Dielectric Properties of Oil Palm Trunk Core. *Journal of Clean Energy Technologies*. **3**(6), pp.422–427.

Jones, D.A., Lelyveld, T.P., Mavrofidis, S.D., Kingman, S.W. and Miles, N.J. 2002. Microwave heating applications in environmental engineering - A review. *Resources, Conservation and Recycling*. **34**(2), pp.75–90.

Jurado, E., Skiadas, I. V. and Gavala, H.N. 2013. Enhanced methane productivity from manure fibers by aqueous ammonia soaking pretreatment. *Applied Energy*. **109**, pp.104–111.

Kaparaju, P., Serrano, M. and Angelidaki, I. 2010. Optimization of biogas production from wheat straw stillage in UASB reactor. *Applied Energy*. **87**(12), pp.3779–3783.

Kaparaju, P.L.N. and Rintala, J.A. 2005. The effects of post-treatments and temperature on recovering the methane potential of >2 mm solid fraction of digested cow manure. *Environmental Technology*. **26**(6), pp.625–632.

Kappe, C.O. 2013. How to measure reaction temperature in microwave-heated transformations. *Chemical Society Reviews*. **42**(12), pp.4977–4990.

Karthikeyan, O. P., Nguyen Hao, H.T., Razaghi, A. and Heimann, K. 2018. Recycling of food waste for fuel precursors using an integrated bio-refinery approach. *Bioresource Technology*. **248**, pp.194–198.

Karthikeyan, O. P, Trably, E., Mehariya, S., Bernet, N., Wong, J.W.. and Carrere, H. 2018. Pretreatment of food waste for methane and hydrogen recovery: A review. *Bioresource Technology*. **249**(September 2017), pp.1025–1039.

Karthikeyan, O.P. and Visvanathan, C. 2013. Bio-energy recovery from high-solid organic substrates by dry anaerobic bio-conversion processes: A review. *Reviews in Environmental Science and Biotechnology*. **12**(3), pp.257–284.

Kaspar, H.F. and Wuhrmann, K. 1978. Kinetic parameters and relative turnovers of some important catabolic reactions in digesting sludge. *Applied and Environmental Microbiology*. **36**(1), pp.1–7.

Katami, T., Yasuhara, A. and Shibamoto, T. 2004. Formation of Dioxins from Incineration of Foods Found in Domestic Garbage. *Environmental Science and Technology*. **38**(4), pp.1062–1065.

Kawai, M., Nagao, N., Tajima, N., Niwa, C., Matsuyama, T. and Toda, T. 2014. The effect of the labile organic fraction in food waste and the substrate/inoculum ratio on anaerobic digestion for a reliable methane yield. *Bioresource Technology*. **157**, pp.174–180.

Khalid, A., Arshad, M., Anjum, M., Mahmood, T. and Dawson, L. 2011. The anaerobic digestion of solid organic waste. *Waste Management*. **31**(8), pp.1737–1744.

Khanal, S.K., Grewell, D., Sung, S. and Van Leeuwen, J. 2007. Ultrasound applications in wastewater sludge pretreatment: A review. *Critical Reviews in Environmental Science and Technology*. **37**(4), pp.277–313.

Kitagawa, K., Kanuma, Y., Oguro, T. and Harada, A. 1986. Reliability of Magnetrons for Microwave Ovens. *Journal of Microwave Power and Electromagnetic Energy*. **21**(3), pp.149–158.

Köksoy, G.T. and Sanin, F.D. 2010. Effect of digester F/M ratio on gas production and sludge minimization of ultrasonically treated sludge. *Water Science and Technology*. **62**(7), pp.1510–1517.

Kong, X., Xu, S., Liu, J., Li, H., Zhao, K. and He, L. 2016. Enhancing anaerobic digestion of high-pressure extruded food waste by inoculum optimization. *Journal of Environmental Management*. **166**, pp.31–37.

Kostas, E.T., Beneroso, D. and Robinson, J.P. 2017. The application of microwave heating in bioenergy: A review on the microwave pre-treatment and upgrading technologies for biomass. *Renewable and Sustainable Energy Reviews*. **77**(March), pp.12–27.

Koster, I.W. and Lettinga, G. 1988. Anaerobic digestion at extreme ammonia concentrations. *Biological Wastes*. **25**(1), pp.51–59.

Kratky, L. and Jirout, T. 2011. Biomass Size Reduction Machines for Enhancing Biogas Production. *Chemical Engineering and Technology*. **34**(3), pp.391–399.

Kratzeisen, M., Starcevic, N., Martinov, M., Maurer, C. and Müller, J. 2010. Applicability of biogas digestate as solid fuel. *Fuel*. **89**(9), pp.2544–2548.

Kudakasseril Kurian, J., Raveendran Nair, G., Hussain, A. and Vijaya Raghavan, G.S. 2013. Feedstocks, logistics and pre-treatment processes for sustainable lignocellulosic biorefineries: A comprehensive review. *Renewable and Sustainable Energy Reviews*. **25**, pp.205–219.

Kumar, D. and Murthy, G.S. 2011. Impact of pretreatment and downstream processing technologies on economics and energy in cellulosic ethanol production. *Biotechnology for Biofuels*. **4**.

Kumar, M., Ou, Y.L. and Lin, J.G. 2010. Co-composting of green waste and food waste at low

C/N ratio. *Waste Management*. **30**(4), pp.602–609.

Kwon, S.H. and Lee, D.H. 2004. Evaluation of Korean food waste composting with fed-batch operations I: Using water extractable total organic carbon contents (TOCw). *Process Biochemistry*. **39**(10), pp.1183–1194.

De la Rubia, M.Á., Walker, M., Heaven, S., Banks, C.J. and Borja, R. 2010. Preliminary trials of in situ ammonia stripping from source segregated domestic food waste digestate using biogas: Effect of temperature and flow rate. *Bioresource Technology*. **101**(24), pp.9486–9492.

Labatut, R.A., Angenent, L.T. and Scott, N.R. 2011. Biochemical methane potential and biodegradability of complex organic substrates. *Bioresource Technology*. **102**(3), pp.2255–2264.

Lansing, S., Martin, J.F., Botero, R.B., Nogueira da Silva, T. and Dias da Silva, E. 2010. Wastewater transformations and fertilizer value when co-digesting differing ratios of swine manure and used cooking grease in low-cost digesters. *Biomass and Bioenergy*. **34**(12), pp.1711–1720.

Lauf, R. J., D. W. Bible, A. C. Johnson, and C. A. Everleigh. *1993*. 2 to 18 GHz *Broadband Microwave Heating Systems*. *Microwave Journal*. **36** (11):24—34.

Laurent, J., Casellas, M., Pons, M.N. and Dagot, C. 2009. Flocs surface functionality assessment of sonicated activated sludge in relation with physico-chemical properties. *Ultrasonics Sonochemistry*. **16**(4), pp.488–494.

Lee, D.H., Behera, S.K., Kim, J.W. and Park, H.S. 2009. Methane production potential of leachate generated from Korean food waste recycling facilities: A lab-scale study. *Waste Management*. **29**(2), pp.876–882.

Lee, P. and Willis, P. 2010. Waste arisings in the supply of food and drink to households in the UK. . (March), p.85.

Lee, S.H., Kang, H.J., Lee, Y.H., Lee, T.J., Han, K., Choi, Y. and Park, H.D. 2012. Monitoring bacterial community structure and variability in time scale in full-scale anaerobic digesters. *Journal of Environmental Monitoring*. **14**(7), pp.1893–1905.

Lewens, B. 2011. Is Digestate a Cost or Asset? : Andigestion.

Li, J., Dai, J., Liu, G., Zhang, H., Gao, Z., Fu, J., He, Y. and Huang, Y. 2016. Biochar from microwave pyrolysis of biomass: A review. *Biomass and Bioenergy*. **94**, pp.228–244.

Li, L., Diederick, R., Flora, J.R. V and Berge, N.D. 2013. Hydrothermal carbonization of food waste and associated packaging materials for energy source generation. *Waste Management*. **33**(11), pp.2478–2492.

Li, Y. and Jin, Y. 2015. Effects of thermal pretreatment on acidification phase during two-phase batch anaerobic digestion of kitchen waste. *Renewable Energy*. **77**, pp.550–557.

Li, Y., Park, S.Y. and Zhu, J. 2011. Solid-state anaerobic digestion for methane production from organic waste. *Renewable and Sustainable Energy Reviews*. **15**(1), pp.821–826.

Li, Y., Zhang, R., Liu, G., Chen, C., He, Y. and Liu, X. 2013. Comparison of methane production potential, biodegradability, and kinetics of different organic substrates. *Bioresource Technology*. **149**, pp.565–569.

Li, Y., Zhang, Y., Sun, Y., Wu, S., Kong, X., Yuan, Z. and Dong, R. 2017. The performance efficiency of bioaugmentation to prevent anaerobic digestion failure from ammonia and propionate inhibition. *Bioresource Technology*. **231**, pp.94–100.

Liebetrau, J., Kraft, E., Bidlingmaier, W., 2004. The influence of the hydrolysis rate of co-substrates on process behaviour. In: Guiot, S.G. ed., *Proceedings of the Tenth World Congress on Anaerobic.* Canadian Association on Water Quality, Montreal, pp. 1296–1300.

Lin, C.S.K., Pfaltzgraff, L.A., Herrero-Davila, L., Mubofu, E.B., Abderrahim, S., Clark, J.H., Koutinas, A.A., Kopsahelis, N., Stamatelatou, K., Dickson, F., Thankappan, S., Mohamed, Z., Brocklesby, R. and Luque, R. 2013. Food waste as a valuable resource for the production of chemicals, materials and fuels. Current situation and global perspective. *Energy and Environmental Science*. **6**(2), pp.426–464.

Lin, L., Yuan, S., Chen, J., Xu, Z. and Lu, X. 2009. Removal of ammonia nitrogen in wastewater by microwave radiation. *Journal of Hazardous Materials*. **161**(2–3), pp.1063–1068.

Lindmark, J., Leksell, N., Schnürer, A. and Thorin, E. 2012. Effects of mechanical pre-treatment on the biogas yield from ley crop silage. *Applied Energy*. **97**, pp.498–502.

Lindner, J., Zielonka, S., Oechsner, H. and Lemmer, A. 2015. Effects of mechanical treatment of digestate after anaerobic digestion on the degree of degradation. *Bioresource Technology*. **178**, pp.194–200.

Lipinski, B., Hanson, C., Lomax, J., Kitinoja, L., Waite, R. and Searchinger, T. 2013. *Reducing food loss and waste* [Online]. Available from: http://www.worldresourcesreport.org.

Lissens, G., Thomsen, A.B., De Baere, L., Verstraete, W. and Ahring, B.K. 2004. Thermal wet oxidation improves anaerobic biodegradability of raw and digested biowaste. *Environmental Science and Technology*. **38**(12), pp.3418–3424.

Liu, G. 2014. Food Losses and Food Waste in China: A First Estimate. . (66).

Liu, K., Chen, Y., Xiao, N., Zheng, X. and Li, M. 2015. Effect of humic acids with different characteristics on fermentative short-chain fatty acids production from waste activated sludge. *Environmental Science and Technology*. **49**(8), pp.4929–4936.

Liu, T. and Sung, S. 2002. Ammonia inhibition on thermophilic aceticlastic methanogens. *Water science and technology : a journal of the International Association on Water Pollution Research*. **45**(10), pp.113–120.

Liu, T., Zhou, X., Li, Z., Wang, X. and Sun, J. 2019. Effects of liquid digestate pretreatment on biogas production for anaerobic digestion of wheat straw. *Bioresource Technology*. **280**(January), pp.345–351.

Liu, X., Wang, W., Gao, X., Zhou, Y. and Shen, R. 2012. Effect of thermal pretreatment on the physical and chemical properties of municipal biomass waste. *Waste Management*. **32**(2), pp.249–255.

Liu, Z. and Zhang, F.S. 2008. Effects of various solvents on the liquefaction of biomass to produce fuels and chemical feedstocks. *Energy Conversion and Management*. **49**(12), pp.3498–3504.

López Torres, M. and Espinosa Lloréns, M. del C. 2008. Effect of alkaline pretreatment on anaerobic digestion of solid wastes. *Waste Management*. **28**(11), pp.2229–2234.

Loupy, A. ed. 2002. *Microwaves in Organic Synthesis.* Weinheim, Germany: Wiley-VCH.

Lü, F., Zhou, Q., Wu, D., Wang, T., Shao, L. and He, P. 2015. Dewaterability of anaerobic digestate from food waste: Relationship with extracellular polymeric substances. *Chemical Engineering Journal*. **262**, pp.932–938.

Lu, X., Jordan, B. and Berge, N.D. 2012. Thermal conversion of municipal solid waste via hydrothermal carbonization: Comparison of carbonization products to products from current waste management techniques. *Waste Management*. **32**(7), pp.1353–1365.

Lu, Z., Fan, L., Wu, Z., Zhang, H., Liao, Y., Zheng, D. and Wang, S. 2015. Efficient liquefaction of woody biomass in polyhydric alcohol with acidic ionic liquid as a green catalyst. *Biomass and Bioenergy*. **81**, pp.154–161.

Luste, S., Luostarinen, S. and Sillanpää, M. 2009. Effect of pre-treatments on hydrolysis and methane production potentials of by-products from meat-processing industry. *Journal of Hazardous Materials*. **164**(1), pp.247–255.

Ma, J., Duong, T.H., Smits, M., Verstraete, W. and Carballa, M. 2011. Enhanced biomethanation of kitchen waste by different pre-treatments. *Bioresource Technology*. **102**(2), pp.592–599.

Maaike, H.V.G., 2014. *Food Waste or Wasted Food: an Empirical Investigation of the Determinants of Food Waste.* Master of Science in Business Administration, University of Stavanger Business School, Norway.

Madsen, M., Holm-Nielsen, J.B. and Esbensen, K.H. 2011. Monitoring of anaerobic digestion processes: A review perspective. *Renewable and Sustainable Energy Reviews*. **15**(6), pp.3141–3155.

Malamis, D., Moustakas, K. and Haralambous, K.J. 2016. Evaluating in-vessel composting in treating sewage sludge and agricultural waste by examining and determining the kinetic reactions of the process. *Clean Technologies and Environmental Policy*. **18**(8), pp.2493–2502.

Mao, T. and Show, K.Y. 2006. Performance of high-rate sludge digesters fed with sonicated sludge. *Water Science and Technology*. **54**(9), pp.27–33.

Di Maria, F., Gigliotti, G., Sordi, A., Micale, C., Zadra, C. and Massaccesi, L. 2013. Hybrid solid anaerobic digestion batch: Biomethane production and mass recovery from the organic fraction of solid waste. *Waste Management and Research*. **31**(8), pp.869–873.

Marin, J., Kennedy, K.J. and Eskicioglu, C. 2010. Effect of microwave irradiation on anaerobic degradability of model kitchen waste. *Waste Management*. **30**(10), pp.1772–1779.

Mata-Alvarez, J., Llabrés, P., Cecchi, F. and Pavan, P. 1992. Anaerobic digestion of the Barcelona central food market organic wastes: Experimental study. *Bioresource Technology*. **39**(1), pp.39–48.

Matsuda, T., Yano, J., Hirai, Y. and Sakai, S.I. 2012. Life-cycle greenhouse gas inventory analysis of household waste management and food waste reduction activities in Kyoto, Japan. *International Journal of Life Cycle Assessment*. **17**(6), pp.743–752.

Mawioo, P.M., Garcia, H.A., Hooijmans, C.M., Velkushanova, K., Simonič, M., Mijatović, I. and Brdjanovic, D. 2017. A pilot-scale microwave technology for sludge sanitization and drying. *Science of the Total Environment*. **601–602**, pp.1437–1448.

Meghan, S., 2014. *Caribbean Biogas Company to Provide Energy from Waste to Housing Development in Jamaica*. [online] GVEP International . [Accessed 06 August 2020]. Available from: https://www.energy4impact.org/news/caribbean-biogas-company-provide-energy-waste-housing-development-jamaica

Mena, C., Adenso-Diaz, B. and Yurt, O. 2011. The causes of food waste in the supplier-retailer interface: Evidences from the UK and Spain. *Resources, Conservation and Recycling*. **55**(6), pp.648–658.

Menardo, S., Balsari, P., Dinuccio, E. and Gioelli, F. 2011. Thermal pre-treatment of solid fraction from mechanically-separated raw and digested slurry to increase methane yield. *Bioresource Technology*. **102**(2), pp.2026–2032.

Menéndez, J.A., Arenillas, A., Fidalgo, B., Fernández, Y., Zubizarreta, L., Calvo, E.G. and Bermúdez, J.M. 2010. Microwave heating processes involving carbon materials. *Fuel Processing Technology*. **91**(1), pp.1–8.

Meredith, R. 1998. *Engineers' Handbook of Industrial Microwave Heating* [Online]. The Institution of Engineering and Technology, Michael Faraday House, Six Hills Way, Stevenage SG1 2AY, UK: IET. Available from: https://digital-library.theiet.org/content/books/po/pbpo025e.

Michele, P., Giuliana, D., Carlo, M., Sergio, S. and Fabrizio, A. 2015. Optimization of solid state anaerobic digestion of the OFMSW by digestate recirculation: A new approach. *Waste Management*. **35**, pp.111–118.

Minowa, T., Murakami, M., Dote, Y., Ogi, T. and Yokoyama, S. ya 1995. Oil production from garbage by thermochemical liquefaction. *Biomass and Bioenergy*. **8**(2), pp.117–120.

Misi, S.N. and Forster, C.F. 2001. Batch co-digestion of multi-component agro-wastes. *Bioresource Technology*. **80**(1), pp.19–28.

Miura, M., Kaga, H., Sakurai, A., Kakuchi, T. and Takahashi, K. 2004. Rapid pyrolysis of wood block by microwave heating. *Journal of Analytical and Applied Pyrolysis*. **71**(1), pp.187–199.

Mohamad, Z. and Keng, J. 2013. Opportunities and Challenges in Sustainable Waste Management Transition in Malaysia: A multi-level socio-technical perspective. *Globelics Seminar on Low Carbon Development ….*, pp.1–14.

Molinuevo-Salces, B., Gómez, X., Morán, A. and García-González, M.C. 2013. Anaerobic co-digestion of livestock and vegetable processing wastes: Fibre degradation and digestate stability. *Waste Management*. **33**(6), pp.1332–1338.

Møller, H.B., Lund, I., Sommer, S.G., 2000. Solid–liquid separation of livestock slurry: efficiency and cost. Bioresour. Technol. 74, 223– 229.

Møller, H.B., Sommer, S.G. and Ahring, B.K. 2002. Separation efficiency and particle size distribution in relation to manure type and storage conditions. *Bioresource Technology*. **85**(2), pp.189–196.

Møller, J., Boldrin, A. and Christensen, T.H. 2009. Anaerobic digestion and digestate use: Accounting of greenhouse gases and global warming contribution. *Waste Management and Research*. **27**(8), pp.813–824.

Möller, K. and Müller, T. 2012. Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. *Engineering in Life Sciences*. **12**(3), pp.242–257.

Mönch-Tegeder, M., Lemmer, A., Oechsner, H. and Jungbluth, T. 2013. Investigation of the methane potential of horse manure. *Agricultural Engineering International: CIGR Journal*. **15**(2), pp.161–172.

Monlau, F., Barakat, A., Trably, E., Dumas, C., Steyer, J.P. and Carrère, H. 2013. Lignocellulosic materials into biohydrogen and biomethane: Impact of structural features and pretreatment. *Critical Reviews in Environmental Science and Technology*. **43**(3), pp.260–322.

Monlau, F., Sambusiti, C., Ficara, E., Aboulkas, A., Barakat, A. and Carrère, H. 2015. New opportunities for agricultural digestate valorization: Current situation and perspectives. *Energy and Environmental Science*. **8**(9), pp.2600–2621.

Montavon, P., Mauron, A.F. and Duruz, E. 2003. Changes in green coffee protein profiles during roasting. *Journal of Agricultural and Food Chemistry*. **51**(8), pp.2335–2343.

Montecchio, D., Gallipoli, A., Gianico, A., Mininni, G., Pagliaccia, P. and Braguglia, C.M. 2016. Biomethane potential of food waste: modeling the effects of mild thermal pretreatment and digestion temperature. *Environmental Technology*. **38**(11), pp.1452–1464.

Morales, F.J. and Jiménez-Pérez, S. 2004. Peroxyl radical scavenging activity of melanoidins in aqueous systems. *European Food Research and Technology*. **218**(6), pp.515–520.

Moreno-Andrade, I. and Buitrón, G. 2003. Influence of the initial substrate to microorganisms concentration ratio on the methanogenic inhibition test. *Water Science and Technology*. **48**(6), pp.17–22.

Moset, V., Al-zohairi, N. and Møller, H.B. 2015. The impact of inoculum source, inoculum to substrate ratio and sample preservation on methane potential from different substrates. *Biomass and Bioenergy*. **83**, pp.474–482.

Motasemi, F. and Afzal, M.T. 2013. A review on the microwave-assisted pyrolysis technique. *Renewable and Sustainable Energy Reviews*. **28**, pp.317–330.

Motasemi, F., Afzal, M.T., Salema, A.A., Mouris, J. and Hutcheon, R.M. 2014. Microwave dielectric characterization of switchgrass for bioenergy and biofuel. *Fuel*. **124**, pp.151–157.

Motasemi, F., Salema, A.A. and Afzal, M.T. 2015. Dielectric characterization of corn stover for microwave processing technology. *Fuel Processing Technology*. **131**, pp.370–375.

Mottet, A., Habouzit, F. and Steyer, J.P. 2014. Anaerobic digestion of marine microalgae in different salinity levels. *Bioresource Technology*. **158**, pp.300–306.

Mottet, A., Steyer, J.P., Déléris, S., Vedrenne, F., Chauzy, J. and Carrère, H. 2009. Kinetics of thermophilic batch anaerobic digestion of thermal hydrolysed waste activated sludge. *Biochemical Engineering Journal*. **46**(2), pp.169–175.

Mouat, A., Mistry, A.B., P., Webb, J. 2010. Digestate market development in Scotland. [online]. vol. OPR080-801. Zero waste Scotland Stirling, UK. [Accessed 06 August 2020]. Available from: http://www.wrap.org.uk/sites/files/wrap/Zero_Waste_Scotland_Digestate_Market_Development.pdf

Murphy, J.D., McKeogh, E. and Kiely, G. 2004. Technical/economic/environmental analysis of biogas utilisation. *Applied Energy*. **77**(4), pp.407–427.

Nakajima-Kambe, T., Shimomura, M., Nomura, N., Chanpornpong, T. and Nakahara, T. 1999. Decolorization of molasses wastewater by Bacillus sp. under Thermophilic and anaerobic conditions. *Journal of Bioscience and Bioengineering*. **87**(1), pp.119–121.

Naran, E., Toor, U.A. and Kim, D.J. 2016. Effect of pretreatment and anaerobic co-digestion of food waste and waste activated sludge on stabilization and methane production. *International Biodeterioration and Biodegradation*. **113**, pp.17–21.

National Environment Agency (NEA), 2013. *Food Wastage in Singapore over the Years.*

Naturvårdsverket, 2010. Personal Communication on Waste Generation (Stockholm, Sweden).

Naylor, R. 2011. Expanding the boundaries of agricultural development. *Food Security*. **3**(2), pp.233–251.

Nayono, S.E., Gallert, C. and Winter, J. 2010. Co-digestion of press water and food waste in a biowaste digester for improvement of biogas production. *Bioresource Technology*. **101**(18), pp.6987–6993.

Neumann, J., Binder, S., Apfelbacher, A., Gasson, J.R., Ramírez García, P. and Hornung, A. 2015. Production and characterization of a new quality pyrolysis oil, char and syngas from digestate - Introducing the thermo-catalytic reforming process. *Journal of Analytical and Applied Pyrolysis*. **113**, pp.137–142.

Neumann, J., Meyer, J., Ouadi, M., Apfelbacher, A., Binder, S. and Hornung, A. 2016. The conversion of anaerobic digestion waste into biofuels via a novel Thermo-Catalytic Reforming process. *Waste Management*. **47**, pp.141–148.

Neves, L., Oliveira, R. and Alves, M.M. 2004. Influence of inoculum activity on the bio-methanization of a kitchen waste under different waste/inoculum ratios. *Process Biochemistry*. **39**(12), pp.2019–2024.

Neyens, E. and Baeyens, J. 2003. A review of thermal sludge pre-treatment processes to improve dewaterability. *Journal of Hazardous Materials*. **98**(1–3), pp.51–67.

Nielfa, A., Cano, R. and Fdz-Polanco, M. 2015. Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge. *Biotechnology Reports*. **5**(1), pp.14–21.

Nkoa, R. 2013. Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. *Agronomy for Sustainable Development*. **34**(2), pp.473–492.

NNFCC 2012. NNFCC Market Review. . (August), pp.1–8.

Ogwueleka, T. 2009. Municipal solid waste characteristics and management in Nigeria. *Iranian Journal of Environmental Health Science & Engineering*. **6**.

Ohmomo, S., KANEKO, Y., SIRIANUNTAPIBOON, S., SOMCHAI, P., ATTHASAMPUNNA, P. and NAKAMURA, I. 1987. Research on decolorization of melanoidin by microorganisms. Part V. Decolorization of molasses waste water by a thermophilic strain, Aspergillus fumigatus G-2-6. *Agricultural and Biological Chemistry*. **51**(12), pp.3339–3346.

Ohmomo, S., Yoshkawa, H., Yui, M., Nakamura, I., Daengsubha, W., Nozaki, K. and Nakajima, T. 1988. Screening of Anaerobic Bacteria with the Ability to Decolorize Molasses Melanoidin. *Agricultural and Biological Chemistry*. **52**(10), pp.2429–2435.

Pain, B.F., Hepherd, R.Q., Pittman, R.J., 1978. Factors affecting the performance of four slurry separating machines. J. Agric. Eng. Res. 23, 231–242.

Painter, T.J. 1998. Carbohydrate polymers in food preservation: An integrated view of the Maillard reaction with special reference to discoveries of preserved foods in Sphagnum-dominated peat bogs. *Carbohydrate Polymers*. **36**(4), pp.335–347.

Palmowski, L., Simons, L. and Brook, R. 2006. Ultrasonic treatment to improve anaerobic digestibility of dairy waste streams. *Water Science and Technology*. **53**(8), pp.281–288.

Palmowski, L.M. and Muller, J.A. 2000. Influence of the size reduction of organic waste on their anaerobic digestion. . **41**(3), pp.155–162.

Pan, J., Chen, X., Sheng, K., Yu, Y., Zhang, C. and Ying, Y. 2013. Effect of ammonia on biohydrogen production from food waste via anaerobic fermentation *In*: *International Journal of Hydrogen Energy*. Elsevier Ltd, pp.12747–12754.

Pantelopoulos, A., Magid, J. and Jensen, L.S. 2016. Thermal drying of the solid fraction from biogas digestate: Effects of acidification, temperature and ventilation on nitrogen content. *Waste Management*. **48**, pp.218–226.

Parawira, W., Murto, M., Read, J.S. and Mattiasson, B. 2004. Volatile fatty acid production during anaerobic mesophilic digestion of solid potato waste. *Journal of Chemical Technology and Biotechnology*. **79**(7), pp.673–677.

Parfitt, J., Barthel, M. and MacNaughton, S. 2010. Food waste within food supply chains: Quantification and potential for change to 2050. *Philosophical Transactions of the Royal Society B: Biological Sciences*. **365**(1554), pp.3065–3081.

Park, B., Ahn, J.H., Kim, J. and Hwang, S. 2004. Use of microwave pretreatment for enhanced anaerobiosis of secondary slugde. *Water Science and Technology*. **50**(9), pp.17–23.

Park, W.J. and Ahn, J.H. 2011. Effects of microwave pretreatment on mesophilic anaerobic digestion for mixture of primary and secondary sludges compared with thermal pretreatment. *Environmental Engineering Research*. **16**(2), pp.103–109.

Parra-Orobio, B.A., Torres-Lozada, P. and Marmolejo-Rebellón, L.F. 2017. Anaerobic digestion of municipal biowaste for the production of renewable energy: Effect of particle size. *Brazilian Journal of Chemical Engineering*. **34**(2), pp.481–491.

Pellera, F.M. and Gidarakos, E. 2016. Effect of substrate to inoculum ratio and inoculum type on the biochemical methane potential of solid agroindustrial waste. *Journal of Environmental Chemical Engineering*. **4**(3), pp.3217–3229.

Penaud, V., Delgenès, J.P. and Moletta, R. 1999. Thermo-chemical pretreatment of a microbial biomass: Influence of sodium hydroxide addition on solubilization and anaerobic biodegradability. *Enzyme and Microbial Technology*. **25**(3–5), pp.258–263.

Peng, W. and Pivato, A. 2017. Sustainable Management of Digestate from the Organic Fraction of Municipal Solid Waste and Food Waste Under the Concepts of Back to Earth Alternatives and Circular Economy. *Waste and Biomass Valorization*. **10**(2), pp.465–481.

Peng, X., Nges, I.A. and Liu, J. 2016. Improving methane production from wheat straw by digestate liquor recirculation in continuous stirred tank processes. *Renewable Energy*. **85**, pp.12–18.

Peng, X., Zhang, S., Li, L., Zhao, X., Ma, Y. and Shi, D. 2018. Long-term high-solids anaerobic digestion of food waste: Effects of ammonia on process performance and microbial community. *Bioresource Technology*. **262**(174), pp.148–158.

Pérez-Elvira, S.I., Sapkaite, I. and Fdz-Polanco, F. 2016. Separate digestion of liquid and solid fractions of thermally pretreated secondary sludge. assessment and global evaluation. *Brazilian Journal of Chemical Engineering*. **33**(4), pp.699–704.

Pham, T.P.T., Kaushik, R., Parshetti, G.K., Mahmood, R. and Balasubramanian, R. 2015. Food waste-to-energy conversion technologies: Current status and future directions. *Waste Management*. **38**(1), pp.399–408.

Pilli, S., Bhunia, P., Yan, S., LeBlanc, R.J., Tyagi, R.D. and Surampalli, R.Y. 2011. Ultrasonic pretreatment of sludge: A review. *Ultrasonics Sonochemistry*. **18**(1), pp.1–18.

Pind, P.F., Angelidaki, I. and Ahring, B.K. 2003. Dynamics of the anaerobic process: Effects of volatile fatty acids. *Biotechnology and Bioengineering*. **82**(7), pp.791–801.

Plazl, I., Leskovšek, S. and Koloini, T. 1995. Hydrolysis of sucrose by conventional and microwave heating in stirred tank reactor. *The Chemical Engineering Journal and The Biochemical Engineering Journal*. **59**(3), pp.253–257.

Pöschl, M., Ward, S. and Owende, P. 2010. Evaluation of energy efficiency of various biogas production and utilization pathways. *Applied Energy*. **87**(11), pp.3305–3321.

Price, J.L. 2001. The landfill directive and the challenge ahead: demands and pressures on the UK householder. *Resources, Conservation and Recycling*. **32**(3–4), ’.

Prorot, A., Julien, L., Christophe, D. and Patrick, L. 2011. Sludge disintegration during heat treatment at low temperature: A better understanding of involved mechanisms with a multiparametric approach. *Biochemical Engineering Journal*. **54**(3), pp.178–184.

Puchajda, B. and Oleszkiewicz, J. 2006. Thermophilic anaerobic acid digestion of biosolids: Hydrolysis, acidification, and optimization of retention time of acid digestion. *Journal of Environmental Engineering and Science*. **5**(3), pp.187–195.

Pullammanappallil, P.C., Chynoweth, D.P., Lyberatos, G. and Svoronos, S. a 2001. Stable performance of anaerobic digestion in the presence of a high concentration of propionic acid. *Bioresource technology*. **78**(2), pp.165–9.

Pullen, T. 2015. *Anaerobic digestion: making biogas, making energy*. New York: Routledge.

Qiao, W., Wang, W., Xun, R., Lu, W. and Yin, K. 2008. Sewage sludge hydrothermal treatment by microwave irradiation combined with alkali addition. *Journal of Materials Science*. **43**(7), pp.2431–2436.

Ramanathan, V., Allison, J., Auffhammer, M., Auston, D., Barnosky, A.D., Chiang, L., Collins, W.D., Davis, S.J., Forman, F., Hecht, S.B., Kammen, D.M., Lin Lawell, C.-Y.C., Matlock, T., Press, D., Rotman, D., Samuelsen, S., Solomon, G., Victor, D., Washom, B. and Christensen, J. 2016. Chapter 1. Bending the Curve: Ten Scalable Solutions for Carbon Neutrality and Climate Stability. *Collabra*. **2**(1), pp.1–17.

Ramasamy, S. and Moghtaderi, B. 2010. Dielectric properties of typical australian wood-based biomass materials at microwave frequency. *Energy and Fuels*. **24**(8), pp.4534–4548.

Ranjith, K.A. 2012. Sustainable solid waste management in India. *Columbia University*., pp.6–157.

Raposo, F., Borja, R., Martín, M.A., Martín, A., de la Rubia, M.A. and Rincón, B. 2009. Influence of inoculum-substrate ratio on the anaerobic digestion of sunflower oil cake in batch mode: Process stability and kinetic evaluation. *Chemical Engineering Journal*. **149**(1–3), pp.70–

77.

RECO Baltic 21 Tech project, 2012. National Waste Management Review Belarus and Russia.

Rehl, T. and Müller, J. 2011. Life cycle assessment of biogas digestate processing technologies. *Resources, Conservation and Recycling*. **56**(1), pp.92–104.

Remme, U., Blesl, M. and Fahl, U. 2008. Future European gas supply in the resource triangle of the Former Soviet Union, the Middle East and Northern Africa. *Energy Policy*. **36**(5), pp.1622–1641.

Reza, M.T., Werner, M., Pohl, M. and Mumme, J. 2014. Evaluation of integrated anaerobic digestion and hydrothermal carbonization for bioenergy production. *Journal of visualized experiments : JoVE*. (88), pp.1–9.

Riding, M.J., Herbert, B.M.J., Ricketts, L., Dodd, I., Ostle, N. and Semple, K.T. 2015. Harmonising conflicts between science, regulation, perception and environmental impact: The case of soil conditioners from bioenergy. *Environment International*. **75**, pp.52–67.

Risberg, K., Cederlund, H., Pell, M., Arthurson, V. and Schnürer, A. 2017. Comparative characterization of digestate versus pig slurry and cow manure – Chemical composition and effects on soil microbial activity. *Waste Management*. **61**, pp.529–538.

Robinson, J., Dodds, C., Stavrinides, A., Kingman, S., Katrib, J., Wu, Z., Medrano, J. and Overend, R. 2015. Microwave pyrolysis of biomass: Control of process parameters for high pyrolysis oil yields and enhanced oil quality. *Energy and Fuels*. **29**(3), pp.1701–1709.

Robinson, J.P., Kingman, S.W., Barranco, R., Snape, C.E. and Al-Sayegh, H. 2010. Microwave Pyrolysis of Wood Pellets. *Industrial & Engineering Chemistry Research*. **49**(2), pp.459–463.

Rodriguez, C., Alaswad, A., Benyounis, K.Y. and Olabi, A.G. 2017. Pretreatment techniques used in biogas production from grass. *Renewable and Sustainable Energy Reviews*. **68**, pp.1193–1204.

Romania, 2018. Romania to Develop National Strategy to Reduce Food Waste: Minister. [online] [Accessed 06 August 2020]. Available from: http://dezvoltaredurabila.gov.ro/web/wp-content/uploads/2019/03/Romanias-Sustainable-Development-Strategy-2030.pdf

Ruile, S., Schmitz, S., Mönch-Tegeder, M. and Oechsner, H. 2015. Degradation efficiency of agricultural biogas plants - A full-scale study. *Bioresource Technology*. **178**, pp.341–349.

Salema, A.A., Yeow, Y.K., Ishaque, K., Ani, F.N., Afzal, M.T. and Hassan, A. 2013. Dielectric properties and microwave heating of oil palm biomass and biochar. *Industrial Crops and Products*. **50**, pp.366–374.

Salemdeeb, R., Font Vivanco, D., Al-Tabbaa, A. and zu Ermgassen, E.K.H.J. 2017. A holistic approach to the environmental evaluation of food waste prevention. *Waste Management*. **59**, pp.442–450.

Salminen, E., Einola, J. and Rintala, J. 2003. The methane production of poultry slaughtering residues and effects of pre-treatments on the methane production of poultry feather. *Environmental Technology (United Kingdom)*. **24**(9), pp.1079–1086.

Salsabil, M.R., Laurent, J., Casellas, M. and Dagot, C. 2010. Techno-economic evaluation of thermal treatment, ozonation and sonication for the reduction of wastewater biomass volume before aerobic or anaerobic digestion. *Journal of Hazardous Materials*. **174**(1–3), pp.323–333.

Sambusiti, C., Monlau, F., Ficara, E., Musatti, A., Rollini, M., Barakat, A. and Malpei, F. 2015. Comparison of various post-treatments for recovering methane from agricultural digestate. *Fuel Processing Technology*. **137**, pp.359–365.

Sanders, J., Scott, E., Weusthuis, R. and Mooibroek, H. 2007. Bio-refinery as the bio-inspired process to bulk chemicals. *Macromolecular Bioscience*. **7**(2), pp.105–117.

Saveyn, H. and Eder, P. 2014. *European Commission, Joint Research Centre – Institute for Prospective Technological Studies - End-of-waste criteria for biodegradable waste subjected to biological treatment (compost & digestate): Technical proposals* [Online]. Available from: https://ec.europa.eu/jrc/sites/default/files/eow_biodegradable_waste_final_report.pdf.

Sawayama, S., Inoue, S., Minowa, T., Tsukahara, K. and Ogi, T. 1997. Thermochemical liquidization and anaerobic treatment of kitchen garbage. *Journal of Fermentation and Bioengineering*. **83**(5), pp.451–455.

Schieder, D., Schneider, R. and Bischof, F. 2000. Thermal hydrolysis (TDH) as a pretreatment method for the digestion of organic waste. *Water Science and Technology*. **41**(3), pp.181–187.

Schmit, K.H. and Ellis, T.G. 2001. Comparison of Temperature-Phased and Two-Phase Anaerobic Co-Digestion of Primary Sludge and Municipal Solid Waste. *Water Environment Research*. **73**(3), pp.314–321.

Serna-Maza, A., Heaven, S. and Banks, C.J. 2015. Biogas stripping of ammonia from fresh digestate from a food waste digester. *Bioresource Technology*. **190**, pp.66–75.

Serrano, A., Siles, J.A., Martín, M.A., Chica, A.F., Estévez-Pastor, F.S. and Toro-Baptista, E. 2016. Improvement of anaerobic digestion of sewage sludge through microwave pre-treatment. *Journal of Environmental Management*. **177**, pp.231–239.

Sezer, K. and Arikan, O. 2011. Waste characterization at mixed municipal solid waste composting and recycling facility units. *Desalination and Water Treatment*. **26**(1–3), pp.92–97.

Shah, S., 2006. Mind the energy gap - experts query need for nuclear. The Independent 24 January 2006. [Accessed 11 August 2020]. Available from: https://www.independent.co.uk/news/business/analysis-and-features/mind-the-energy-gap-experts-query-need-for-nuclear-340642.html

Shahriari, H. 2011. Enhancement of Anaerobic Digestion of Organic Fraction of Municipal Solid Waste by Microwave Pretreatment. , p.175.

Shahriari, H., Warith, M., Hamoda, M. and Kennedy, K. 2013. Evaluation of single vs. staged mesophilic anaerobic digestion of kitchen waste with and without microwave pretreatment. *Journal of Environmental Management*. **125**, pp.74–84.

Shahriari, H., Warith, M., Hamoda, M. and Kennedy, K.J. 2012. Anaerobic digestion of organic fraction of municipal solid waste combining two pretreatment modalities, high temperature microwave and hydrogen peroxide. *Waste Management*. **32**(1), pp.41–52.

Shahriari, H., Warith, M. and Kennedy, K.J. 2011. Effect of microwave temperature, intensity and moisture content on solubilisation of organic fraction of municipal solid waste. *International Journal of Environmental Technology and Management*. **14**(1–4), pp.67–83.

Sharma, V.K., Testa, C., Lastella, G., Cornacchia, G. and Comparato, M.P. 2000. Inclined-plug-flow type reactor for anaerobic digestion of semi-solid waste. *Applied Energy*. **65**(1–4), pp.173–185.

Sharpley, A. 2016. Managing agricultural phosphorus to minimize water quality impacts. *Scientia Agricola*. **73**(1), pp.1–8.

Shin, H.S., Youn, J.H. and Kim, S.H. 2004. Hydrogen production from food waste in anaerobic mesophilic and thermophilic acidogenesis. *International Journal of Hydrogen Energy*. **29**(13), pp.1355–1363.

Show, K.Y., Mao, T. and Lee, D.J. 2007. Optimisation of sludge disruption by sonication. *Water Research*. **41**(20), pp.4741–4747.

Siebert, S. 2007. Quality requirements and quality assurance of digestion residuals in Germany.

Siegert, I. and Banks, C. 2005. The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors. *Process Biochemistry*. **40**(11), pp.3412–3418.

Sirianuntapiboon, S., Phothilangka, P. and Ohmomo, S. 2004. Decolorization of molasses wastewater by a strain No.BP103 of acetogenic bacteria. *Bioresource Technology*. **92**(1), pp.31–39.

Sirianuntapiboon, S., Somchai, P., Atthasampunna, P., Sihanonth, P. and Hayashida, S. 1995. An Absorption Mechanism for the Decolorization of Melanoidin by Rhizoctonia sp. D-90. *Bioscience, Biotechnology, and Biochemistry*. **59**(7), pp.1185–1189.

Skiadas, I. V., Gavala, H.N., Lu, J. and Ahring, B.K. 2005. Thermal pre-treatment of primary and secondary sludge at 701 °C prior to anaerobic digestion. *Water Science and Technology*. **52**(1–2), pp.161–166.

Soares, B., Gama, N., Freire, C., Barros-Timmons, A., Brandão, I., Silva, R., Pascoal Neto, C. and Ferreira, A. 2014. Ecopolyol production from industrial cork powder via acid liquefaction using polyhydric alcohols. *ACS Sustainable Chemistry and Engineering*. **2**(4), pp.846–854.

Somiya, I., 2001. Development of Organic Acid Fermentation and Collection process. Daiki Ataka Engineering Co. Ltd., *Research Report*, pp. 97–102.

Stadtman, T.C. and Barker, H.A. 1951. Studies on the methane fermentation. X. A new formate-decomposing bacterium, Methanococcus vannielii. *Journal of bacteriology*. **62**(3), pp.269–80.

Stoknes, K., Scholwin, F., Krzesiński, W., Wojciechowska, E. and Jasińska, A. 2016. Efficiency of a novel "Food to waste to food" system including anaerobic digestion of food waste and cultivation of vegetables on digestate in a bubble-insulated greenhouse. *Waste Management*. **56**, pp.466–476.

Sturm, G.S.J., Verweij, M.D., Gerven, T. Van, Stankiewicz, A.I. and Stefanidis, G.D. 2013. On the parametric sensitivity of heat generation by resonant microwave fields in process fluids. *International Journal of Heat and Mass Transfer*. **57**(1), pp.375–388.

Suchada, P., Tränkler, J., Cholada, K. and Schöll, W. 2003. The role of formal and informal sectors in solid waste management of developing countries. *Proceedings Sardinia 2003, Ninth International Waste Management and Landfill Symposium*. (October).

Sun, F., Mao, Z., Zhang, Jianhua, Zhang, H., Tang, L., Zhang, C., Zhang, Jing and Zhai, F. 2010. Water-recycled cassava bioethanol production integrated with two-stage UASB treatment. *Chinese Journal of Chemical Engineering*. **18**(5), pp.837–842.

Taherzadeh, M.J. and Karimi, K. 2008. *Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review*.

Taiwan EPA, 2008. Taiwan' Zero Waste Policy to Meet 2008 Goal in Advance.

Tamil Nadu Agricultural University (TNAU), 2008. *Entrepreneurial training manual*. Tamil Nadu Agricultural University, Coimbatore.

Tampio, E., Ervasti, S. and Rintala, J. 2015. Characteristics and agronomic usability of digestates from laboratory digesters treating food waste and autoclaved food waste. *Journal of Cleaner Production*. **94**, pp.86–92.

Tang J., Chan T.V. 2007. Microwave and radio frequency in sterilization and pasteurization applications. In: Yanniots ES and Sunden B, editors. *Heat transfer in food processing-recent developments and applications*. Southampton, UK: Wessex Institute of Technology (WIT) Press. pp. 101–157.

Tang, B., Yu, L., Huang, S., Luo, J. and Zhuo, Y. 2010. Energy efficiency of pre-treating excess sewage sludge with microwave irradiation. *Bioresource Technology*. **101**(14), pp.5092–5097.

Tembhurkar A.R., Mhaisalkar V.A. J 2007. Studies on Hydrolysis and Acidogenesis of kitchen waste in two-phase anaerobic digestion. *Journal of the Institution of Public Health Engineers*, **2**.

Thi, N.B.D., Kumar, G. and Lin, C.Y. 2015. An overview of food waste management in developing countries: Current status and future perspective. *Journal of Environmental Management*. **157**, pp.220–229.

Thostenson, E.T. and Chou, T.W. 1999. Microwave processing: fundamentals and applications. *Composites Part A: Applied Science and Manufacturing*. **30**(9), pp.1055–1071.

Tiehm, A., Nickel, K. and Neis, U. 1997. The use of ultrasound to accelerate the anaerobic digestion of sewage sludge. *Water Science and Technology*. **36**(11), pp.121–128.

Tomei, M.C., Braguglia, C.M. and Mininni, G. 2008. Anaerobic degradation kinetics of particulate organic matter in untreated and sonicated sewage sludge: Role of the inoculum. *Bioresource Technology*. **99**(14), pp.6119–6126.

Toreci, I., Kennedy, K.J. and Droste, R.L. 2010. Effect of high-temperature microwave irradiation on municipal thickened waste activated sludge solubilization. *Heat Transfer Engineering*. **31**(9), pp.766–773.

Toreci, I., Kennedy, K.J. and Droste, R.L. 2009. Evaluation of continuous mesophilic anaerobic sludge digestion after high temperature microwave pretreatment. *Water Research*. **43**(5), pp.1273–1284.

Totlani, V.M. and Peterson, D.G. 2007. Influence of epicatechin reactions on the mechanisms of maillard product formation in low moisture model systems. *Journal of Agricultural and Food Chemistry*. **55**(2), pp.414–420.

Tripathi, M., Sahu, J.N., Ganesan, P., Monash, P. and Dey, T.K. 2015. Effect of microwave frequency on dielectric properties of oil palm shell (OPS) and OPS char synthesized by microwave pyrolysis of OPS. *Journal of Analytical and Applied Pyrolysis*. **112**, pp.306–312.

Trzcinski, A.P. and Stuckey, D.C. 2011. Parameters affecting the stability of the digestate from a two-stage anaerobic process treating the organic fraction of municipal solid waste. *Waste Management*. **31**(7), pp.1480–1487.

Tsafrakidou, P., Bekatorou, A., Koutinas, A.A., Kordulis, C., Banat, I.M., Petsi, T. and Sotiriou, M. 2018. Acidogenic Fermentation of Wheat Straw After Chemical and Microbial Pretreatment for Biofuel Applications. *Energy Conversion and Management*. **160**(February), pp.509–517.

Tsapekos, P., Kougias, P.G., Kuthiala, S. and Angelidaki, I. 2018. Co-digestion and model simulations of source separated municipal organic waste with cattle manure under batch and continuously stirred tank reactors. *Energy Conversion and Management*. **159**(January), pp.1–6.

Tyagi, V.K. and Lo, S.L. 2013. Microwave irradiation: A sustainable way for sludge treatment and resource recovery. *Renewable and Sustainable Energy Reviews*. **18**(71), pp.288–305.

Uçkun Kiran, E., Trzcinski, A.P., Ng, W.J. and Liu, Y. 2014. Bioconversion of food waste to energy: A review. *Fuel*. **134**(10), pp.389–399.

UNEP 2011. *Towards a Green Economy. Pathways to Sustainable Development and Poverty Eradication*.

Vavilin, V.A. and Angelidaki, I. 2005. Anaerobic degradation of solid material: Importance of initiation centers for methanogenesis, mixing intensity, and 2D distributed model. *Biotechnology and Bioengineering*. **89**(1), pp.113–122.

Vavilin, V.A., Fernandez, B., Palatsi, J. and Flotats, X. 2008. Hydrolysis kinetics in anaerobic degradation of particulate organic material: An overview. *Waste Management*. **28**(6), pp.939–951.

Vavilin, V.A., Lokshina, L.Y., Rytov, S. V., Kotsyurbenko, O.R., Nozhevnikova, A.N. and Parshina, S.N. 1997. Modelling methanogenesis during anaerobic conversion of complex organic matter at low temperatures. *Water Science and Technology*. **36**(6–7), pp.531–538.

Veeken, A. and Hamelers, B. 1999. Effect of temperature on hydrolysis rates of selected biowaste components. *Bioresource Technology*. **69**(3), pp.249–254.

Vela, G.R., Wu, J.F., 1979. Mechanism of lethal action of 2450mHz radiation on microorganisms. *Appl. Environ. Microbiol*. **37**, pp. 550–553.

Van de Velden, M., Baeyens, J., Brems, A., Janssens, B. and Dewil, R. 2010. Fundamentals, kinetics and endothermicity of the biomass pyrolysis reaction. *Renewable Energy*. **35**(1), pp.232–242.

Venir, E., Pittia, P., Giavon, S. and Maltini, E. 2009. Structure and water relations of melanoidins investigated by thermal, rheological, and microscopic analysis. *International Journal of Food Properties*. **12**(4), pp.819–833.

Véronique, M., Shailendra, M., Victoire, E., Clementine, O.C., Thomas, G., Gina, A., Hortense, M., Hubert, R., Phil, D., Steve, O., Gareth, M., 2010. *Preparation Study on Food Waste across EU 27*. European Commission, Paris, France.

Vietnam, Ministry of Natural Resources and Environment of Vietnam, 2011. National State of Environment Report 2011-solid Waste (Vietnam).

De Vrieze, J., De Lathouwer, L., Verstraete, W. and Boon, N. 2013. High-rate iron-rich activated sludge as stabilizing agent for the anaerobic digestion of kitchen waste. *Water Research*. **47**(11), pp.3732–3741.

Wahlen, S. and Winkel, T. 2017. Household Food Waste. *Reference Module in Food Science*. **46**, pp.1–9.

Wall, D.M., O'Kiely, P. and Murphy, J.D. 2013. The potential for biomethane from grass and slurry to satisfy renewable energy targets. *Bioresource Technology*. **149**, pp.425–431.

Wang, H.Y., Qian, H. and Yao, W.R. 2011. Melanoidins produced by the Maillard reaction: Structure and biological activity. *Food Chemistry*. **128**(3), pp.573–584.

Wang, J.Y., Liu, X.Y., Kao, J.C.M. and Stabnikova, O. 2006. Digestion of pre-treated food waste in a hybrid anaerobic solid-liquid (HASL) system. *Journal of Chemical Technology and Biotechnology*. **81**(3), pp.345–351.

Wang, X., Selvam, A. and Wong, J.W.C. 2016. Influence of lime on struvite formation and nitrogen conservation during food waste composting. *Bioresource Technology*. **217**, pp.227–232.

Wang, Y., Zhang, Y., Wang, J. and Meng, L. 2009. Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria. *Biomass and Bioenergy*. **33**(5), pp.848–853.

Ward, A.J., Hobbs, P.J., Holliman, P.J. and Jones, D.L. 2008. Optimisation of the anaerobic digestion of agricultural resources. *Bioresource Technology*. **99**(17), pp.7928–7940.

Waste & Resources Action Programme (WRAP) 2009. Anaerobic digestate. *WRAP Publications*., p.44.

Watanabe, Y., SUGI, R., TANAKA, Y. and HAYASHIDA, S. 1982. Enzymatic decolorization of melanoidin by Coriolus sp. No. 20. *Agricultural and Biological Chemistry*. **46**(6), pp.1623–1630.

Wei, Y., Van Houten, R.T., Borger, A.R., Eikelboom, D.H. and Fan, Y. 2003. Minimization of excess sludge production for biological wastewater treatment. *Water Research*. **37**(18), pp.4453–4467.

Weiland, P. 2010. Biogas production: Current state and perspectives. *Applied Microbiology and Biotechnology*. **85**(4), pp.849–860.

Wellinger, A., Murphy, J.D., Baxter, D. 2013. *The biogas handbook: science, production and applications*. New Delhi: Woodhead Publishing limited.

Westerman, P.W. and Bicudo, J.R. 2000. Tangential flow separation and chemical enhancement to recover swine manure solids, nutrients and metals. *Bioresource Technology*. **73**(1), pp.1–11.

Wilkinson, S., Smart, K.A. and Cook, D.J. 2015. Optimising the (Microwave) Hydrothermal Pretreatment of Brewers Spent Grains for Bioethanol Production. *Journal of Fuels*. **2015**, pp.1–13.

World Energy Council 2016. World Energy Resources 2016. *World Energy Council 2016*.

WRAP 2011. Compost & Anaerobic Digestate Quality for Welsh Agriculture. WRAP. Banbury: Written by: Taylor, M., Rollett, A., Chambers, B.

WRAP 2012. *Using quality anaerobic digestate to benefit crops* [Online]. [Accessed 06 August 2020]. Available from: http://www.wrap.org.uk/sites/files/wrap/Quality digestate - using quality anaerobic digestate to benefit crops.pdf.

WRAP 2013. *Household Food and Drink Waste in the United Kingdom 2012* [Online]. [Accessed 06 August 2020]. Available from: http://www.wrap.org.uk/sites/files/wrap/hhfdw-2012-main.pdf.pdf.

WRAP 2016. *Field experiments for quality digestate and compost in agriculture - Work Package 2 Report – Digestate Nitrogen Supply and Environmental Emissions* [Online]. [Accessed 06 August 2020]. Available from: http://www.wrap.org.uk/sites/files/wrap/WRAP_DC-Agri_research_summary.pdf.

WRAP 2020. Food Surplus and Waste in the UK – key facts. (January), p.14.

Wu, D., Lü, F., Gao, H., Shao, L. and He, P. 2011. Mesophilic bio-liquefaction of lincomycin manufacturing biowaste: The influence of total solid content and inoculum to substrate ratio. *Bioresource Technology*. **102**(10), pp.5855–5862.

Wu, D., Lü, F., Shao, L. and He, P. 2017. Effect of cycle digestion time and solid-liquid separation on digestate recirculated one-stage dry anaerobic digestion: Use of intact polar lipid analysis for microbes monitoring to enhance process evaluation. *Renewable Energy*. **103**, pp.38–48.

Xiao, K., Zhou, Y., Guo, C., Maspolim, Y. and Ng, W.J. 2015. Dynamics of propionic acid degradation in a two-phase anaerobic system. *Chemosphere*. **140**, pp.47–53.

Xie, S., Lawlor, P.G., Frost, J.P., Hu, Z. and Zhan, X. 2011. Effect of pig manure to grass silage ratio on methane production in batch anaerobic co-digestion of concentrated pig manure and grass silage. *Bioresource Technology*. **102**(10), pp.5728–5733.

Xu, H.L., Wang, J.Y., Zhang, H. and Tay, J.H. 2003. A comparative study of anaerobic digestion of food waste in a single pass, a leachate recycle and coupled solid/liquid reactors. *Water science and technology : a journal of the International Association on Water Pollution Research*. **47**(1), pp.319–324.

Xu, Z., Sun, D.-W., Zeng, X.-A., Liu, D. and Pu, H. 2015. Research Developments in Methods to Reduce the Carbon Footprint of the Food System: A Review. *Critical Reviews in Food Science and Nutrition*. **55**(9), pp.1270–1286.

Yadvika, Santosh, Sreekrishnan, T.R., Kohli, S. and Rana, V. 2004. Enhancement of biogas production from solid substrates using different techniques - A review. *Bioresource Technology*. **95**(1), pp.1–10.

Yang, C., Song, H.L., Chen, F. and Zou, T.T. 2011. Response Surface Methodology for Meat-Like Odorants from the Maillard Reaction with Glutathione II: The Tendencies Analysis of Meat-Like Donors. *Journal of Food Science*. **76**(9).

Yang, C., Yang, M. and Yu, Q. 2012. An Analytical Study on the Resource Recycling Potentials of Urban and Rural Domestic Waste in China. *Procedia Environmental Sciences*. **16**, pp.25–

33.

Yang, X., Wang, X. and Wang, L. 2010. Transferring of components and energy output in industrial sewage sludge disposal by thermal pretreatment and two-phase anaerobic process. *Bioresource Technology*. **101**(8), pp.2580–2584.

Yang, Z., Wang, W., He, Y., Zhang, R. and Liu, G. 2018. Effect of ammonia on methane production, methanogenesis pathway, microbial community and reactor performance under mesophilic and thermophilic conditions. *Renewable Energy*. **125**, pp.915–925.

Yin, J., Liu, J., Chen, T., Long, Y. and Shen, D. 2019. Influence of melanoidins on acidogenic fermentation of food waste to produce volatility fatty acids. *Bioresource Technology*. **284**(January), pp.121–127.

Yirong, C., Heaven, S. and Banks, C.J. 2014. Effect of a Trace Element Addition Strategy on Volatile Fatty Acid Accumulation in Thermophilic Anaerobic Digestion of Food Waste. *Waste and Biomass Valorization*. **6**(1), pp.1–12.

Yu, Y., Chan, W.I., Liao, P.H. and Lo, K. V. 2010. Disinfection and solubilization of sewage sludge using the microwave enhanced advanced oxidation process. *Journal of Hazardous Materials*. **181**(1–3), pp.1143–1147.

Yuan, H., Li, R., Zhang, Y., Li, X., Liu, C., Meng, Y., Lin, M. and Yang, Z. 2015. Anaerobic digestion of ammonia-pretreated corn stover. *Biosystems Engineering*. **129**, pp.142–148.

Yuan, J.S., Tiller, K.H., Al-Ahmad, H., Stewart, N.R. and Stewart, C.N. 2008. Plants to power: bioenergy to fuel the future. *Trends in Plant Science*. **13**(8), pp.421–429.

Yuan, Z., Zhu, Y., Wang, Y. and Zhang, L. 2012. Conserving energy by optimizing the heat exchanger networks of glyphosate production with pinch technology. *Clean Technologies and Environmental Policy*. **14**(4), pp.749–757.

Zhang, C., Su, H., Baeyens, J. and Tan, T. 2014. Reviewing the anaerobic digestion of food waste for biogas production. *Renewable and Sustainable Energy Reviews*. **38**, pp.383–392.

Zhang, J., Lv, C., Tong, J., Liu, Jianwei, Liu, Jibao, Yu, D., Wang, Y., Chen, M. and Wei, Y. 2016. Optimization and microbial community analysis of anaerobic co-digestion of food waste and sewage sludge based on microwave pretreatment. *Bioresource Technology*. **200**, pp.253–261.

Zhang, L. and Jahng, D. 2012. Long-term anaerobic digestion of food waste stabilized by trace elements. *Waste Management*. **32**(8), pp.1509–1515.

Zhang, R., Elmashad, H., Hartman, K., WangANG, F., Liu, G., Choate, C. and Gamble, P. 2007. Characterization of food waste as feedstock for anaerobic digestion. *Bioresource Technology*. **98**(4), pp.929–935.

Zhang, Y. and Banks, C.J. 2013. Impact of different particle size distributions on anaerobic digestion of the organic fraction of municipal solid waste. *Waste Management*. **33**(2), pp.297–307.

Zhang, Y., Banks, C.J. and Heaven, S. 2012. Co-digestion of source segregated domestic food waste to improve process stability. *Bioresource Technology*. **114**, pp.168–178.

Zheng, K., Kennedy, K.J. 2006. Effect of mild microwave pretreatment on digestion of primary sludge. 41st Central Canadian Symp. Water Qual. Res. 12-15 February, Burlington, ON Canada.

Zheng, J., Kennedy, K.J. and Eskicioglu, C. 2009. Effect of low temperature microwave pretreatment on characteristics and mesophilic digestion of primary sludge. *Environmental Technology*. **30**(4), pp.319–327.

Zhou, Y., Zhang, Z., Nakamoto, T., Li, Y., Yang, Y., Utsumi, M. and Sugiura, N. 2011. Influence of substrate-to-inoculum ratio on the batch anaerobic digestion of bean curd refuse-okara under mesophilic conditions. *Biomass and Bioenergy*. **35**(7), pp.3251–3256.

Zhu, B., Gikas, P., Zhang, R., Lord, J., Jenkins, B. and Li, X. 2009. Characteristics and biogas production potential of municipal solid wastes pretreated with a rotary drum reactor. *Bioresource Technology*. **100**(3), pp.1122–1129.

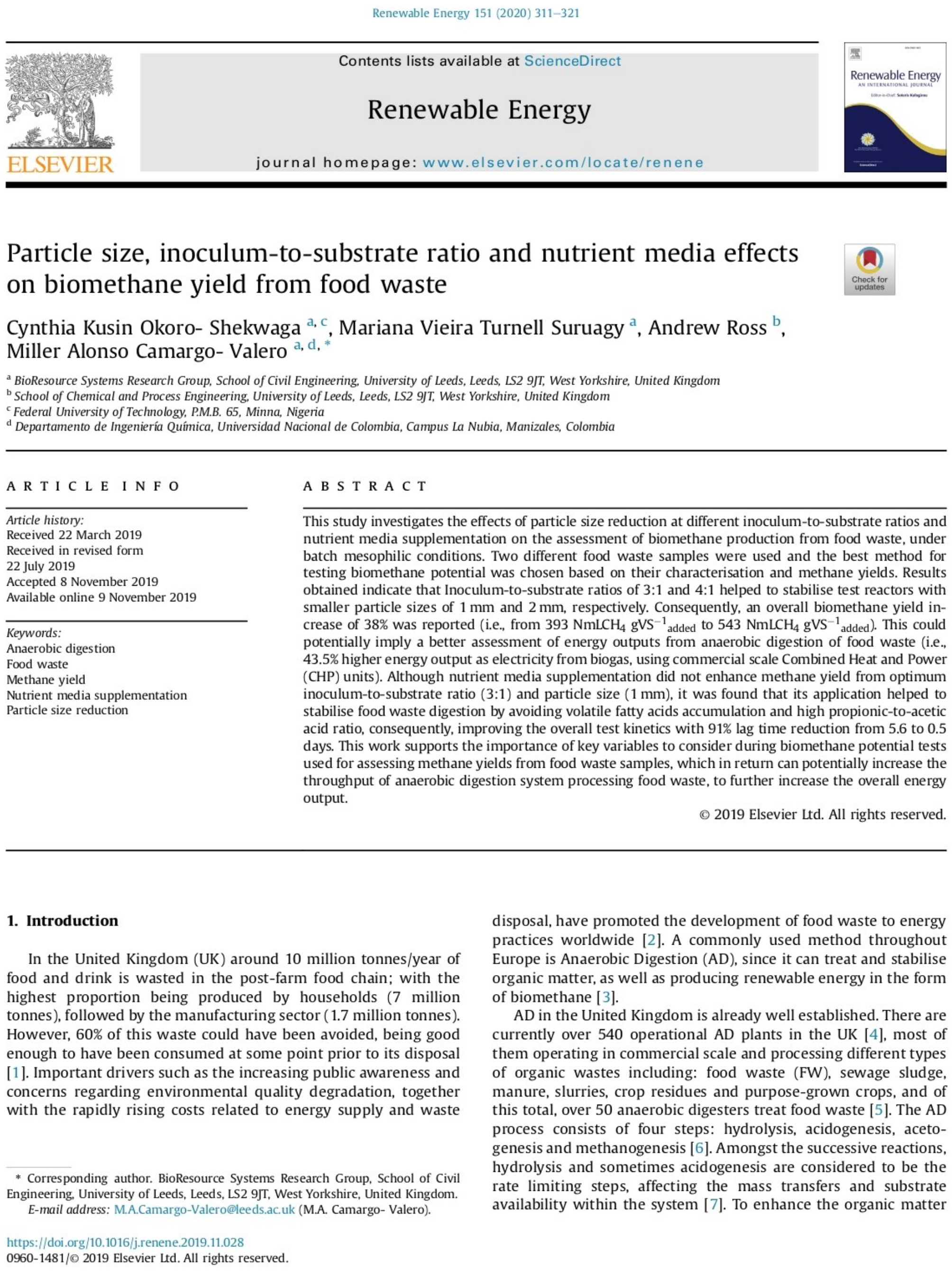
Zi, W., Zhang, X., Wang, L., Peng, J. and Chen, L. 2013. Study on the dielectric properties of biomass material of tobacco stems. *Advanced Materials Research*. **634–638**(1), pp.2570–2578.

Zirkler, D., Peters, A. and Kaupenjohann, M. 2014. Elemental composition of biogas residues: Variability and alteration during anaerobic digestion. *Biomass and Bioenergy*. **67**, pp.89–98.

Zwietering, M.H., Jongenburger, I., Rombouts, F.M. and Van't Riet, K. 1990. Modeling of the bacterial growth curve. *Applied and Environmental Microbiology*. **56**(6), pp.1875–1881.

APPENDICES

A.



Renewable Energy 151 (2020) 311–321

Contents lists available at ScienceDirect

Renewable Energy

journal homepage: www.elsevier.com/locate/renene

Particle size, inoculum-to-substrate ratio and nutrient media effects on biomethane yield from food waste

Cynthia Kusin Okoro- Shekwaga [a, c], Mariana Vieira Turnell Suruagy [a], Andrew Ross [b], Miller Alonso Camargo- Valero [a, d, *]

[a] BioResource Systems Research Group, School of Civil Engineering, University of Leeds, Leeds, LS2 9JT, West Yorkshire, United Kingdom
[b] School of Chemical and Process Engineering, University of Leeds, Leeds, LS2 9JT, West Yorkshire, United Kingdom
[c] Federal University of Technology, P.M.B. 65, Minna, Nigeria
[d] Departamento de Ingeniería Química, Universidad Nacional de Colombia, Campus La Nubia, Manizales, Colombia

ARTICLE INFO

Article history:
Received 22 March 2019
Received in revised form
22 July 2019
Accepted 8 November 2019
Available online 9 November 2019

Keywords:
Anaerobic digestion
Food waste
Methane yield
Nutrient media supplementation
Particle size reduction

ABSTRACT

This study investigates the effects of particle size reduction at different inoculum-to-substrate ratios and nutrient media supplementation on the assessment of biomethane production from food waste, under batch mesophilic conditions. Two different food waste samples were used and the best method for testing biomethane potential was chosen based on their characterisation and methane yields. Results obtained indicate that Inoculum-to-substrate ratios of 3:1 and 4:1 helped to stabilise test reactors with smaller particle sizes of 1 mm and 2 mm, respectively. Consequently, an overall biomethane yield increase of 38% was reported (i.e., from 393 $\text{NmLCH}_4\ \text{gVS}^{-1}_{\text{added}}$ to 543 $\text{NmLCH}_4\ \text{gVS}^{-1}_{\text{added}}$). This could potentially imply a better assessment of energy outputs from anaerobic digestion of food waste (i.e., 43.5% higher energy output as electricity from biogas, using commercial scale Combined Heat and Power (CHP) units). Although nutrient media supplementation did not enhance methane yield from optimum inoculum-to-substrate ratio (3:1) and particle size (1 mm), it was found that its application helped to stabilise food waste digestion by avoiding volatile fatty acids accumulation and high propionic-to-acetic acid ratio, consequently, improving the overall test kinetics with 91% lag time reduction from 5.6 to 0.5 days. This work supports the importance of key variables to consider during biomethane potential tests used for assessing methane yields from food waste samples, which in return can potentially increase the throughput of anaerobic digestion system processing food waste, to further increase the overall energy output.

1. Introduction

In the United Kingdom (UK) around 10 million tonnes/year of food and drink is wasted in the post-farm food chain; with the highest proportion being produced by households (7 million tonnes), followed by the manufacturing sector (1.7 million tonnes). However, 60% of this waste could have been avoided, being good enough to have been consumed at some point prior to its disposal [1]. Important drivers such as the increasing public awareness and concerns regarding environmental quality degradation, together with the rapidly rising costs related to energy supply and waste disposal, have promoted the development of food waste to energy practices worldwide [2]. A commonly used method throughout Europe is Anaerobic Digestion (AD), since it can treat and stabilise organic matter, as well as producing renewable energy in the form of biomethane [3].

AD in the United Kingdom is already well established. There are currently over 540 operational AD plants in the UK [4], most of them operating in commercial scale and processing different types of organic wastes including: food waste (FW), sewage sludge, manure, slurries, crop residues and purpose-grown crops, and of this total, over 50 anaerobic digesters treat food waste [5]. The AD process consists of four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis [6]. Amongst the successive reactions, hydrolysis and sometimes acidogenesis are considered to be the rate limiting steps, affecting the mass transfers and substrate availability within the system [7]. To enhance the organic matter

---

* Corresponding author. BioResource Systems Research Group, School of Civil Engineering, University of Leeds, Leeds, LS2 9JT, West Yorkshire, United Kingdom.
E-mail address: M.A.Camargo-Valero@leeds.ac.uk (M.A. Camargo- Valero).

https://doi.org/10.1016/j.renene.2019.11.028
0960-1481/© 2019 Elsevier Ltd. All rights reserved.

solubilisation and avoid any impact from the rate-limiting steps, several pre-treatments methods have been applied to food waste prior to anaerobic digestion process including: chemical [8], biological [9], and physical strategies [10].

As part of the physical pre-treatments for FW there is the mechanical gridding, which allows Particle Size (PS) reduction. Smaller particles ultimately increase biodegradability by expanding the surface area and subsequently, food availability to the microbial community, thus improving methane production [11,12]. In agreement [13], reported that by reducing food waste PS from 2.14 to 1.02 mm the maximum substrate utilisation doubled, thus improving process performance. Meanwhile, in some cases PS reduction can have a detrimental effect as suggested by Ref. [12]; stating a negative relationship between excessive PS reduction and methane production.

Moreover, methane production from food waste can also be enhanced using different inoculum-to-substrate ratio (ISR) [14]. assessed the biomethane potential of kitchen waste by testing a range of ISR (2, 1, 0.74 and 0.43), along with two inoculum types (granular and suspended). The authors concluded that acidification was successfully prevented over the tested ISR range when granular inoculum was used. Suspended sludge on the other hand, only avoided acidification at the highest ISR. Similarly [15], applied a bovine fluid inoculum at ISR 0.17, 0.11, and 0.05 to assay the biostabilisation of the organic fraction of municipal solid waste, revealing a straight-forward relation between higher amounts of inoculum and process performance improvement. Although previous studies have investigated the individual effect of PS and ISR on biomethane yield, a further combination of PS with ISR towards biomethane improvement from AD, as at the time of conducting this study, have not yet been reported in the literature.

Despite the various methods to improve biodegradability and biomethane production from FW, it has been shown that digestion of this substrate alone has often proven difficult and rarely reported as successful [16], especially in a single-stage process. The main difficulty is related to the fact that most food waste are trace-element deficient substrates. Thus, important nutrients to the AD biochemical pathways, especially to the methanogenesis step such as Co, Ni, W, Se and Mo are often found in very low concentrations or even absent [17]. However, with appropriate nutrient supplementation the AD process of FW becomes more resistant to environmental changes, hence more efficient [18–21].

Therefore, the principal aims of this paper were as follows: a) determine whether the combination of PS reduction and ISR could enhance process stability and the assessment of biomethane yield from food waste anaerobic digestion, and b) to investigate if nutrient media supplementation can enhance even further the biomethane yield of food waste under the optimum PS and ISR, using batch biochemical methane potential (BMP) assays at mesophilic temperatures.

2. Material and methods

2.1. Food waste collection, processing and particle size characterization

Food waste was collected from the Leeds University Refectory, Leeds, United Kingdom, on two different occasions. The first collection occurred during a single visit to the establishment. Due to its composition reflecting mainly raw, uncooked ingredients from the kitchen area of the refectory, this sample was denominated Kitchen Waste (KW). The second collection happened over five consecutive days and composite samples consisted of both plate waste (from the eating area) and kitchen wastes, hence denominated as Composite Food Waste (CFW) samples (Table 2). The two sampling streams were conducted to understand the effect of particle size, inoculum-to-substrate ratio and nutrient media on the effective biomethane potential of different food waste streams likely to be produced at household level (i.e., uncooked food waste and food waste), using food waste from the refectory as a proxy.

Samples were collected on the same day they were discarded, as suggested by Ref. [2]; thus avoiding dealing with putrescible waste and consequently, underestimating Total Solids (TS) and/or Volatile Solids (VS) results. The collected waste was manually sorted for any unwanted impurities such as glass, paper, cardboard, plastic and bones. Sorted food waste substrate was thoroughly mixed, chopped and ground with a mincer. To allow further substrate size reduction and better homogenisation, the sample was blended with a food liquidizer. During this process, no water was added so the moisture content would not be affected. After the homogenisation and particle reduction step, the PS for the raw food waste was characterised by sieving a known amount of sample through a series of sieves with aperture between 1 and 10 mm and comparing the recovered solids to the reject to achieve a solids recovery of not less than 95%. Below an aperture of 5 mm the solids recovery was less than 95%, hence, the raw homogenised food waste PS was characterised as $\leq$5 mm. Subsequently, food waste samples with a PS of 1 mm and 2 mm were achieved by sieving the raw homogenised food waste sample through the respective sieve. Due to the dense and paste nature of the sample, it was not possible to allow it to drain freely through the sieves, therefore, manual pressure was applied during the sieving process using a flat metal bar. Hence, the first food waste PS was the undersize of the processed sample from 1 mm sieve, the second PS was the undersize of the processed sample from a 2 mm sieve and the last was the raw homogenised sample after processing with PS $\leq$ 5 mm; having 95% solids recovery from a 5 mm sieve.

To generate representative sub-samples, the food waste sample for each PS group was individually mixed and divided into four samples. Subsequently, smaller samples of 500 g were weighed into refrigerator bags, labelled and stored at $-20\,^{\circ}$C until required for the experiments; one bag from each sample was however stored at $4\,^{\circ}$C to carry out the characterisation. Frozen samples used for the experiments were thawed at $4\,^{\circ}$C prior to BMP experiments; such that no heat was added to defrost the samples.

2.2. Inoculum

The inoculum used in this study was obtained from a mesophilic anaerobic digester, treating sewage sludge at Esholt Wastewater Treatment Plant in Yorkshire, UK. Before each experimental set-up the inoculum was passed through a 1 mm sieve to remove any large particles or grit and then incubated at $37\,^{\circ}$C. Acclimation of the inoculum to food waste was done over a 30 days period, by adding 3 $g_{FW}\ L^{-1}{}_{inoculum}$ once every two weeks, equivalent to 0.2 $gVS_{FW}\ L^{-1}\ day^{-1}$. Since the experiments were carried out in distinct timeline, the adapted inoculum (henceforth referred to as inoculum) was characterised regarding its main physical-chemical properties two days before each BMP set-up.

2.3. Experimental design

2.3.1. Anaerobic biodegradability (BMP) tests

This step consisted of two sets of experiments. Experiment 1 tested the effect of combining different PS and ISR on the biomethane yield of KW. Once the optimal conditions of ISR and PS for improved biomethane yield were established with KW, the biomethane yield at the same conditions were conducted with CFW in comparison with KW. Considering that KW and CFW samples had similar biomethane yields, Experiment 2 was conducted to test the effect of nutrient media supplementation to further improve the biomethane yield using CFW samples only. The decision of applying nutrient media supplementation on CFW was based on the results from food waste characterisation – having higher theoretical methane potential (TMP), but less metal content than KW. BMP trials were conducted in batches using 500 ml Duran bottles, with 400 ml working volume, under mesophilic conditions (37 °C). The temperature was maintained by means of a water bath as part of the automatic methane potential test system (AMPTS II) by Bioprocess Control as described by Ref. [22]. To determine the biomethane originating from the inoculum, blank samples were prepared for each set of experiment, containing only inoculum and distilled water. A 3^2 factorial design was employed for Experiment 1; that is three levels of food waste PS and three levels of ISR (Table 1). All BMP assays were conducted in triplicates.

2.3.1.1. Experiment 1: Applying different food waste particle size and inoculum-to-substrate ratios. The food waste samples were blended with a Nutribullet homogeniser and characterised as ≤ 5 mm; having >95% recovery of the food waste from a 5 mm screen. They were then sieved through 1 mm and 2 mm screens to obtain the respective PS, as such the three PS (≤ 1 mm, ≤ 2 mm and ≤ 5 mm); hereafter denoted as 1 mm, 2 mm and 5 mm, were added to each reactor as a substrate, at different concentrations, depending on the ISR used. These sizes were chosen because smaller PS below 1 mm could encourage high volatile fatty acids (VFAs) concentration, due to enhanced fermentation [12], while above 5 mm lower biogas yield could be obtained, due to poor substrate degradation. Three ISR were tested; 2:1, 3:1 and 4:1 based on VS content.

When assembling the reactors, a fixed volume of 300 ml of inoculum was used for all assays and the VS concentration in this amount of inoculum was calculated. For each ISR, the required amount of food waste was determined. Hence, the calculated FW amount was added to 300 ml of inoculum and made up to 1 L with distilled water. Bulk samples were prepared with constant manual mixing and divided into aliquots of 500 ml; out of which 400 ml was used for the BMP analysis, while the 100 ml samples remaining were used to conduct the experimental analysis for day 0 (when the reactors were assembled). The reactors were continuously flushed with pure N_2 gas for 1 min to ensure anaerobic conditions of the reactors and capped tightly with rubber stoppers.

2.3.1.2. Experiment 2: Applying nutrient media to improve methane yield. The CFW was used in Experiment 2 and tested at ISR of 3:1. Although, the KW and CFW had similar biomethane yields at optimum conditions of PS and ISR, the lower C/N ratio and nutrient content, as well as the higher TMP of the CFW, suggested that its supplementation with macro- and micro-nutrient media could further enhance methane production. The nutrient media composition and preparation was based on previous works [23–26]. Four stock solutions A, B and C and D were used to prepare the final nutrient media and the concentration of chemicals in each solution is given below in g L-^{1} in distilled water.

Solution A: NH_4CL (0.53), KH_2PO_4 (0.27), K_2HPO_4 (0.35), $CaCl_2 \cdot 2H2O$ (0.075), $MgCl_2 \cdot 6H_20$ (0.10), $FeCl_2 \cdot 4H_20$ (0.02), $MnCl_2 \cdot 4H_20$ (0.05), H_3BO_4 (0.05), $ZnCl_2$ (0.05), $CuSO_4$ (0.03), $Na_2MoO_4 \cdot 2H_2O$, (0.01), $CoCl_2 \cdot 6H_2O$ (0.50), $NiCl_2 \cdot 6H_2O$ (0.05).

Solution B: Biotin (0.002), Folic Acid (0.002), Riboflavin (0.005), Thiamine (0.005), Nicotinic Acid (0.005), Cobalamin (0.0001), p-aminobenzoic acid (0.005).

Solution C: 500 g of $Na_2S \cdot 9H_2O$ in 1 L of distilled water.

Solution D: 0.5 g of Resazurin in 1 L of distilled water as an oxidation-reduction indicator.

Solution A was used as a base solution and autoclaved for 15 min at 121 °C and 103.4 KPa. Then the other solutions were added to it in the following volumes: 10 ml of solution B; 1 ml of solution C and 1 ml of solution D. Finally, the pH was corrected to 7.0 ± 0.2 by gradually adding $NaHCO_3$; up to a maximum of 1.20 g. When assembling the reactors, 15 g of VS of inoculum was used and the required amount of food waste (in g of VS) was established by dividing it by the respective ISR (3:1). The volume of media used in the reactor was determined by deducting the inoculum and food waste volumes from the 400 ml reactor working volume. No water was used in the reactors with nutrient media, thus possibly avoiding important nutrients becoming a limiting factor on the system. The media was transferred to each reactor, followed by the inoculum and food waste. A Resazurim solution was added to indicate the presence of oxygen inside the reactors. During the media inoculation, the bottles were continuously flushed with pure N_2 gas to ensure anaerobic conditions of the reactors and capped tightly with rubber stoppers.

2.3.2. BMP test monitoring

Liquid samples were analysed on day 0 and then on day 4 (except for Experiment 2 where samples were also analysed on day 7). After this period, sampling was carried out once a week, until the last day of digestion; when the digestate was also characterised. All analytical monitoring during the BMP test was conducted in duplicates.

Daily methane production from each reactor was automatically measured and converted to Standard Temperature and Pressure (STP) conditions (1 atm and 0 °C) by the AMPTS II system. Methane yield was calculated based on the amount of VS added as described in the AMPTS II manual. The total digestion period was 28 days, or when the daily methane production was less than 1% of the total cumulative methane produced by the reactor since the beginning of the experiment – [27].

2.3.3. Analytical methods

Standard analytical methods used for the examination of wastewaters and sludge were employed [28] to characterise liquid samples, including the following parameters: total solids - TS

Table 1
Experimental set-up for Experiment 1.

Particle size, PS (mm)	Inoculum to Substrate Ratio, ISR	Volatile Solids (VS) content (g/Reactor)
1	2	8.10
1	3	11.38
1	4	6.75
2	2	9.05
2	3	8.04
2	4	7.54
5	2	5.72
5	3	5.08
5	4	4.76

(Method 2540 B), volatile solids - VS (2540 E) and chemical oxygen demand - COD (5220 C). The pH of all reactors was measured using a pH meter (HACH, 40d). Elemental carbon, hydrogen, nitrogen and sulphur (CHNS) were measured using Thermo Scientific FLASH2000 Organic Elemental Analyser. Samples were first dried at 40 °C for two days and ground to a powder using a mortar and pestle.

Protein content was performed by determining the nitrogen using the Kjeldahl method, and the lipid content by the Soxhlet extraction method at 40–60 °C, using petroleum Spirit as solvent (Nielsen, 2010). Carbohydrate values were obtained by differential method; deducting lipid, protein, ash and moisture content from the total weight of the samples. Volatile Fatty Acids (acetic; propionic; i-butyric, butyric, valeric and i-valeric acid) were measured using a Gas Chromatographer - GC (Agilent Technologies, 7890A) equipped with a flame ionization detector (FID), an auto-sampler and a DB-FFAP column (length 30 m, diameter 0.32 mm and film thickness 0.5 μm), and using Helium as a carrier gas. The operating conditions of the GC-FID detector were: 150 °C inlet temperature and 200 °C FID temperature. Liquid samples were adjusted to pH 2.0 using phosphoric acid and allowed to rest for 30 min and then centrifuged at 14,000 RPM (16,000×g) for 5 min, using a Technico Maxi Microcentrifuge. After centrifuging, the supernatant was filtered through a 0.2 μm filter and the liquid analysed for VFAs. The GC was calibrated with SUPELCO Volatile Acid Standard Mix, which includes acetic-, propionic-, iso-butyric-, butyric-, iso-valeric-, valeric-, iso-caproic-, caproic- and heptanoic-acids. The concentration of the various trace elements and metals were determined by AOAC Method 2015.01, for heavy metals in food, by Inductively Coupled Plasma – Mass Spectrometry (ICP-MS), using microwave-assisted acid digestion (nitric acid and hydrogen peroxide) [29].

2.3.4. Data processing and statistical analysis

The estimation of the theoretical methane potential (TMP) was calculated based on the Buswell equation [30]. A kinetic analysis of the methane production and soluble COD degradation was conducted. The modified Gompertz (MGompertz) growth model (Equation (1)) was used to fit the methane production curves, according to Ref. [31]; to estimate the lag phase and maximum specific methane production rate for each assay, using Origin-Pro® 2018 graphical and statistics software.

$$y = Aexp\left\{ - exp\left[\frac{\mu_m \cdot e}{A}(\lambda - t) + 1\right]\right. \tag{1}$$

where; $y = Cumulative\ methane\ yield\ (mLCH_4 g\ VS^{-1}{}_{added})$, $A = Maximum\ methane\ yield\ (mLCH_4\ g\ VS^{-1}{}_{added})\ at\ time\ t$, $\mu m = Maximum\ specific\ methane\ yield\ per\ day\ (mLCH_4(gVS^{-1}{}_{added}\ Day^{-1}))$, λ=Lag phase (Days),e = exp(1)

Coupled with the kinetic fitting, a full factorial design of experiment (DOE) was constructed using Minitab18 statistical software to analyse the variance between the BMP data from Experiment 1, using a 2 factor and 3 levels (3^2) factorial design. A surface regression analysis was also conducted with the structured DOE to further examine the effect of intermediate PS (3 and 4) effect on the biomethane yield at a confidence level (α) of 0.05.

3. Results and discussion

The composition of both KW and CFW are described in Table 2. CFW samples had a broader composition than KW, possibly because of a longer collection period compared to KW. The physical and biochemical characteristics of both samples are shown in Table 3.

Regarding the composition of the substrate, both samples had a high VS/TS ratio; 95.58 and 92.91% for the KW and CFW respectively, indicating that most components of the wastes are organic matter susceptible of biodegradation, thus its viability as a feedstock for biogas production via anaerobic digestion. Food waste is a substrate known for having low pH ranges. The results found in this study were in consonant with others FW studies, which found a pH range between 4.0 and 5.2 [22]; Elbeshbishy et al., 2012; [35–39].

Nevertheless, CFW contained higher concentrations of lipids (27.62%) compared to other food waste samples, including KW, hence, suggesting a likely higher biomethane potential [7]. However, the C/N ratio at 5 mm PS (10.95–17.19) was lower than the recommended value range of 20–30 [40]. An optimum C/N is required for bacteria to allow their growth and maintain a stable environment, as well as being an important indicator of potential ammonium/ammonia toxicity and inhibition. The significantly lower C/N ratio of the CFW sample (10.95) could hinder the AD process, by decreasing the COD (chemical oxygen demand) removal and VS destruction rates, thus negatively affecting the reactor performance and further methane production (Musa et al., 2014).

The TS content in the KW and CFW were mainly constituted of carbohydrates at 57.52 and 42.75%, followed by lipids at 24.25 and 27.62%, respectively. Protein content was significantly higher in the CFW sample, than the KW sample (1.7 times greater) and other reported elsewhere (1.4 times greater – Table 3). This implies the CFW has a higher potential for high ammonia loads and related toxicity.

Based on the inorganic composition of the wastes here studied, the KW sample contained higher concentrations of trace elements compared to the CFW, except for Selenium, which was absent in the former. Overall, based on different waste compositions published in the literature, it is possible to corroborate the representativeness of both samples used in this study, and their suitability for anaerobic biodegradability.

3.1. Experiment 1: Influence of particle size and inoculum-to-substrate ratio

3.1.1. Influence of particle size reduction on food waste elemental characteristics

Mechanical pre-treatment, which mainly involves size reduction, is widely employed in anaerobic digestion, with reported increase in methane yield, especially due to enhanced hydrolysis [[41]7]. The reduction in PS and subsequent sample preparation of the 2 mm and 1 mm kW samples resulted in a change in TS from 214.2 g/kg at 5 mm to 209.0 g/kg and 205.9 g/kg at 2 mm and 1 mm respectively. The VS content also slightly changed from 205 g/kg at 5 mm to 200 g/kg at 2 mm and 197 g/kg at 1 mm. These negligible changes in TS and VS contents due to sample preparation (larger, heavier samples could have been rejected during sieving) may have impacted on the elemental characteristics of the samples.

Reducing the PS in this study resulted in an increase in C/N ratio. The C/N ratio increased by 29% and 32% when the KW PS was reduced from 5 mm to 2 mm and 5 mm–1 mm respectively. It is possible that due to fractionation the solids reject from the sieve when the PS were reduced, influenced the detainment of some of the elemental components, thus, altering the elemental characteristics of the smaller PS.

According to the *p*-values from two sample t-tests conducted at $\alpha = 0.05$ (Table 4), reduction in KW PS from 5 mm significantly affected the elemental characteristics especially the carbon and nitrogen content. However, further reduction in PS from 2 mm to 1 mm did not significantly affect the elemental characteristics (except for hydrogen). The significant changes in elemental composition observed in the KW sample following PS reduction can be attributed to the fact that these elements are largely chemically

Table 2
Composition of food waste samples.

Sample	Component
Kitchen Waste (KW)	Pineapple, water melon, casaba melon, strawberry, red, green and yellow pepper, carrot, cucumber, lettuce, tomato, white rice, potatoes (harsh brown) and white buns.
Composite Food Waste (CFW)	Tomato, chickpeas, cucumber, green peas, mushroom, carrot, fried and cooked potatoes, potatoes peels, rocket leaves, onions, broccoli, green beans, corn, red pepper, okra, bread, pizza, spaghetti, Yorkshire pudding, rice, fried and boiled eggs, bacon, beef, fish chicken, sausages, minced meat, baked beans and butter.

Table 3
Physical and Biochemical Characteristics of food waste samples and comparison with published literature*.

Parameter/Sample	Present work		References				
	Average Value (standard deviation)		Vavouraki et al. (2013)	[2]	[32]	[33]	[34]
	KW	CFW	Kitchen Waste	Food Waste	Food Waste	Food Waste	Food Waste
Moisture Content %	78.58 (0.25)	68.11 (0.30)	81.5(0.66)	–	–	–	–
Total Solids (TS), mg/kg (wet base = w.b.)	21.4 (2.52)	31.9 (3.01)	18.5(0.71)	30.90(0.07)	18.1(0.6)	23.1(0.3)	14.3 (1.75)
Volatile Solids (VS), mg/kg (w.b.)	20.5 (1.36)	29.6 (4.05)	_	26.35(0.14)	17.1 (0.6)	21.0(0.3)	13.1 (1.71)
VS/TS % (dry base = d.b.)	95.58	92.91	94.1 (0.35)	85.30 (0.65)	0.94(0.01)	90.9(0.2)	–
C %TS	50.87 (0.07)	53.06 (0.37)	–	46.78(1.15)	46.67	56.3(1.1)	47.4(0.01)
H %TS	7.21 (0.14)	7.79 (0.10)	–	–	–	–	6.65(0.28)
N %TS	2.96 (0.03)	4.85 (0.07)	–	3.16(0.22)	3.54	2.3(0.3)	1.90(0.09)
O %TS	38.83 (0.24)	34.18 (0.51)	–	–	–	–	43.7(0.28)
S %TS	0.13 (0.01)	0.13 (0.03)	–	–	–	–	0.41(0.06)
C/N	17.19	10.95	–	14.80	13.2	24.5(1.1)	24.94
Lipid % TS	24.25 (0.44)	27.62 (1.36)	14.0(0.51)	–	23.3(0.45)	–	–
Protein % TS	14.33 (0.68)	24.31 (1.00)	16.9(0.69)	–	–	–	–
Carbohydrate % TS	57.52 (0.48)	42.75 (1.97)	24.0 (1.06)	–	61.9	–	–
Calcium (Ca), mg/kg TS	154.2 (3.8)	227.3 (20.4)	–	–	–	–	–
Cobalt (Co), µg/kg TS	3.6 (1.1)	2.8 (0.5)	–	–	–	–	–
Cooper (Cu), mg/kg TS	1.7 (0.2)	1.3 (0.1)	–	–	–	–	–
Chromium (Cr), mg/kg TS	N.D.**	N.D.	–	–	–	–	–
Iron (Fe), mg/kg TS	3.6 (0.4)	4.3 (0.6)	–	–	–	–	–
Nickel (Ni), µg/kg TS	219.1 (58.8)	156.9 (28.1)	–	–	–	–	–
Magnesium (Mg), mg/kg TS	42.8 (2.2)	40.5 (1.1)	–	–	–	–	–
Manganese (Mn), mg/kg TS	1.1 (0.04)	0.6 (0.08)	–	–	–	–	–
Molybdenum (Mo), µg/kg TS	24.6 (4.0)	33.8 (3.5)	–	–	–	–	–
Selenium (Se), µg/kg TS	n.d	391.2 (103.2)	–	–	–	–	–
Potassium (K), mg/kg TS	586.1 (11.5)	773.5 (22.0)	–	–	–	–	–
Tungsten (W), µg/kg TS	5.9 (1.9)	4.5 (0.9)	–	–	–	–	–
Zinc (Zn), mg/kg TS	2.1 (0.5)	4.9 (0.8)	–	–	–	–	–
Total Chemical Oxygen Demand (TCOD), g/L	264.55	327.46 (22.13)	–	–	–	–	–
Total VFAs, mg/L	412.49(25.82)	746.82 (2.65)	–	–	–	–	–
pH	4.20	4.85	–	–		–	–

*Data reported as mean values with standard deviation in brackets, where available.
**N.D. = Not Detectable.

Table 4
P-values of from two sample *t*-test analysis of elemental characteristics of KW sample at different PS.

PS interaction	N	C	H	C/N
5 mm vs 1 mm	0.000	0.009	0.017	0.000
5 mm vs 2 mm	0.000	0.002	0.164	0.001
2 mm vs 1 mm	0.896	0.093	0.014	0.086

bound within the solids.

3.1.2. Volatile fatty acids profile

Considering that each experiment for the respective PS were set up differently with differing initial VFA concentration, the VFA degradation profile was normalised against the initial concentration on the day of set up (Day0) as shown in Fig. 1. Hence, each experiment had a starting value of 1 and higher values in any experimental setup could imply either of two things; (i) the rate of VFA consumption was lower than the rate of VFA accumulation; such that, an increased rate of VFA consumption would bring this value closer to or lower than 1 and (ii) the amount of VFA produced during fermentation was relatively higher; such that, the higher values become more a function of initial VFA produced.

The latter implies that such reactors would yield more methane if all the VFA were eventually consumed. But this was hardly the case with higher food waste PS (especially 5 mm), which although had the highest VFA peaks, produced the least amount of methane (see Section 3.1.3). Therefore, the reduction in PS is believed to have influenced faster VFA consumption, according to the former assumption.

In Fig. 1, we observe that VFA accumulated up to as much as 30 times the starting concentration when 5 mm PS was employed. This reduced significantly with 2 mm PS treatment, which had VFA accumulation measuring up to 13 times its starting concentration. Further reduction to 1 mm PS resulted in VFA accumulating only less than 3 times its initial concentration. This is also supported by the lag in methane production within the early days of digestion at 5 mm PS for each corresponding ISR (discussed in Section 3.1.4). This means with 5 mm PS, methane production progressed at an ‘inhibited steady-state’; whereby, the process continued at a stable

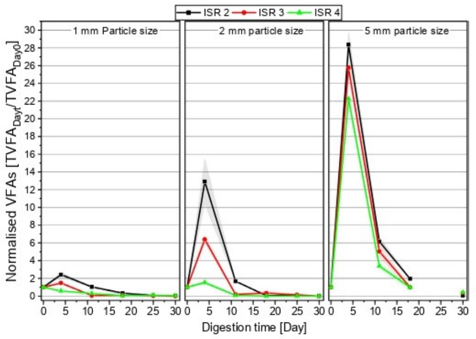


Fig. 1. Total VFA degradation curves for PS and ISR optimisation experiments, normalised against the initial concentration at Day0. Disconnection between Day 30 and the rest of the data sets for the 5 mm particle size curves was due to missing data as a result of lab closure for that time period. Shaded area around lines represent standard deviation from mean.

rate, but with low methane production [24]. It was not surprising to observe higher VFA accumulation at lower ISR for all three PS in the ISR order 2 > 3 > 4. Considering lower ISR meant relatively more food waste loading within the same PS experiments, the VFA levels increased at lower ISR during fermentation. The variation in ISR within each PS treatment was beneficial in identifying possible PS and ISR combinations that could help decrease the lag in methane production.

Acetic (A) and propionic (P) acids are the main precursors to methane production [7]. To minimise the VFA-induced inhibition, a P/A ratio of 1.4 have been set as a benchmark [42,43]. The P/A trends for all BMP assays are shown in Fig. 2.

While the total VFAs at lower particle sizes of 1 mm and 2 mm were relatively lower than the levels measured at 5 mm PS (Fig. 1), the corresponding P/A ratios at lower particle sizes were comparatively higher than the levels measured at 5 mm (Fig. 2). This suggests that acetic acid degradation progressed at a faster rate than acetogenesis for lower PS of 2 mm and 1 mm, which is also supported by relatively lower lag times.

The P/A peaks observed at ISR 2 relative to ISR3 and ISR4 for all PS ranges could be due to the higher food waste loading at that ISR compared to the other ISR assayed. Interestingly, for 1 mm and 2 mm PS, the P/A levels at an ISR of 4 rose slightly above the threshold of 1.4. This was possibly due to a higher rate of acetic acid degradation following a higher availability of microorganism at that ISR.

Therefore, with PS reduction, the rate of acetic acid degradation was perceived to be increased, which is also supported by lower lag times recorded for smaller PS compared to a PS of 5 mm (Section 3.1.4) and at an ISR of 3, the P/A level was maintained below the threshold value at all PS.

3.1.3. Biomethane yield from experiment 1

The biomethane yield from Experiment 1 ranged from 393 $NmLCH_4\ gVS^{-1}_{added}$ to 543 $NmLCH_4\ gVS^{-1}_{added}$ (Fig. 3). The highest biomethane yield was obtained with a combination of 1 mm PS and 3:1 ISR, while the least yield was obtained with a combination of 5 mm PS and 4:1 ISR. The methane yield from this study is similar to values reported in literature in the range of 211–581 ml $CH_4\ gVS^{-1}_{added}$, for food-based anaerobic digestion [33,44,45]. From Fig. 3, we observe that the high biomethane yields were obtained at 1 mm PS and decreased with increasing PS. This suggests that PS reduction does affect the BMP from food waste and is believed to be related to the improved VFA degradation rate. An overall biomethane increase of 38% was observed in this study with PS reduction. Similarly [11], reported 23% increase in methane yield from sisal fibre waste when it was reduced from 100 mm to 2 mm. [12]; also stated that smaller mean PS of food waste increased overall methane yield by 28%, when the mean PS was reduced from 0.843 to 0.391 mm using a bead mill, because of enhanced solubilisation. In a study on the effect of PS and sodium ion concentration on anaerobic thermophilic food waste digestion [13], concluded that PS is one of the most important factors of food waste anaerobic digestion. Furthermore, they observed an inverse relationship between food waste and maximum substrate utilisation rate, with PS reduction from 2 mm to 1.02 mm. Although, these studies were conducted at largely varied PS ranges, they all attributed PS reduction with increase in biomethane yield due to enhanced substrate solubilisation.

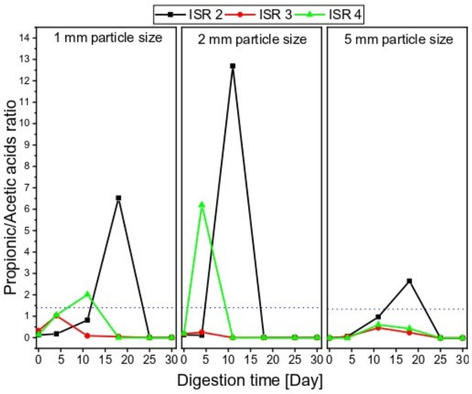


Fig. 2. Propionic to acetic acid ratios for PS and ISR optimisation experiments using the grab sample; dotted lines indicate the acceptable limit of 1.4.

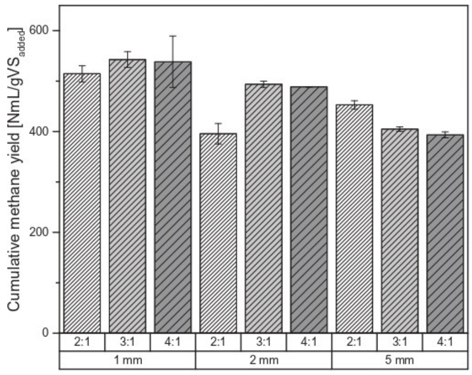


Fig. 3. Overall methane yield from Experiment 1, with error bars indicating standard deviations.

Arguably, PS reduction would seemingly increase the energy demand in AD systems, however, at the time of conducting this study, there was no data on energy required for PS reduction to support whether the increased energy output achieved in this study can sufficiently cover the energy input. Nevertheless, a potential increase in methane yield such as the one obtained in this study, could increase the energy output to make up for the energy demand from size reduction. For instance, the gross calorific value of methane is 39.8 MJ m^{-3}, as such, the energy value of the methane yield from 5 mm to 1 mm PS was 76,376 and 109,649 MJ $tonne^{-1}$, equivalent to 21,216 and 30,458 kWh $tonne^{-1}$ respectively (where 1 kWh = 3.6 MJ). The efficiency for methane conversion to electricity was estimated to be 35% [46], hence, without further PS reduction (5 mm), an energy output of 7,426 kWh $tonne^{-1}$ can be obtained. Meanwhile, with further PS reduction to 1 mm, the energy output increases to 10,660 kWh $tonne^{-1}$, which is 43.5% higher than the energy output at 5 mm.

Biomethane yield increased when the ISR was increased for smaller PS of 1 mm and 2 mm, while the opposite was observed at 5 mm PS. From the VFA profiles presented in Section 3.1.2 and the cumulative methane yield in Fig. 3, it might be useful to accompany PS reduction with ISR increase for improved yield. This is because reducing the PS results in enhanced solubilisation; owing to an increased surface area. Consequently, the microorganisms (inoculum) should be increased to consume the high amount of solubilised materials. This factor is often neglected, which could be responsible for the contrasting findings by different studies on ISR and food-related waste BMPs. For instance, in a study with soybean curd residue - SCR (or okara) [47], reported an increase in methane yield with an increase in ISR, while [45] concluded there was no significant difference in the methane production coefficient from the BMP of maize at ISR 3, 2, 1.5 and 1 respectively.

3.1.4. Kinetic assessment

The MGompertz model was used in fitting the experimental data, being widely adopted for fitting cumulative methane production [48–58]. In agreement with the VFAs profile (Section 3.1.1), reduction in lag time was observed when PS was reduced from 5 mm to 2 mm and 1 mm (Table 5), as a result of an increase in the degradation rate. Although, shorter lag times were observed with PS 2 mm, it did not necessarily culminate in the highest methane yield. Thus, it can be inferred, that combining a low PS (such as 1 mm and 2 mm) with a low ISR of 2:1 might not be suitable due to an increase in lag time. A similar effect was observed with the combination of high PS of 5 mm and a high ISR of 4:1. Overall, the lag time reduced from 7 days with 5 mm PS to as low as 0.1 day with PS reduction. Hence, the choice of PS and ISR could greatly impact the kinetic parameters for food waste anaerobic digestion.

The overall percentage biodegradability was highest at 1 mm PS and ratio 3:1. Based on the results shown in Table 5, it is possible to infer that PS reduction improves the anaerobic biodegradability of food waste and hence, the ability to better assess methane production under BMP test conditions [27]. also reported similar high percentage degradability (≥100%) for organic fraction of municipal solid waste.

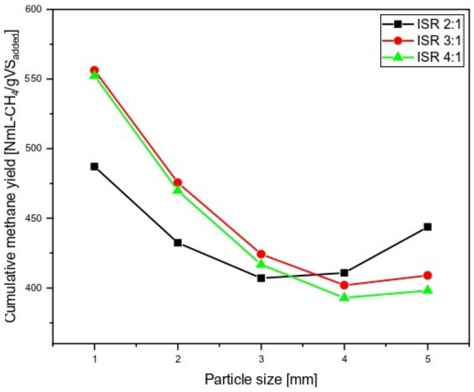


Fig. 4. Interaction plot for cumulative methane yield versus food waste PS at different ISR.

3.1.5. Statistical analysis

A response surface regression was conducted for the cumulative methane yield versus the ISR using obtained yields from the 3^2 factorial DOE ($n = 18$) to establish Equations (2)–(4) (where $P = PS$). These equations were then used to predict the cumulative methane yields at PS 3 mm and 4 mm shown in Fig. 4.

$$\textit{Cumulative methane yield at ISR } 2:1 = p^2 - 6.74p + 39.08 \tag{2}$$

$$\textit{Cumulative methane yield at ISR } 3:1 = P^2 - 8.52p + 45.60 \tag{3}$$

$$\textit{Cumulative methane yield at ISR } 4:1 = P^2 - 8.46p + 45.44 \tag{4}$$

Fig. 4 further demonstrates that increase in methane yield is inversely proportional to increase in PS at all tested ISR. The ISR of 3:1 enriched higher biomethane yield (especially at lower PS) than 2:1 and 4:1; the reason being a relatively balanced fraction of substrate to acting microbial load, which enabled non-inhibitory

Table 5
Particle Size (PS) and Inoculum-to-Substrate ratio (ISR) influence on process kinetics and biodegradability.

PS	ISR	k-value (Day^{-1})	R^2	Lag phase (Day)	Theoretical methane potential ($NmLCH_4$ $gVS^{-1}{}_{FW}$)	Experimental yield ($NmLCH_4$ $gVS^{-1}{}_{FW}$)	Percentage biodegradability (%)
1 mm	2:1	0.27	0.99	3.5	515.65	514.63	99.8
	3:1	0.43	0.99	0.2	515.65	542.79	105.3
	4:1	0.40	0.98	0.4	515.65	538.33	104.4
2 mm	2:1	0.33	0.99	0.9	483.91	395.73	81.8
	3:1	0.53	0.99	0.1	483.91	493.84	102.1
	4:1	0.74	0.99	0.1	483.91	488.47	100.9
5 mm	2:1	0.25	0.98	5.8	547.90	452.89	82.7
	3:1	0.39	0.99	6.3	547.90	404.72	73.9
	4:1	0.46	0.99[a]	7.0	547.90	393.42	71.8

VFA production and consumption trend. It is established here that PS pre-treatment was the more influencing factor on the methane yield obtained.

3.2. Experiment 2: Influence of trace elements concentration towards methane production

3.2.1. Food waste and inoculum contribution towards trace element content

According to Ref. [59]; the concentration and presence/absence of trace elements in food waste is a consequence of various factors, including environmental aspects, such as nutrient availability in soil. Therefore, for a better means of comparison, the trace elements present in CFW were juxtaposed to food waste samples from across the UK. Nevertheless, the values were significantly lower, and could be a result of the metal analysis methodology and/or sample composition, amongst other factors.

The inoculum used for this experiment showed values for most metals below range of those reported elsewhere in the literature for seeds treating food waste (Table 6). The trace element content from the inoculum is a relevant information, since it can sometimes counter-balance the lack of nutrients presents on food waste, thus stabilising the anaerobic digestion process [60]. Based on the recommended concentrations of the trace metals for anaerobic biomass by Ref. [17]; it is clearly seen from Table 6 that the CFW sample would not provide enough nutrient content on its own for the biomass, even with the inoculum contribution, corroborating that the sample could benefit from nutrient media supplementation.

Therefore, the amount of trace elements to be added was determined by the combination of different metal mixtures [23–26] as an attempt to supply the biomass with all the necessary nutrients for the stable anaerobic digestion process. Differently from previous studies in the literature, there was no individual metal concentration value calculation to meet the specific requirements of the studied food waste sample.

3.2.2. Process stability and methane yield in the batch trial under media supplementation

The nutrient media supplemented reactor exhibited a more stable anaerobic digestion of food waste when compared to the control (no media supplementation) (Fig. 5a). The absence of sharp pH drops because of no VFAs accumulation during fermentation (expected to be intensified on the first week of digestion), demonstrates the possible benefit of nutrient supplementation. As opposed to the control where an uncoupling between production and consumption of VFAs occurred, resulting in its accumulation and simultaneous pH drop between day 4 and 7. The control behaviour was already anticipated, as the single stage anaerobic digestion performance of food waste is usually reported as unsuccessful, mainly due to the rapid consumption of the labile fraction of the waste, which ultimately leads to the described scenario [8].

[63] treated food waste on a single-stage mesophilic anaerobic digestion and demonstrated that when supplemented with Co, Fe, Mo and Ni, the digestion became more stable in terms of pH values and lower VFAs levels when compared to the control, suggesting that these nutrients have an important role for improving methanogens and the overall process performance. Similarly, in this study, the total VFAs levels were also higher for the control than for the supplemented reactor between day 4 and 7, where a concentration of 2,101.4 mg L^{-1} was observed as opposed to only 548.7 mg L^{-1} for the same period in the nutrient treated reactor. This substantiates the rapid consumption of the readily degradable fraction of food waste faster in a nutrient balanced digestion, as well as the maintenance of a lower concentration levels of VFAs by the presence of certain metals.

As previously mentioned, P/A ratio can be used as a tool for detecting digestion imbalance, with values above 1.4 suggesting digester failure [64]. On the fourteenth day of experiment the control showed a P/A of 4.6 (Fig. 5b). Conversely, the reactor supplied with nutrient media did not show any P/A values above 1.4 throughout the digestion period (Fig. 5b). According to Ref. [34]; when the digestion of food waste is nutrient-sufficient, the propionic acid degradation rate is constant and therefore, there is no VFAs/propionic acid accumulation. On the contrary, under insufficient amounts Ni, Co and Fe, the anaerobic digestion becomes unstable, thus more susceptible to failures. Additionally [18], concluded that Se and Mo and W are essential when performing batch trials of mesophilic anaerobic digestion on food waste, improving the acetic and propionic acid degradation respectively.

It is clearly seen from the results that although the composite food waste sample and the inoculum used in this study did not provide enough concentration of nutrients for the anaerobic biomass, the trace elements supplementation in a form of a predetermined media, containing amongst other elements: Co, Mo, Fe and Ni counterbalanced the lack of nutrients. This offered protection against VFAs accumulation/propionic acid build-up, hence, avoiding a likely esteemed digestion failure.

The cumulative methane yields for the reactors with and without the influence of nutrient media supplementation is depicted in Fig. 6. Notably, the control exhibited higher methane yield (544.6 NmL $gVS^{-1}{}_{added}$) compared with the supplemented reactor (490.5 NmL $gVS^{-1}{}_{added}$). However, methane production rate differed significantly between them, with the nutrient media

Table 6
Trace elements on CFW, inoculum, nutrient media and recommended values for anaerobic biomass*.

Element/Reference	Co mg/KgTS	Fe mg/KgTS	Ni mg/KgTS	Mn mg/KgTS	Mo mg/KgTS	Se mg/KgTS	W mg/KgTS
Food Waste (Composite Sample)							
Ludlow, UK 2015$^{(a)}$	0.1	89	n.a.	92	0.37	0.17	n.a.
Ludlow, UK 1998$^{(b)}$	>0.25	229	n.a.	85 (14)	0.46 (0.05)	>0.30	n.a.
Luton, UK$^{(b)}$	0.07 (0.01)	148 (1)	n.a.	97.7 (1.6)	1.1 (0.2)	1.2 (0.6)	n.a.
Hackney, UK$^{(b)}$	0.35 (0.19)	175 (58)	n.a.	94.5 (4.1)	1.2 (0.2)	0.4 (0.3)	n.a.
Present Study	0.030 (0.005)	4.2 (0.6)	0.20 (0.03)	0.60 (0.08)	0.030 (0.004)	0.4 (0.1)	0.005 (0.001)
Inoculum							
[17]	2.9	n.a	24.2	n.a	4	<1	2.7
[18]	0.083	n.a	2.9	n.a	0.29	0.050	<0.035
Present Study	0.003	n.a	0.01	n.a	0	0.03	0.002
Recommended – Anaerobic Biomass							
[17]	9	–	11	–	7	1.5	<0.1

(a) [61]; (b) [62].
*Figures are reported as mean values with standard deviation in brackets, where available.
n.a. - not analysed.

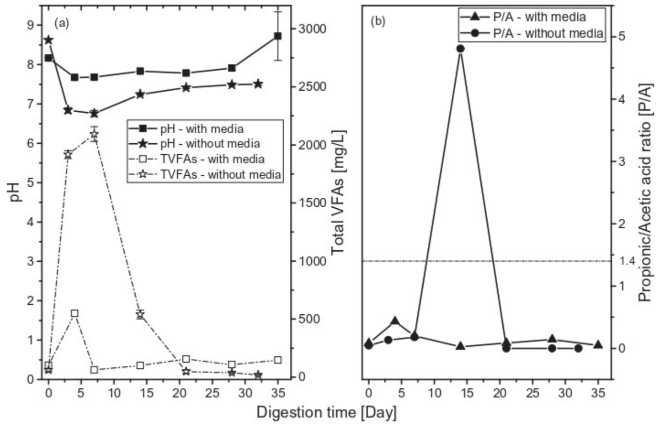


Fig. 5. a) Total VFAs concentration and pH for the nutrient media supplemented reactor and control (no nutrient media supplementation); b) Propionic to Acetic Ratio for the nutrient media supplemented reactor and control.

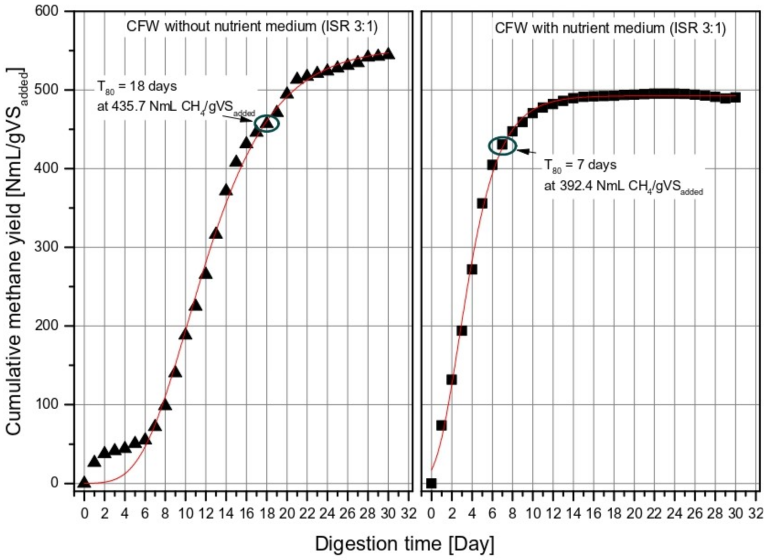


Fig. 6. Cumulative Methane yield (dotted points) with Modified Gompertz fitting (red line) for CFW with and without media supplementation, showing the influence of nutrient media on attaining T80.

supplemented reactor presenting a much faster rate than the reactor without media on the first days of anaerobic process. This behaviour was already expected, as the VFAs accumulation between the 4 – 7th days of digestion negatively influenced methane production for the same period in the reactor without media. For this reason, methane production was hindered, only significantly increasing from the 8th day of digestion, as opposed to the media supplemented reactor, in which the first week was the most relevant period for methane generation. The improved process performance in this case is also confirmed by the technical digestion time (T80), which corresponds to the period (in days) taken by the digestion process to achieve 80% of the cumulative yield [56]. The nutrient supplemented reactor, reached the T80 at the 7th day of digestion, as opposed to the reactor without media, which only

Table 7
Kinetics for experiment 2.

Sample	ISR	k-value (Day^{-1})	R^2	Lag phase (Day)	Theoretical potential ($NmLCH_4$ $gVS^{-1}{}_{FW}$)	Experimental yield ($NmLCH_4$ $gVS^{-1}{}_{FW}$)	Percentage biodegradability (%)
Nutrient media supplemented	3:1	0.45	0.998	0.5	588.78	490.48	83.3
Control	3:1	0.22	0.994	5.6	588.78	544.62	92.5

reached at the 18th day, representing a 2.57 times faster rate, when the process is under nutrient-sufficient conditions.

The observed delay of methane production for the control at the first week of digestion was also reflected on the lag phase, which was 11.58 times longer than for the nutrient enriched reactor; once more validating the better performance of the anaerobic digestion of food waste on the first week when nutrient media was added (Table 7). Additionally, the process kinetics for the control was also negatively affected, exhibiting a k-value of 0.215, equivalent 2.10 times lower than the nutrient enriched reactor.

Biodegradability rate of the different conditions were analysed according to Ref. [65]. As it can be seen from Table 7, biodegradability was not related to the process stability, but to its performance (methane yield). Therefore, the reactor without media showed the highest percentage biodegradability than the nutrient enriched reactor, meaning that the experimental values obtained by the BMP test were closer to the theoretical methane values obtained by Buswell equation [30].

4. Conclusions

The results presented in this paper suggests that PS reduction improved the anaerobic degradability of food waste, which consequently improved the assessment of methane production under BMP test conditions. Although, excessive food waste PS reduction increases the tendency for VFAs build-up, this was overcome by a proper selection of ISR, thus, stabilising the digestion process and avoiding this common finding when anaerobically digesting food waste as a sole substrate, in a single-stage process.

For smaller PS of 1 mm and 2 mm, a combination with an ISR of 3:1 and 4:1 helped to stabilise the systems, while with larger PS of 5 mm, an ISR of 2:1 was most suitable. Consequently, lower lag times were observed at ISR of 3:1 and 4:1 for 1 mm and 2 mm PS treatments and at ISR of 2:1 for 5 mm PS respectively. In general, for PS $\leq$ 3 mm the highest methane yield was obtainable at ISR of 3:1, while for PS $\geq$ 3 mm, the highest methane yield was obtainable at ISR 2:1. As a result of improved degradability and a balanced PS and ISR combination, an overall methane increase of 38% was obtained with a PS reduction from 5 mm to 1 mm, which corresponds to a potential rise in the energy output from 7,426 kWh $tonne^{-1}$ to 10,660 kWh $tonne^{-1}$.

Differently from the combined PS reduction and ISR effects, which heralded a positive effect on the final methane yield of food waste, nutrient media supplementation did not enhance the ultimate methane yield. On the other hand, it was found that its application helped to stabilise food waste digestion process by avoiding: a) VFAs accumulation and high P/A ratio and b) reducing the lag time (8.9% less time needed), thus strongly suggesting that nutrient media supplementation could significantly reduce the hydraulic retention time (HRT) of food waste anaerobic digestion, thus increasing the throughput and biomethane recovery.

Further investigation needs to be done on the bioavailability of essential nutrients such as Ni, Co, Mo, Se, W, Fe and Mn during the digestion process of food waste, hence, enabling a better understanding of these nutrients utilisation in batch systems, offering a possibility for further adjustments and improvement of the media here tested.

As documented by this study, there is not a clear winner strategy for methane yield enhancement from food waste as a sole substrate in AD. All the applied methods (PS, ISR and nutrient media), have benefits, and costs related to energy input that need to be estimated for large scale operational systems. However, the authors believe that the findings here discussed could benefit the AD industry by emphasising the importance of better testing conditions and combining already existing methods to try to maximise this sector efficiency.

Acknowledgements

This research was supported by the University of Leeds, through the Leeds International Research Scholarship and the Brazilian government through CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

References

[1] WRAP, Household Food Waste in the UK, Final Report, WRAP, Banbury, UK, 2015.
[2] R. Zhang, H. Elmashad, K. Hartman, F. Wang, G. Liu, C. Choate, P. Gamble, Characterization of food waste as feedstock for anaerobic digestion, Bioresour. Technol. 98 (4) (2007) 929–935.
[3] T. Pullen, Anaerobic Digestion : Making Biogas, Making Energy, Routledge, New York, 2015.
[4] REA [Online], The Authorative Annual Report on the UK Energy Sector, vol. 2, Renewable Energy Association, 2017, pp. 0–1, 2, https://www.mendeley.com/viewer/?fileId=7ef5375f-b025-6411-6b15-48f4543dd9d4&documentId=5e16bc10-7314-340d-998b-9648b361ad9b.
[5] WRAP, Household Food and Drink Waste in the United Kingdom, WRAP, Banbury, UK, 2012.
[6] L. Appels, A.V. Assche, K. Willems, J. Dègreve, J.V. Impe, R. Dewil, Peracetic Acid Oxidation as an alternative pre-treatment for anaerobic digestion of waste activate sludge, Bioresour. Technol. 102 (2) (2011) 4124–4130.
[7] C. Zhang, H. Su, J. Baeyens, T. Tan, Reviewing the anaerobic digestion of food waste for biogas production, Renew. Sustain. Energy Rev. 38 (2014) 383–392, https://doi.org/10.1016/j.rser.2014.05.038 [Online].
[8] J. Ma, T.H. Duong, M. Smits, W. Verstraete, M. Carballa, Enhanced biomethanation of kitchen waste by different pre-treatments, Bioresour. Technol. 102 (2011) 592–599.
[9] H.B. Gonzales, K. Takyu, H. Sakashita, Y. Nakano, W. Nishijima, M. Okada, Biological solubilization and mineralization as novel approach for the pretreatment of food waste, Chemosphere 58 (2005) 57–63, https://doi.org/10.1016/j.chemosphere.2004.08.092.
[10] H. Shahriari, M. Warith, M. Hamoda, K. Kennedy, Evaluation of single vs. staged mesophilic anaerobic digestion of kitchen waste with and without microwave pretreatment, J. Environ. Manag. 125 (2013) 74–84.
[11] A. Mshandete, L. Björnsson, A.K. Kivaisi, M.S.T. Rubindamayugi, B. Mattiasson, Effect of particle size on biogas yield from sisal fibre waste, Renew. Energy 31 (14) (2006) 2385–2392.
[12] K. Izumi, Y. Okishio, ki, N. Nagao, C. Niwa, S. Yamamoto, T. Toda, Effects of particle size on anaerobic digestion of food waste, Int. Biodeterior. Biodegrad. 64 (7) (2010) 601–608, https://doi.org/10.1016/j.ibiod.2010.06.013 [Online].
[13] I.S. Kim, D.H. Kim, S.H. Hyun, Effect of particle size and sodium ion concentration on anaerobic thermophilic food waste digestion, Water Sci. Technol. 41 (3) (2000) 67–73.
[14] L. Neves, R. Oliveira, M.M. Alves, Influence of inoculum activity on the biomethanization of a kitchen waste under different waste/inoculum ratios, Process Biochem. 39 (12) (2004) 2019–2024.
[15] W.S. Lopes, V.D. Leite, S. Prasad, Influence of inoculum on performance of anaerobic reactors for treating municipal solid waste, Bioresour. Technol. 94 (2004), 261–26.
[16] E. Tampio, S. Ervasti, T. Paavola, S. Heaven, C. Banks, J. Rintala, Anaerobic digestion of autoclaved and untreated food waste, Waste Manag. 34 (2014) 370–377.

[17] V. Facchin, C. Cavinato, F. Fatone, P. Pavan, F. Cecchi, D. Bolzonella, Effect of trace element supplementation on the mesophilic anaerobic digestion of food waste in batch trials: the influence of inoculums origin, Biochem. Eng. J. 70 (2013) 71–77.

[18] C.J. Banks, Y. Zhang, Y. Jiang, S. Heaven, Trace elements requirement for stable food waste digestion at elevated ammonia concentrations, Bioresour. Technol. 104 (2012) 127–135.

[19] M. Climenhaga, C. Banks, Anaerobic digestion of catering wastes: effect of micronutrients and retention time, Water Sci. Technol. 57 (2008) 687–692.

[20] H.M. El-Mashad, J.A. McGarvey, R. Zhang, Performance and microbial analysis of anaerobic digesters treating food waste and dairy manure, Biol. Eng. 1 (2008) 233–242.

[21] M. Lee, T. Hidaka, W. Hagiwara, H. Tsuno, Comparative performance and microbial diversity of hyperthermophilic and thermophilic co-digestion of kitchen garbage and excess sludge, Bioresour. Technol. 100 (2009) 578–585.

[22] J.D. Browne, J.D. Murphy, Assessment of the resource associated with biomethane from food waste, Appl. Energy 104 (2013) 170–177.

[23] O.A. Adewale, Enhancing Methane Production in the UK WWTP via Co-Digestion of Micro-algae and Sewage Sludge, PhD thesis, Leeds University. School of Civil Engineering, 2014, p. 218.

[24] I. Angelidaki, M. Alves, D. Bolzonella, L. Borzacconi, J.L. Campos, A.J. Guwy, S. Kalyuzhnyi, P. Jenicek, J.B. Van Lier, Defining the biomethane potential (BMP) of solid organic waste and energy crops: a proposed protocol for batch assays, Water Sci. Technol. 59 (5) (2009) 927–934.

[25] W.F. Owen, D.C. Stuckey, J.B. Healy, L.Y. Young, P.L. McCarty, Biossay for monitoring biochemical methane potential and anaerobic toxicity, Water Res. 13 (6) (1979) 485–492.

[26] J. Kim, C. Park, T. Kim, M. Lee, S. Kim, S. Kim, J. Lee, Effects of various pretreatments for enhanced anaerobic digestion with waste activated sludge, Issue Series Title: J. Biosci. Bioeng. 95 (3) (2003) 271–275.

[27] A. Nielfa, R. Cano, M. Fdz-Polanco, Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge, Biotechnology Reports 5 (1) (2015) 14–21.

[28] APHA, in: A.D. Eaton, M.A.H. Franson (Eds.), Standard Methods for the Examination of Water and Wastewater 21st, American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC, 2005.

[29] AOAC, AOAC official method 2015.01 heavy metals in food: inductively coupled plasma–mass Spectrometry first action 2015 [Online], J. AOAC Int. 96 (4) (2013), 704–704, http://www.ingentaconnect.com/content/10.5740/jaoac.int.2012.007.

[30] A.M. Buswell, H.F. Muller, Mechanism of methane fermentation, Ind. Eng. Chem. 44 (3) (1952) 550–552.

[31] M.H. Zwietering, I. Jongenburger, F.M. Rombouts, K. Van't Riet, Modeling of the bacterial growth curve, Appl. Environ. Microbiol. 56 (6) (1990) 1875–1881.

[32] L. Zhang, Y.W. Lee, D. Jahng, Anaerobic co-digestion of food waste and piggery wastewater: focusing on the role of trace elements, Bioresour. Technol. 102 (2011) 5048–5059, https://doi.org/10.1016/j.biortech.2011.01.082.

[33] C. Zhang, H. Su, T. Tan, Batch and semi-continuous anaerobic digestion of food waste in a dual solid-liquid system, Bioresour. Technol. 145 (2013) 10–16, https://doi.org/10.1016/j.biortech.2013.03.030 [Online].

[34] H. Qiang, Q. Niu, Y. Chi, Y. Li, Trace metals requirements for continuous thermophilic methane fermentation of high-solid food waste, Chem. Eng. J. 222 (2013) 330–336.

[35] J. De Vrieze, L. De Lathouwer, W. Verstraete, N. Boon, High-rate iron-rich activated sludge as stabilizing agent for the anaerobic digestion of kitchen waste, Water Res. 47 (11) (2013) 3732–3741, https://doi.org/10.1016/j.watres.2013.04.020 [Online].

[36] Defra, Optimising Inputs and Outputs from Anaerobic Digestion Processes, 2010.

[37] K. Paritosh, S.K. Kushwaha, M. Yadav, N. Pareek, A. Chawade, V. Vivekanand, Food waste to energy: an overview of sustainable approaches for food waste management and nutrient recycling, BioMed Res. Int. 2017 (2017).

[38] X. Wang, X. Lu, F. Li, G. Yang, Effects of temperature and Carbon-Nitrogen (C/N) ratio on the performance of anaerobic co-digestion of dairy manure, chicken manure and rice straw: focusing on ammonia inhibition, PLoS One 9 (5) (2014) 1–7.

[39] W. Zhang, S. Wu, J. Guo, J. Zhou, R. Dong, Performance and kinetic evaluation of semi-continuously fed anaerobic digesters treating food waste: role of trace elements, Bioresour. Technol. 178 (2015) 297–305, https://doi.org/10.1016/j.biortech.2014.08.046 [Online].

[40] B. Puyuelo, S. Ponsá, T. Gea, A. Sánchez, Determining C/N ratios for typical organic wastes using biodegradable fractions, Chemosphere 85 (2011) 653–659.

[41] M. Carlsson, A. Lagerkvist, F. Morgan-Sagastume, The effects of substrate pre-treatment on anaerobic digestion systems: a review, Waste Manag. 32 (9) (2012) 1634–1650, https://doi.org/10.1016/j.wasman.2012.04.016 [Online].

[42] N. Buyukkamaci, A. Filibeli, Volatile fatty acid formation in an anaerobic hybrid reactor, Process Biochem. 39 (2004) 1491–1494.

[43] U. Marchaim, C. Krause, Propionic to acetic acid ratios in overloaded anaerobic digestion, Bioresour. Technol. 43 (3) (1993) 195–203.

[44] T.P.T. Pham, R. Kaushik, G.K. Parshetti, R. Mahmood, R. Balasubramanian, Food waste-to-energy conversion technologies: current status and future directions, Waste Manag. 38 (1) (2015) 399–408, https://doi.org/10.1016/j.wasman.2014.12.004 [Online].

[45] F. Raposo, C.J. Banks, I. Siegert, S. Heaven, R. Borja, Influence of inoculum to substrate ratio on the biochemical methane potential of maize in batch tests, Process Biochem. 41 (6) (2006) 1444–1450.

[46] N. Scarlat, J.F. Dallemand, F. Fahl, Biogas: developments and perspectives in Europe, Renew. Energy 129 (2018) 457–472, https://doi.org/10.1016/j.renene.2018.03.006 [Online].

[47] Y. Zhou, Z. Zhang, T. Nakamoto, Y. Li, Y. Yang, M. Utsumi, N. Sugiura, Influence of substrate-to-inoculum ratio on the batch anaerobic digestion of bean curd refuse-okara under mesophilic conditions, Biomass Bioenergy 35 (7) (2011) 3251–3256, https://doi.org/10.1016/j.biombioe.2011.04.002 [Online].

[48] X. Kong, S. Xu, J. Liu, H. Li, K. Zhao, L. He, Enhancing anaerobic digestion of high-pressure extruded food waste by inoculum optimization, J. Environ. Manag. 166 (2016) 31–37, https://doi.org/10.1016/j.jenvman.2015.10.002 [Online].

[49] F.M. Pellera, E. Gidarakos, Effect of substrate to inoculum ratio and inoculum type on the biochemical methane potential of solid agroindustrial waste, Journal of Environmental Chemical Engineering 4 (3) (2016) 3217–3229, https://doi.org/10.1016/j.jece.2016.05.026 [Online].

[50] F. Lü, Q. Zhou, D. Wu, T. Wang, L. Shao, P. He, Dewaterability of anaerobic digestate from food waste: relationship with extracellular polymeric substances, Chem. Eng. J. 262 (2015) 932–938, https://doi.org/10.1016/j.cej.2014.10.051 [Online].

[51] V. Moset, N. Al-zohairi, H.B. MØller, The impact of inoculum source, inoculum to substrate ratio and sample preservation on methane potential from different substrates, Biomass Bioenergy 83 (2015) 474–482, https://doi.org/10.1016/j.biombioe.2015.10.018 [Online].

[52] A. Mottet, F. Habouzit, J.P. Steyer, Anaerobic digestion of marine microalgae in different salinity levels, Bioresour. Technol. 158 (2014) 300–306, https://doi.org/10.1016/j.biortech.2014.02.055 [Online].

[53] J. Pan, X. Chen, K. Sheng, Y. Yu, Effect of ammonia on biohydrogen production from food waste via anaerobic fermentation, Int. J. Hydrogen Energy 38 (29) (2013) 12747–12754, https://doi.org/10.1016/j.ijhydene.2013.06.093 [Online].

[54] D.M. Wall, P.O. Kiely, J.D. Murphy, The potential for biomethane from grass and slurry to satisfy renewable energy targets, Bioresour. Technol. 149 (2013) 425–431, https://doi.org/10.1016/j.biortech.2013.09.094 [Online].

[55] A. Boulanger, E. Pinet, M. Bouix, T. Bouchez, A.A. Mansour, Effect of inoculum to substrate ratio (I/S) on municipal solid waste anaerobic degradation kinetics and potential, Waste Manag. 32 (12) (2012) 2258–2265, https://doi.org/10.1016/j.wasman.2012.07.024 [Online].

[56] S. Xie, P.G. Lawlor, J.P. Frost, Z. Hu, X. Zhan, Effect of pig manure to grass silage ratio on methane production in batch anaerobic co-digestion of concentrated pig manure and grass silage, Bioresour. Technol. 102 (10) (2011) 5728–5733.

[57] H.S. Shin, J.H. Youn, S.H. Kim, Hydrogen production from food waste in anaerobic mesophilic and thermophilic acidogenesis, Int. J. Hydrogen Energy 29 (13) (2004) 1355–1363.

[58] V. Hong, R.E. Wrolstad, Use of HPLC separation/photodiode array detection for characterization of anthocyanins, J. Agric. Food Chem. 38 (3) (1990) 708–715 [Online], http://pubs.acs.org/doi/abs/10.1021/jf00093a026.

[59] C. Reilly, Metal Contamination of Food, its Significance for Food Quality and Human Health, Blackwell Science Ltd, 2002, ISBN 0-632-05927-3, p. 266.

[60] H. Qiang, D.L. Lang, Y. Li, High -solid mesophilic methane fermentation of food waste with emphasis on Iron, Cobalt, and Nickel requirements, Bioresour. Technol. 103 (1) (2012) 21–27.

[61] C. Yirong, S. Heaven, C.J. Banks, Effect of Trace Element Addition Strategy on Volatile Fatty Acid Accumulation in Thermophilic Anaerobic Digestion of Food Waste, vol. 6, Waste Biomass Valor, 2015, pp. 1–12.

[62] K.H. Hansen, I. Angelidaki, B.K. Ahring, Anaerobic digestion of swine manure: inhibition by ammonia, Water Res. 32 (1998) 5–12.

[63] L. Zhang, D. Jahng, Long-term anaerobic digestion of food waste stabilized by trace elements, Waste Manag. 32 (8) (2012) 1509–1515.

[64] D.T. Hill, S.A. Cobb, J.P. Bolte, Using volatile fatty acid relationships to predict anaerobic digester failure, Trans. ASAE (Am. Soc. Agric. Eng.) 30 (2) (1987) 496–501.

[65] R.E. Speece, Anaerobic Biotechnology and Odor/Corrosion Control, Archea Press, Nashville, TN, 2008.

B.

ÁREA TEMÁTICA: 3 (Reciclagem)

INFLUÊNCIA DO PRETRATAMENTO POR MICROONDAS NA PRODUÇÃO DE METANO ADVINDOS DE RESÍDUOS ALIMENTARES SOB DIGESTÃO ANAERÓBIA MESOFÍLICA

Mariana Vieira Turnell Suruagy[1] (cnmvts@leeds.ac.uk), Andrew Ross [1] (a.b.ross@leeds.ac.uk), Miller Alonso Camargo-Valero[2] (m.a.camargo-valero@leeds.ac.uk)

[1]BioResource Systems Research Group, School of Civil Engineering, University of Leeds, Leeds LS2 9JT, West Yorkshire, United Kingdom.
[1]School of Chemical and Process Engineering, University of Leeds, Leeds LS2 9JT, West Yorkshire, United Kingdom
[2]Departamento de Ingeniería Química, Universidad Nacional de Colombia, Campus La Nubia, Manizales, Colombia

RESUMO

O presente trabalho tem como objetivo discutir a eficácia do pré-tratamento térmico (por micro-ondas) de resíduos alimentares na produção final de metano através da digestão anaeróbica mesofílica 37°C (tipo batch). Temperaturas finais de 85, 115, 145 e 175°C foram utilizadas, com potência variando entre alta (7.8ramp), média (3.9 ramp) e baixa (1.9 ramp), e 5 minutos para a exposição das amostras à temperatura final desejada. Os resultados indicam que o pré-tratamento térmico dos resíduos alimentares do tipo irradiação micro-ondas foi eficaz na solubilização de material orgânico em todas as condições aqui testadas. Ademais, o pré-tratamento também se mostrou eficiente quanto ao aumento da intensidade da hidrólise para a maioria das condições aqui testadas, com a exceção de 175°C o qual independente da potência e, continuamente exibiu um pobre desempenho da digestão com o subsequente acúmulo de ácidos orgânicos voláteis e baixa produtividade e rendimento de metano, possivelmente relacionado à formação de compostos inibidores de bactérias metanogênicas, tais como fenóis e melanoidinas. Analises estatísticas One-Way ANOVA revelaram uma diferença significativa ($p<0.05$) entre as condições operacionais extremas: (85°C 7.8 °C/min e 175°C 1.9°C/min), sugerindo que em termos econômicos é mais viável a utilização de temperatura mais baixa combinada a um tempo de tratamento mais curto, economizando, portanto, energia necessária à hidrólise e concomitantemente, promovendo um aumento no rendimento do metano (42.37%).

Palavras-chave: Pré-tratamento por micro-ondas; Resíduos alimentares; Biometano.

INFLUENCE OF MICROWAVE PRETREATMENT ON THE BIOMETHANATION OF FOOD WASTE UNDER MESOPHILIC ANAEROBIC CONDITIONS

ABSTRACT

This paper discusses the effectiveness of microwave (MW) pre-treatment to enhance the anaerobic biodegradability of Food Waste, under mesophilic conditions (37°C). Microwave trials were conducted at 85, 115, 145 and 175°C, with ramp rates of 1.9, 3.9 and 7.8 °C/min and a holding time of 5 min. The results indicate that MW irradiation was effective in solubilising organic matter at all temperatures and ramp rates tested. The net hydrolysis rate and subsequent digestion were also improved for most ramp rates and final temperatures, with the exception of the tests conducted at 175 °C, which consistently exhibited a poor performance in the digestion process with accumulation of Total Volatile Fatty Acids (TVFAs) and low methane yields. These results indicate that under microwave temperatures of 175 °C, biomethane formation is negatively affected, possibly due to the formation of methanogenic inhibitory compounds such as melanoids and phenols. One–way ANOVA statistical analysis suggests that there was a significant difference ($p<0.05$) between the two extreme conditions tested (85 °C, 7.8 °C/min and 175°C, 1.9 °C/min). For this reason, it is suggested that in order to improve methane yields in the anaerobic digestion of food waste, microwave pre-treatment should be performed at lower temperature (85°C) with a short exposure time (through a fast heating rate, 7.8 °C/min), thus lowering energy requirements for the hydrolysis process and promoting higher methane yields (42.37% increase).

Keywords: Microwave pretreatment, Food Waste, Biomethane.

1. INTRODUCTION

As of 2011, the world generated an estimated 2 billion tonnes of municipal solid waste (AMOO and FAGBENLE, 2013). Worryingly, the production of MSW is expected to rise, reaching 9.5 billion tonnes by 2050 (FAO, 2009). As part of the municipal solid waste there is Food Waste (FW); a mixture of organic materials that can originate from the processing, sorting, cooking, preparation and handling of food (ARIUNBAATAR et al., 2015). Its composition varies widely across the globe since it is a result of various factors such as the market economy, climate, legislation, cultural differences, urbanization, supply chain technology as well as dietary habits (PARFITT et al., 2010). Nevertheless, this type of waste is characterized by a high moisture content and low pH, the former classifying it as an energy rich biodegradable residual (BENCH et al., 2005).

On the other hand, its high moisture content catalysis rapid decay under ambient conditions, thus making its management a challenging task. Conventional disposal methods such as landfilling is not desirable because of the fugitive methane (CH_4) emissions to the atmosphere; soil and groundwater pollution; production of toxic leachates, as well as the utilization of huge land areas; odour emanation and vermin attraction (SHIN et al., 2001, ZHANG and JAHNG, 2012). Moreover, according to USEPA (2006), landfilling practice has been associated with the largest human related source of fugitive methane, accounting for 34% of all methane anthropogenic emissions. This unsustainable practice has raised the interest around the globe for alternatives disposal technologies and the development of food- to- waste energy practices (ADHIRKARI and BARRIGTON, 2006).

Anaerobic Digestion (A.D) is a promising approach due to its capability of treating and stabilizing organic matter, as well as producing renewable energy in the form of biomethane (PULLEN, 2015), and hence it has been regarded as an attractive option for food waste management (VEEKEN and HAMELERS, 1999). Nevertheless, when treating this type of substrate, low methane yields (50-60% of the theoretical maximum value) are obtained. This is mainly related to the complex structure and composition of its components (lignocellulosic materials, fats and proteins), which make the hydrolysis of such complex molecules the limiting step for anaerobic processing (MARIN et al.,2010). That limitation can be circumvented by adopting different feedstock pre-treatments, including microwave (MW) irradiation.

MW irradiation can break apart weak hydrogen bonds and has the potential to make complex organic molecules unfold and become smaller, thus promoting a solubilisation effect and potentially making them more readily biodegradable. In fact, it has been identified as a promising option for the treatment of the various solid wastes (SHAHRIARI, 2011).

In this work, microwave irradiation for FW was investigated as a pre-treatment step to enhance anaerobic biodegradability and methane production under mesophilic conditions.

2. METHODOLOGY

2.1 Food Waste collection and processing

Food waste was collected from the Leeds University Refectory (Leeds, United Kingdom), over five consecutive days. Samples consisted of both plate waste (from the eating area) and kitchen waste, which were blended to prepared Composite Food Waste (CFW) samples. The collected samples were manually sorted to remove glass, paper, cardboard, plastic and bones.

Sorted food waste was thoroughly mixed, chopped and ground with a mincer. To allow further substrate size reduction and better homogenisation, the sample was blended with a food processer. During this process, no water was added, so that the moisture content would not be affected. In order to generate representative samples, the blended waste was mixed and divided into four sub-samples. Subsequently, smaller samples of 500 g were weighed into plastic bags, labelled and stored at -20 °C until required for the experiments. The food waste composition and characteristics are shown in Table 1 and Table 2, respectively.

Table 1. Composition of food waste used in this study

	Components
Food Waste	Tomato, chickpeas, cucumber, green peas, mushroom, carrot, fried and cooked potatoes, potato peel, rocket leaf, onions, broccoli, green beans, corn, red pepper, okra, bread, pizza, spaghetti, Yorkshire pudding, rice, fried and boiled eggs, bacon, beef, fish, chicken, sausages, minced meat, baked beans, butter.

Table 2. Characteristics of food waste used in this study

Parameter	Average Value (std)	Units
Total Solids (T.S)	334.50 (0.13)	g/kg
Volatile Solids (V.S)	314.9 (0.12)	g/kg
VS/TS	93.95 (0.01)	%(d.b.)
C/N	10.95	-
Lipid	27.62 (1.36)	%TS
Protein	24.31 (1.00)	%TS
Carbohydrate	42.75 (1.97)	%TS

2.2 Microwave Pre-treatment conditions

A laboratory scale STARTSYNTH® microwave equipment with quartz vessels was used for Food Waste pre-treatment. The equipment has a maximum power output of 2.4 KW, operating at 220V and a frequency of 50-60Hz. Each quartz vessel was filled with 2 g of food waste (composite samples sieved at 1mm particle size) and 5ml of distilled water; resulting in a 10% solids suspension. The selected particle size was chosen according to tests previously carried out. The addition of distilled water to the sample had the purpose of decreasing the solid contents from approximately 32% to 10%, thus transforming the sample from a thick paste into a fluid form.

Four final temperatures were chosen to be applied to the samples (85, 115, 145 and 175°C), in combination with three microwave intensities, which corresponded to slow, medium and fast heating rates or ramps (1.9, 3.9 and 7.8, C°/min, respectively). In order to achieve these conditions, a power of 1000W was applied, based on a previous study by ZHANG et al. (2016). The time required to reach the set temperature (T_{ramp}) was calculated using the corresponding heating rate (Equation 1). Once the final temperature was reached, samples were held at such temperature for 5 min (i.e., hold time - T_{hold}). All tested conditions are reported in Table 3.

Equation 1

$$t_{ramp} = \frac{\boldsymbol{FInal\ Temperature}}{\boldsymbol{Heating\ rate}\ \left(\frac{°C}{min}\right)}$$

Table 3. Microwave Operation conditions tested on food waste sample

Microwave Operational Conditions		**Final Temperatures** **85°C**	**115°C**	**145°C**	**175°C**
7.8 ramp	T_{ramp}	11min	15min	19min	22min
	T_{hold}	5min	5min	5min	5min
	T_{total}	**16min**	**20min**	**24min**	**27min**
3.9 ramp	T_{ramp}	22min	30min	38min	45min
	T_{hold}	5min	5min	5min	5min
	T_{total}	**27min**	**35min**	**43min**	**50min**
1.9 ramp	T_{ramp}	45min	61min	77min	92min
	T_{hold}	5min	5min	5min	5min
	T_{total}	**50min**	**66min**	**82min**	**97min**

2.3 Biochemical methane potential tests (BMP test)

In this study, BMP tests were conducted in batches using 500 ml Duran bottles, with 400 ml working volume, under mesophilic conditions (37 °C) in an automatic methane potential test system (AMPTS

II). The inoculum used in this study was obtained from a mesophilic anaerobic digester, treating sewage sludge at Esholt Wastewater Treatment Plant in Yorkshire, UK. Before each experimental set-up, the inoculum was passed through a 1mm sieve to remove any large particles or grit and then incubated at 37 °C. The adaptation of the seed with the raw food waste (untreated) occurred prior to the experiments, at a concentration of 0.34 g-VS/(L·day).

In order to determine the biomethane originating from the inoculum, blank reactors were prepared for each set of experiments, containing only inoculum and distilled water. The methane volume produced by the seed was further corrected from the volume produced by each reactor, giving the real methane production from the substrate. Moreover, controls were added to the experiment, so that the behaviour of the untreated substrate could be monitored. All the experiments were conducted in triplicates.

2.4 Monitoring Samples

Liquid samples from the mix were collected and analysed on day 0 and then on day 4 and 7. After this period, sampling was carried out once a week, until the last day of digestion when the digestate was also characterised. The total digestion period was 28 days, or whenever the daily methane production was less than 1% of the total cumulative methane volume (NIELFA et al., 2015). Ammonia and Total and Soluble Chemical Oxygen Demand were determined by using the HACH 39000 AP Robotic Water System and the corresponding cuvettes (LCK 302, APC 338, and LCK 514 respectively). Total solids - TS (Method 2540 B) and volatile solids - VS (2540 E) for the substrate and inoculum were examined according to standard methods (APHA, 2005).

Elemental analysis (Carbon and Nitrogen) was conducted using the FLASH 2000 Elemental Analyzer. Protein content was performed by determining organic nitrogen content (KJELDAHL method), and lipid content (Soxhlet extraction method at 40 – 60 °C, using petroleum spirit as solvent) (NIELSEN, 2010). Carbohydrate values were obtained by a differential method; deducting lipid, protein, ash and moisture content from the total weight of the samples. The solubilisation of each sample was calculated based on LOPEZ and ESPINOSA (2008). Origin-Pro® statistical package 18 was used to process the resulting methane yields from the tests conducted at different temperatures and ramps, by a (ANOVA) one- way statistical analysis.

3. RESULTS AND DISCUSSION

3.1 Effects of Microwave Irradiation on Food Waste Characterization

As illustrated in Table VI, the Total Chemical Oxygen Demand increased after microwave treatment in all studied conditions. The fast heating rate (7.8 ramp) revealed the higher TCOD solubilisation, with a 37.18 % increase when compared to the control. The medium heating rate in turn, showed a 28.32% increment on TCOD content, and the slow heating rate exhibited a more discreet increase of this parameter 26.79%. These results suggest that the exposure of food waste to microwave irradiation causes the solubilisation of recalcitrant compounds in the solution. Concomitantly, the Soluble Chemical Oxygen Demand (SCOD) also increased in all studied conditions after microwave irradiation exposure when compared to the control. Additionally, the results from Table 4 evidence that the greater organic matter increase effect from all tested conditions occurred at a higher temperature of 175°C combined with a medium and low heating rate, thus potentially favouring its application in Anaerobic Digestion.

Similar to the current study, SHAHRIARI (2011) also reported 175 °C as being the optimum temperature for increasing the solubilisation of organic matter content, when compared to treatments at 115 and 145°C. There is a straight correlation between solubilisation of organic matter (SCOD) and the final microwave temperature applied, and most importantly, with the total time of exposure to microwave treatment.

Table 4. Composite Food Waste Characteristics before (control) and after microwave irradiation

Heating Rate (°C/min)	Parameter/ Temperature	Samples (n)	Control (untreated)	85°C	115°C	145°C	175°C
7.8 ramp (Fast)	TCOD (g/Kg)	4	327.3 (0.05)	**449.00 (0.20)**	**449.64 (0.35)**	**449.51 (0.34)**	**449.16 (0.22)**
	SCOD (g/Kg)	4	228.3 (0.11)	240.79 (0.11)	275.50 (0.38)	276.86 (0.11)	313.43 (0.45)
	Ammonia (g/Kg)	6	13.36 (0.45)	**13.30 (0.21)**	**21.52 (0.06)**	**23.90 (0.22)**	**24.5 (0.30)**
	TVFAs (mg/L)	4	785.49 (2.65)	2381.0 (3.44)	2908.63 (4.02)	3018.0 (3.11')	3088.16 (4.44)
3.9ramp (Medium)	TCOD (g/Kg)	4	327.3 (0.05)	420.62 (0.96)	420.16 (2.65)	420.41 (2.12)	419.08 (1.15)
	SCOD (g/Kg)	4	228.3 (0.11)	262.33 (2.70)	271.58 (0.98)	314. 62 (4.95)	377.00 (0.60)
	Ammonia (g/Kg)	6	13.36 (0.45)	0.340 (5)	0.473 (2.5)	0.783 (2.10)	1.00 (3.5)
	TVFAs (mg/L)	4	785.49 (2.65)	**4887.34 (5)**	**5111.36 (4.69)**	**3967.53 (3.5)**	**3987.65 (4)**
1.9 ramp (Slow)	TCOD (g/Kg)	4	327.3 (0.05)	415.42 (0.80)	415.1 (0.05)	415.0 (0.07)	415.6 (0.09)
	SCOD (g/Kg)	4	228.3 (0.11)	238.5 (0.05)	**278.66 (0.98)**	**363.87 (1.25)**	**377. 31 (0.08)**
	Ammonia (g/Kg)	6	13.36 (0.45)	**0.157 (2.5)**	**0.166 (0.07)**	0.751 (0.09)	1.69 (0.03)
	TVFAs (mg/L)	4	785.49 (2.65)	2263.25 (3.36)	1265.81 (1.55)	3132.42 (2.5)	3286.49 (1.43)

*Standard deviations are shown in brackets

There was an increase in ammonium concentrations when samples were processed at the fastest heating rate (shorter exposition to MW irradiation), regardless the final temperature used. However, when food waste was exposed to longer periods of microwave treatment (medium and slow heating rates), there was a sharp drop on ammonium concentrations for all temperatures applied. One possible explanation is ammonia volatilization. LIN et al. (2008), have previously reported ammonia volatilization under thermal microwave treatment; however in their case, this was done intentionally and used as a mechanism to remove ammonia from wastewater. According to them, ammonium removal was the result of thermal and athermal effects. The thermal effect is the main mechanism from which ammonia volatilization occurs under MW irradiation. Both H_2O and NH_3 are polar molecules and are connected by intermolecular hydrogen bond: N-H..O and O-H…N, thus having the potential to be polarized by MW irradiation. A similar situation could have occurred during this study. This finding could be rather beneficial when anaerobically digesting food waste, especially in cases where feedstocks are rich in nitrogen compounds, since methanogenic activity can be inhibited by high concentrations of ammonia in the system.

At a fast heating rate, the TVFA concentration increased significantly in relation to the control and the increment was linked to the final temperature. TVFA values ranged from 2381.00 to 3088.16 mg/L, representing a 3.03 and 4.0 fold increase for the lowest and highest temperatures. For the medium heating rate, TVFA concentrations ranged from 3967.53 to 5111.36 mg/L compared to 785.49mg/L from the untreated samples (control). However, differently from the fast heating rate, no specific trend for the TVFAs concentration and temperature was observed. The maximum TVFAs increment occurred at 115 °C, where there was a 6.50 fold increase in relation to the raw waste. On the other hand, at 145 °C there was the lowest TVFAs improvement, with an equivalent to a 5.05 fold. Contrary from what was expected, the TVFAs concentrations of the slow heating rate did not increased in relation to the medium or fast heating rate. In this case, values ranged from 1265.81-

3286.89 mg/L and showed no direct relation with the final temperature applied. Samples treated at 175°C showed the highest increment on TVAS in relation to the controls with a 4.18-fold increase, and treated at 115ºC exhibited the lowest increment with 1.61 fold (it also exhibited the lowest VFAs increment for all tested microwave conditions).

These results contrast with previous findings by MARIN et al. (2010) and SHAHRIARI (2011). Both works reported that after microwaving the whole fraction of kitchen waste at 175 °C, there was a decrease of the TVFAs concentration in relation to the untreated sample for both fast (7.8 °C/min) and medium (3.9 °C/min) heating rates, with 47.87% and 61.18% reduction respectively. The exception happened when the waste was heated at a slow heating rate (1.9°C/min), thus implying that a longer exposition to MW irradiation resulted in a 19.56 % increase of the TVFAs content. The thermal transformation of organic matter into volatile fatty acids by this type of pre-treatment can be very promising for enhancing the anaerobic digestion process. The reasoning behind this statement is that by producing the "right" type of VFAs such as acetic acid, the main biomethane precursor (FENG et al., 2013), the AD process could possibly be improved. Regardless of the final temperature and heating rate, acetic acid was the main VFA produced after thermal treatment. The best microwave operational condition for acetic acid production was at medium heating rate (3.9 °C/min) and final temperature of 115°C.

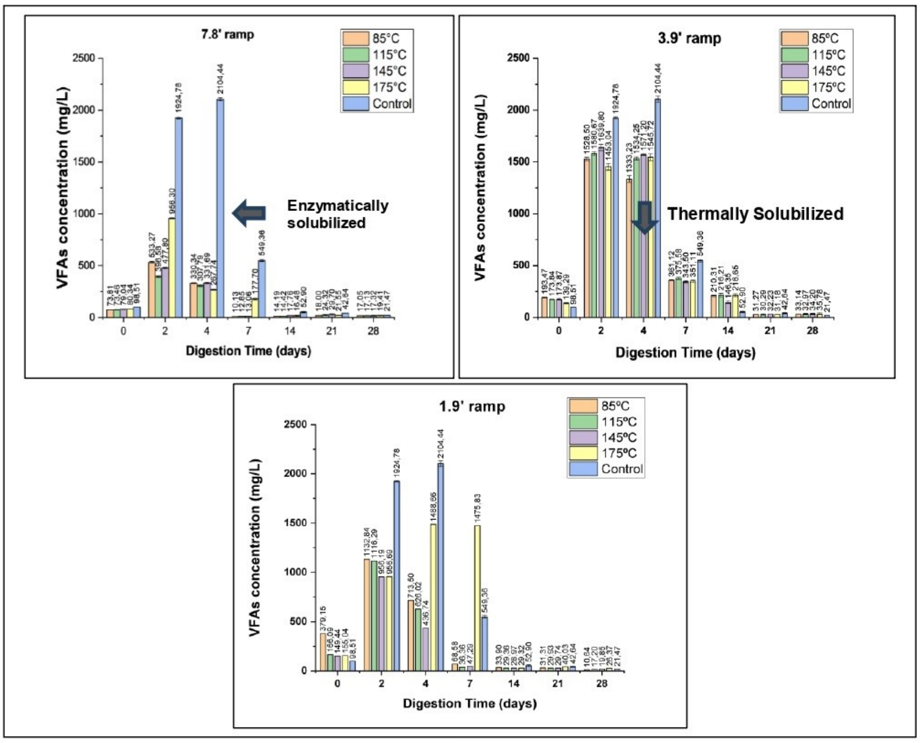
3.2 Effect of microwave treatment on mesophilic anaerobic digestion of Food Waste

3.2.1 Total Volatile Fatty Acids (TVFAs) profile and behaviour

For the untreated samples (control), the greatest TVFAs production occurred between days 2 and 4, period during which hydrolysis was intensified. During the hydrolysis step, the microbial enzymes break down complex molecules into smaller ones, which can be converted into intermediate products (VFAs) in the acidogenesis step (PULLEN, 2015). Therefore, there was an increase on TVFAs concentration equivalent to 19.53 fold on the second day and 21.36 (1924.778mg/L) fold on the fourth day (when VFAs concentration reached its maximum-2010.44mg/L) in relation to when the digestion process started (Figure 01). However, there was a clear kinetic unbalance between VFAs production (acetoclastic bacteria) and consumption (methanogenic bacteria) by the microbial community, thus leading to VFAs accumulation on the control samples between day 2-4 of the digestion period.

Similar to the controls, all the microwaved treated samples also showed an increment on TVFAs concentration on the second day of digestion. However, there was a more discreet TVFAs increment during the whole digestion period. This could have resulted from the thermal treatment breaking down the complex organic molecules (enhancing hydrolysis), thus offering the microorganisms a more readily biodegradable form of substrate, compared to the raw/untreated food waste on which organisms would still have to enzymatically solubilise the substrate. For this reason, VFAs could be consumed at a faster rate avoiding the typical uncoupling between methanogenic and acidogenic populations, which leads to VFAs accumulation on step-reactors treating food waste (MATAALVAREZ et al., 1992). The fast heating rate (7.8 °C/min) exhibited the greatest kinetic balance between TVFAs production (acidogenic bacteria) and consumption (Methanogenic bacteria) with a low concentration of intermediate products throughout the digestion period.

Figure 1. TVFAs concentration during the anaerobic digestion of microwaved food waste treated at different heating rate and temperatures

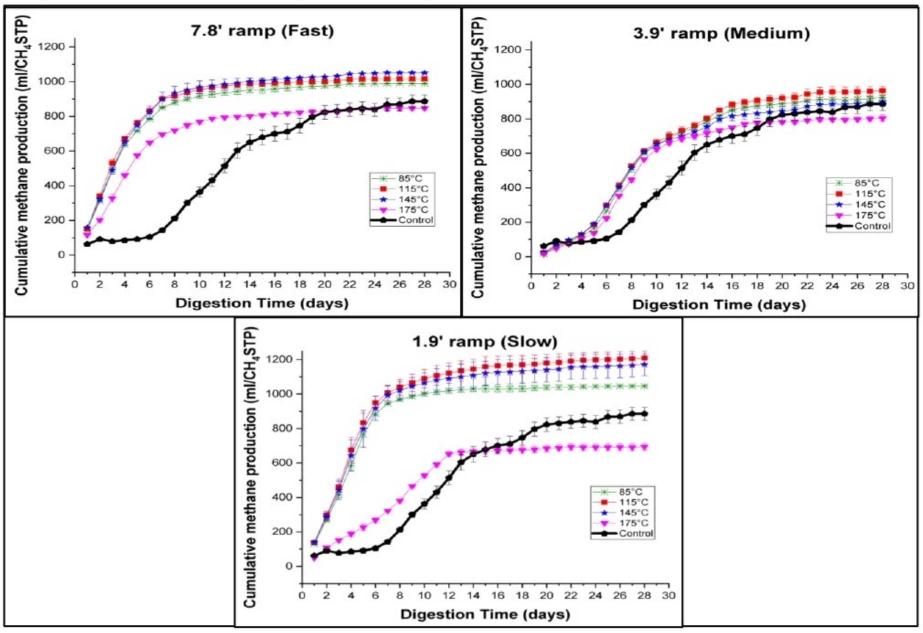


3.2.2 Technical Digestion Time

Microwave irradiation was proof to be also effective in enhancing the methane production from the mesophilic anaerobic digestion of food waste, especially during the first week of digestion (when the hydrolysis rate is greater). The improved process performance in this case can be confirmed by the technical digestion time (T80), which corresponds to the period (time in days) taken by the digestion process to achieve 80% of the maximum yield (XIE et al., 2011).

For the fast heating rate T80 occurred between day 6- 7 days of digestion for most conditions tested. On the other hand, for the medium heating rate (3.9 ramp), it occurred between 11- 13 days of digestion. Lastly, at a slow heating rate (1.9 ramp) this parameter was achieved between days 6-10 of digestion. As a result, for all tested conditions, the volume of methane produced during the first week of anaerobic digestion was greater than the one observed for the controls (T80 at 15 days of digestion), reducing by up to 60% the time required to produce 80% of the total methane (Figure 02). Nevertheless, 175°C regardless of the ramp tested showed a longer technical digestion time varying from 7-13 days.

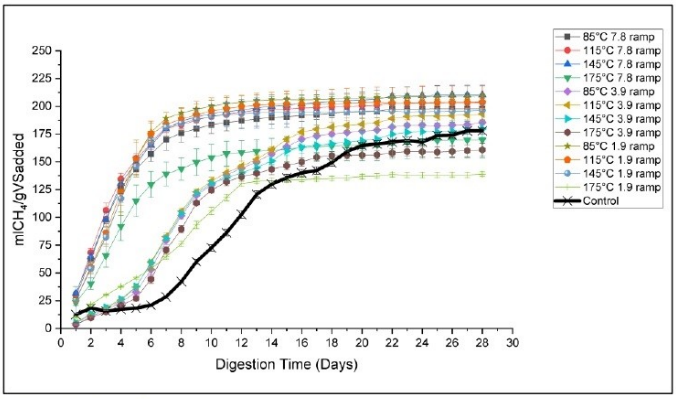
Figure 2. Normalized cumulative methane production (at standard temperature and pressure) of food waste samples treated at different heating rates and temperatures



3.3 Methane yield

Microwave pre-treatment of food waste was most of the times effective in enhancing the methane yield, compared to the controls (Figure 03). On the other hand, applying more heat (175 °C) and irradiation (lower power/ramp) consistently hindered the process performance, most likely due to the formation of hard to digest compounds (phenols, melanoidins) (SHAHRIARI, 2011).

Figure 3. Methane yield from food waste pretreated with microwave irradiation at different temperatures and heating rates



4. CONCLUSIONS

Microwave irradiation promoted some important changes on the food waste parameters compared to the control. One of them was a significant loss of ammonia for most tested conditions in relation to the untreated samples ranging from 92.51- 98.82% (highest loss at 175ºC ,3.9 ramp). Additionally, acetic acid (main methane precursor) was also produced in all tested conditions (115°C, 3.9 ramp showed the highest production). However, these changes did not necessarily imply in a higher methane yield, or better process performance.

Microwave pretreatment of food waste under the final temperatures of 85, 115 and 145°C at different heating rates (fast, medium and slow) was proved to be effective in improving the anaerobic digestion process, by enhancing TVFAS production x consumption rate, methane production during the hydrolysis phase (12.7- 57% more compared to the control), and the methane yield.

On the other hand, the 175°C treated samples regardless of the heating rate consistently showed a poor process performance duration compared to the other temperatures here studied. Although higher temperature promoted higher solubilization (SCOD concentrations), it did not necessarily yield the best hydrolysis rate methane yield, possibly due to the formation of hard to digest compounds, and for this reason would not be recommended.

5. AKNOWLEDGMENTS:

This research was supported by the Brazilian government through its agency CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

REFERENCES

ADHIKARI, B.K., BARRIGTON, S., MARTINEZ, J. Predicted growth of world urban food waste and methane production. **Waste Management Res.** V.24, n.5, p.421-33. 2006.

APHA. **Standard Methods for the Examination of Water and Wastewater** 21st ed. (A. D. Eaton & M. A. H. Franson, eds.). Washington, DC: American Public Health Association, American Water Works Association, Water Environment Federation. 2005.

AMOO, Olaleye M. and FAGBENLE, Richard Layi. Renewable municipal solid waste pathways for energy generation and sustainable development in the Nigerian context. **International Journal of Energy Environmental Engineering**. V. 4, p. 42–59. 2013.

ARIUNBAATAR, J. PANICO, A. YEH, D.H. PIROZZI, F. LENS, P.N.L. and ESPOSITO, Enhanced mesophilic anaerobic digestion of food waste by thermal pretreatment: Substrate versus digestate heating. **Waste Management**. V. 46, p.176–181. 2015.

BENCH, M.L. WOODARD, R. HARDER, M.K. and STANTZOS, N. Waste Minimisation: Home Digestion Trials of Biodegradable Waste. **Res. Conservation and Recycling** V.45, p.84–94. 2005.

FAO – Food and Agriculture Organization. **How to Feed the World in 2050**. [Accessed 13 January 2016]. Available from: http://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf 2009

FENG, L. LI, Y. CHEN, C. LIU, X. XIAO, X. MA, X. ZHANG, R. HE, Y. and LIU, G. Biochemical methane potential (BMP) of vinegar residue and the influence of feed to inoculum ratios on biogas production. **Bio Resources**. V.8, n.2, p.2487–2498. 2013

LIN, L. YUAN, S. LIN, L. YUAN, S. CHEN, J. XU, Z. and LU, X. Removal of ammonia nitrogen in wastewater by microwave radiation. **J. of Hazardous Materials**. V.//161, n.2-3, p.1063-68. 2008.

LÓPEZ TORRES, M. and ESPINOSA Lloréns, M. del C. Effect of alkaline pretreatment on anaerobic digestion of solid wastes. **Waste Management**. V.28, n.11, p.2229–2234. 2008.

MARIN, J. KENNEDY, K.J. and ESKICIOGLU, C. Effect of microwave irradiation on anaerobic degradability of model kitchen waste. **Waste Management**. V.30, n. 10, p.1772–1779. 2010.

MATAALVAREZ, J. CECCHI, F. LLABRES, P. PAVAN, P. Anaerobic-digestion of the Barcelona Central food market organic wastes e plant-design and feasibility study. **Bioresource Technology** V.42, n.1, p.33-42. 1992.

NIELFA, A. CANO, R. and FDZ-POLANCO, M. Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge. **Biotechnology Reports**. V.5, n.1, p.14–21. 2015.

NIELSEN, Suzanne. **Food Analysis Laboratory Manual**. 4th ed.177 pages. 2010.

PARFITT, J. BARTHEL, M. and MACNAUGHTON, S. Food waste within food supply chains: quantification and potential for change to 2050. Philosophical transactions of the Royal Society of London. Series B, **Biological sciences**. V.365, n.1554, p.3065–81. 2010.

PULLEN, T. **Anaerobic digestion**: making biogas, making energy. New York: Routledge. 2015.

SHAHRIARI, H. **Enhancement of Anaerobic Digestion of Organic Fraction of Municipal Solid Waste by Microwave Pretreatment**. p.175. 2011. Thesis (Ph.D.). University of Ottawa.

SHIN, H.S. HAN, S.K. SONG, Y.C. LEE, C.Y. Performance of UASB reactor treating leachate from acidogenic fermenter in the two-phase anaerobic digestion of food waste. **Water Res.** V.35, p3441–3447. 2001.

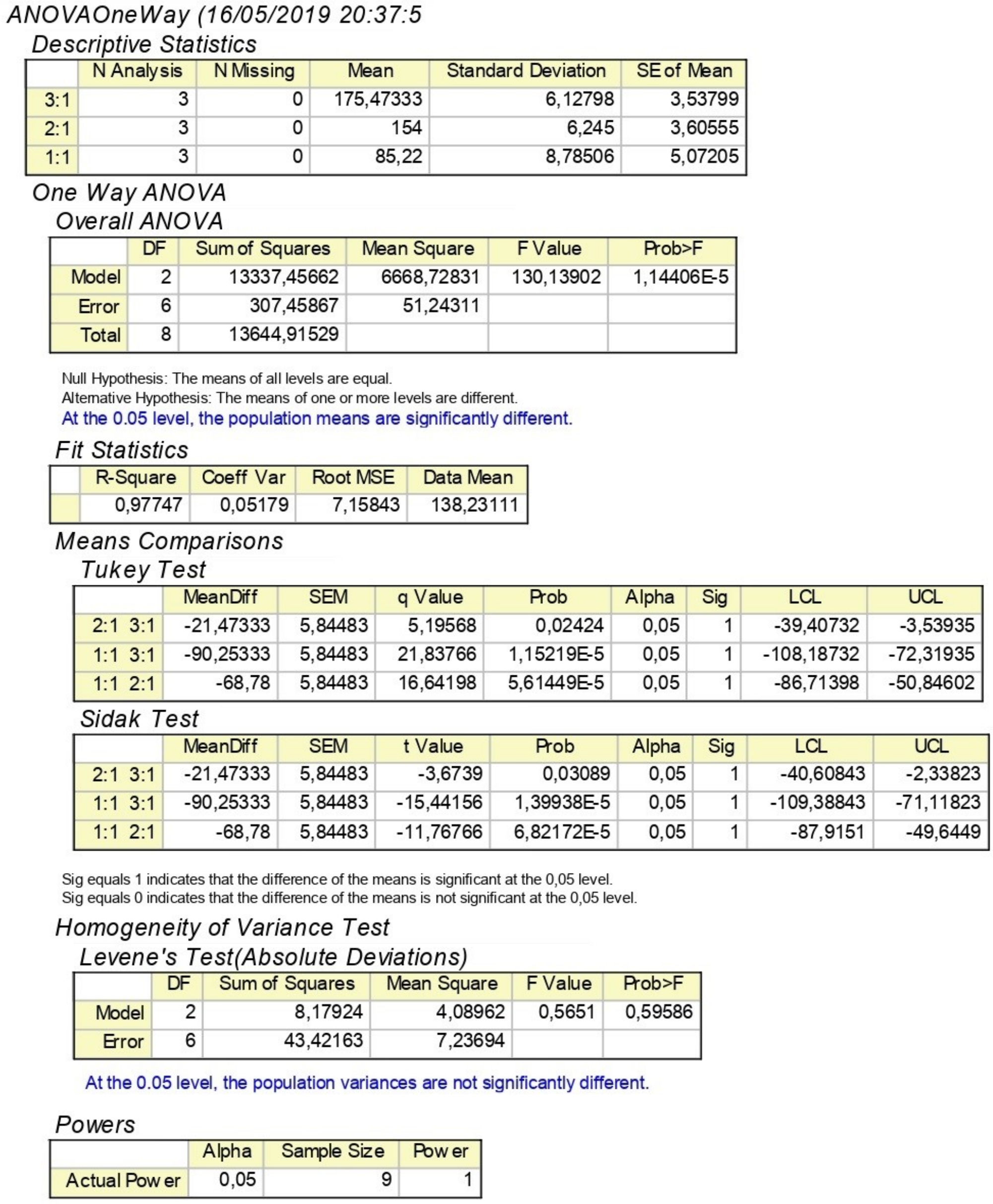
VEEKEN, A. and HAMELERS, B. Effect of temperature on hydrolysis rates of selected biowaste components. **Bioresource Technology**. V. 69, n.3, p.249–254. 1999.

XIE, S. LAWLOR, P.G. FROST, J.P. HU, Z. and ZHAN, X. Effect of pig manure to grass silage ratio on methane production in batch anaerobic co-digestion of concentrated pig manure and grass silage. **Bioresource Technology**. V.102, n. 10, p.5728–5733. 2011.

ZHANG, L. and JAHNG, D. Long-term anaerobic digestion of food waste stabilized by trace elements. **Waste Management.** V. 32, n. 8, p.1509–1515. 2012.

ZHANG, J. LY, C. TONG, J. LIU, J. LIU, J. YU, D. WANG, Y. CHEN, M. and WEI, Y. Optimization and microbial community analysis of anaerobic co-digestion of food waste and sewage sludge based on microwave pretreatment. **Bioresource Technology**. V.200, p.253–261. 2016.

C.



ANOVAOneWay (16/05/2019 20:37:5

Descriptive Statistics

	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean
3:1	3	0	175,47333	6,12798	3,53799
2:1	3	0	154	6,245	3,60555
1:1	3	0	85,22	8,78506	5,07205

One Way ANOVA

Overall ANOVA

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	2	13337,45662	6668,72831	130,13902	1,14406E-5
Error	6	307,45867	51,24311		
Total	8	13644,91529			

Null Hypothesis: The means of all levels are equal.
Alternative Hypothesis: The means of one or more levels are different.
At the 0.05 level, the population means are significantly different.

Fit Statistics

	R-Square	Coeff Var	Root MSE	Data Mean
	0,97747	0,05179	7,15843	138,23111

Means Comparisons

Tukey Test

	MeanDiff	SEM	q Value	Prob	Alpha	Sig	LCL	UCL
2:1 3:1	-21,47333	5,84483	5,19568	0,02424	0,05	1	-39,40732	-3,53935
1:1 3:1	-90,25333	5,84483	21,83766	1,15219E-5	0,05	1	-108,18732	-72,31935
1:1 2:1	-68,78	5,84483	16,64198	5,61449E-5	0,05	1	-86,71398	-50,84602

Sidak Test

	MeanDiff	SEM	t Value	Prob	Alpha	Sig	LCL	UCL
2:1 3:1	-21,47333	5,84483	-3,6739	0,03089	0,05	1	-40,60843	-2,33823
1:1 3:1	-90,25333	5,84483	-15,44156	1,39938E-5	0,05	1	-109,38843	-71,11823
1:1 2:1	-68,78	5,84483	-11,76766	6,82172E-5	0,05	1	-87,9151	-49,6449

Sig equals 1 indicates that the difference of the means is significant at the 0,05 level.
Sig equals 0 indicates that the difference of the means is not significant at the 0,05 level.

Homogeneity of Variance Test

Levene's Test(Absolute Deviations)

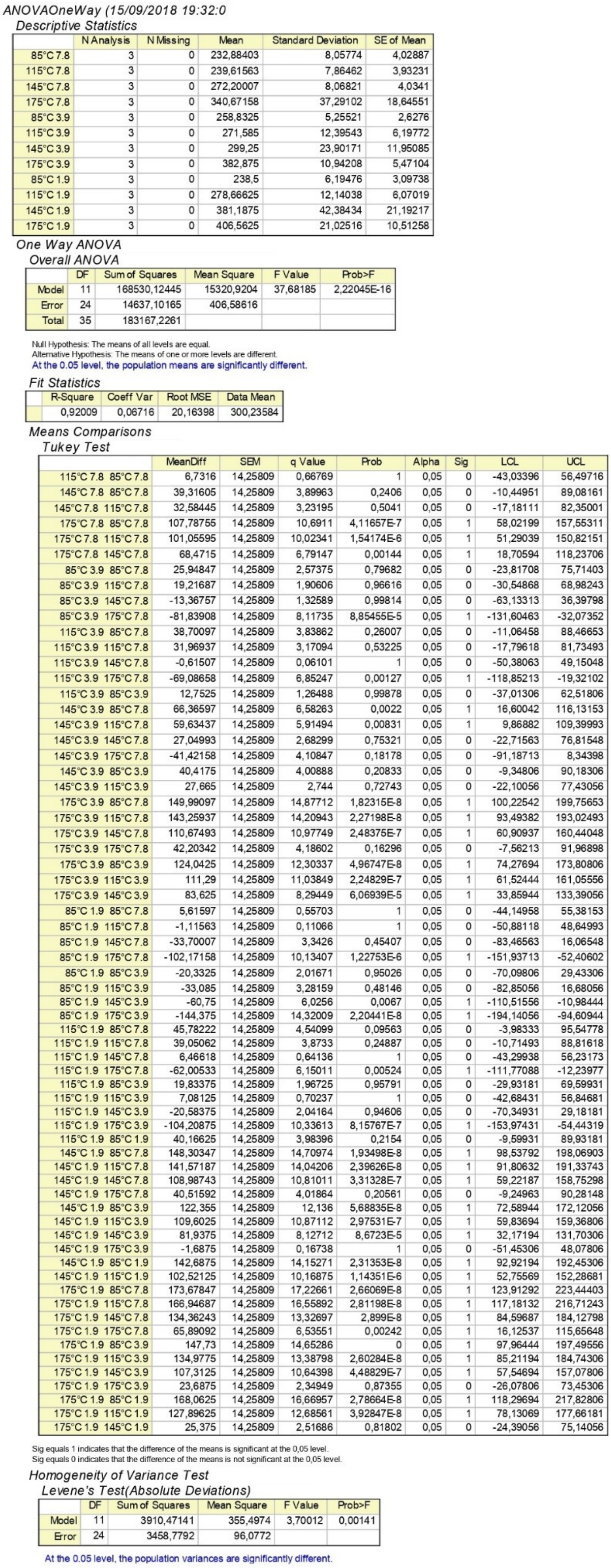
	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	2	8,17924	4,08962	0,5651	0,59586
Error	6	43,42163	7,23694		

At the 0.05 level, the population variances are not significantly different.

Powers

	Alpha	Sample Size	Power
Actual Power	0,05	9	1

D.



ANOVAOneWay (15/09/2018 19:32:0

Descriptive Statistics

	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean
85°C 7.8	3	0	232,88403	8,05774	4,02887
115°C 7.8	3	0	239,61563	7,86462	3,93231
145°C 7.8	3	0	272,20007	8,06821	4,0341
175°C 7.8	3	0	340,67158	37,29102	18,64551
85°C 3.9	3	0	258,8325	5,25521	2,6276
115°C 3.9	3	0	271,585	12,39543	6,19772
145°C 3.9	3	0	299,25	23,90171	11,95085
175°C 3.9	3	0	382,875	10,94208	5,47104
85°C 1.9	3	0	238,5	6,19476	3,09738
115°C 1.9	3	0	278,66625	12,14038	6,07019
145°C 1.9	3	0	381,1875	42,38434	21,19217
175°C 1.9	3	0	406,5625	21,02516	10,51258

One Way ANOVA

Overall ANOVA

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	11	168530,12445	15320,9204	37,68185	2,22045E-16
Error	24	14637,10165	406,58616		
Total	35	183167,2261			

Null Hypothesis: The means of all levels are equal.
Alternative Hypothesis: The means of one or more levels are different.
At the 0.05 level, the population means are significantly different.

Fit Statistics

	R-Square	Coeff Var	Root MSE	Data Mean
	0,92009	0,06716	20,16398	300,23584

Means Comparisons

Tukey Test

	MeanDiff	SEM	q Value	Prob	Alpha	Sig	LCL	UCL
115°C 7.8 85°C 7.8	6,7316	14,25809	0,66769	1	0,05	0	-43,03396	56,49716
145°C 7.8 85°C 7.8	39,31605	14,25809	3,89963	0,2406	0,05	0	-10,44951	89,08161
145°C 7.8 115°C 7.8	32,58445	14,25809	3,23195	0,5041	0,05	0	-17,18111	82,35001
175°C 7.8 85°C 7.8	107,78755	14,25809	10,6911	4,11657E-7	0,05	1	58,02199	157,55311
175°C 7.8 115°C 7.8	101,05595	14,25809	10,02341	1,54174E-6	0,05	1	51,29039	150,82151
175°C 7.8 145°C 7.8	68,4715	14,25809	6,79147	0,00144	0,05	1	18,70594	118,23706
85°C 3.9 85°C 7.8	25,94847	14,25809	2,57375	0,79682	0,05	0	-23,81708	75,71403
85°C 3.9 115°C 7.8	19,21687	14,25809	1,90606	0,96616	0,05	0	-30,54868	68,98243
85°C 3.9 145°C 7.8	-13,36757	14,25809	1,32589	0,99814	0,05	0	-63,13313	36,39798
85°C 3.9 175°C 7.8	-81,83908	14,25809	8,11735	8,85455E-5	0,05	1	-131,60463	-32,07352
115°C 3.9 85°C 7.8	38,70097	14,25809	3,83862	0,26007	0,05	0	-11,06458	88,46653
115°C 3.9 115°C 7.8	31,96937	14,25809	3,17094	0,53225	0,05	0	-17,79618	81,73493
115°C 3.9 145°C 7.8	-0,61507	14,25809	0,06101	1	0,05	0	-50,38063	49,15048
115°C 3.9 175°C 7.8	-69,08658	14,25809	6,85247	0,00127	0,05	1	-118,85213	-19,32102
115°C 3.9 85°C 3.9	12,7525	14,25809	1,26488	0,99878	0,05	0	-37,01306	62,51806
145°C 3.9 85°C 7.8	66,36597	14,25809	6,58263	0,0022	0,05	1	16,60042	116,13153
145°C 3.9 115°C 7.8	59,63437	14,25809	5,91494	0,00831	0,05	1	9,86882	109,39993
145°C 3.9 145°C 7.8	27,04993	14,25809	2,68299	0,75321	0,05	0	-22,71563	76,81548
145°C 3.9 175°C 7.8	-41,42158	14,25809	4,10847	0,18178	0,05	0	-91,18713	8,34398
145°C 3.9 85°C 3.9	40,4175	14,25809	4,00888	0,20833	0,05	0	-9,34806	90,18306
145°C 3.9 115°C 3.9	27,665	14,25809	2,744	0,72743	0,05	0	-22,10056	77,43056
175°C 3.9 85°C 7.8	149,99097	14,25809	14,87712	1,82315E-8	0,05	1	100,22542	199,75653
175°C 3.9 115°C 7.8	143,25937	14,25809	14,20943	2,27198E-8	0,05	1	93,49382	193,02493
175°C 3.9 145°C 7.8	110,67493	14,25809	10,97749	2,48375E-7	0,05	1	60,90937	160,44048
175°C 3.9 175°C 7.8	42,20342	14,25809	4,18602	0,16296	0,05	0	-7,56213	91,96898
175°C 3.9 85°C 3.9	124,0425	14,25809	12,30337	4,96747E-8	0,05	1	74,27694	173,80806
175°C 3.9 115°C 3.9	111,29	14,25809	11,03849	2,24829E-7	0,05	1	61,52444	161,05556
175°C 3.9 145°C 3.9	83,625	14,25809	8,29449	6,06939E-5	0,05	1	33,85944	133,39056
85°C 1.9 85°C 7.8	5,61597	14,25809	0,55703	1	0,05	0	-44,14958	55,38153
85°C 1.9 115°C 7.8	-1,11563	14,25809	0,11066	1	0,05	0	-50,88118	48,64993
85°C 1.9 145°C 7.8	-33,70007	14,25809	3,3426	0,45407	0,05	0	-83,46563	16,06548
85°C 1.9 175°C 7.8	-102,17158	14,25809	10,13407	1,22753E-6	0,05	1	-151,93713	-52,40602
85°C 1.9 85°C 3.9	-20,3325	14,25809	2,01671	0,95026	0,05	0	-70,09806	29,43306
85°C 1.9 115°C 3.9	-33,085	14,25809	3,28159	0,48146	0,05	0	-82,85056	16,68056
85°C 1.9 145°C 3.9	-60,75	14,25809	6,0256	0,0067	0,05	1	-110,51556	-10,98444
85°C 1.9 175°C 3.9	-144,375	14,25809	14,32009	2,20441E-8	0,05	1	-194,14056	-94,60944
115°C 1.9 85°C 7.8	45,78222	14,25809	4,54099	0,09563	0,05	0	-3,98333	95,54778
115°C 1.9 115°C 7.8	39,05062	14,25809	3,8733	0,24887	0,05	0	-10,71493	88,81618
115°C 1.9 145°C 7.8	6,46618	14,25809	0,64136	1	0,05	0	-43,29938	56,23173
115°C 1.9 175°C 7.8	-62,00533	14,25809	6,15011	0,00524	0,05	1	-111,77088	-12,23977
115°C 1.9 85°C 3.9	19,83375	14,25809	1,96725	0,95791	0,05	0	-29,93181	69,59931
115°C 1.9 115°C 3.9	7,08125	14,25809	0,70237	1	0,05	0	-42,68431	56,84681
115°C 1.9 145°C 3.9	-20,58375	14,25809	2,04164	0,94606	0,05	0	-70,34931	29,18181
115°C 1.9 175°C 3.9	-104,20875	14,25809	10,33613	8,15767E-7	0,05	1	-153,97431	-54,44319
115°C 1.9 85°C 1.9	40,16625	14,25809	3,98396	0,2154	0,05	0	-9,59931	89,93181
145°C 1.9 85°C 7.8	148,30347	14,25809	14,70974	1,93498E-8	0,05	1	98,53792	198,06903
145°C 1.9 115°C 7.8	141,57187	14,25809	14,04206	2,39626E-8	0,05	1	91,80632	191,33743
145°C 1.9 145°C 7.8	108,98743	14,25809	10,81011	3,31328E-7	0,05	1	59,22187	158,75298
145°C 1.9 175°C 7.8	40,51592	14,25809	4,01864	0,20561	0,05	0	-9,24963	90,28148
145°C 1.9 85°C 3.9	122,355	14,25809	12,136	5,68835E-8	0,05	1	72,58944	172,12056
145°C 1.9 115°C 3.9	109,6025	14,25809	10,87112	2,97531E-7	0,05	1	59,83694	159,36806
145°C 1.9 145°C 3.9	81,9375	14,25809	8,12712	8,6723E-5	0,05	1	32,17194	131,70306
145°C 1.9 175°C 3.9	-1,6875	14,25809	0,16738	1	0,05	0	-51,45306	48,07806
145°C 1.9 85°C 1.9	142,6875	14,25809	14,15271	2,31353E-8	0,05	1	92,92194	192,45306
145°C 1.9 115°C 1.9	102,52125	14,25809	10,16875	1,14351E-6	0,05	1	52,75569	152,28681
175°C 1.9 85°C 7.8	173,67847	14,25809	17,22661	2,66069E-8	0,05	1	123,91292	223,44403
175°C 1.9 115°C 7.8	166,94687	14,25809	16,55892	2,81198E-8	0,05	1	117,18132	216,71243
175°C 1.9 145°C 7.8	134,36243	14,25809	13,32697	2,899E-8	0,05	1	84,59687	184,12798
175°C 1.9 175°C 7.8	65,89092	14,25809	6,53551	0,00242	0,05	1	16,12537	115,65648
175°C 1.9 85°C 3.9	147,73	14,25809	14,65286	0	0,05	1	97,96444	197,49556
175°C 1.9 115°C 3.9	134,9775	14,25809	13,38798	2,60284E-8	0,05	1	85,21194	184,74306
175°C 1.9 145°C 3.9	107,3125	14,25809	10,64398	4,48829E-7	0,05	1	57,54694	157,07806
175°C 1.9 175°C 3.9	23,6875	14,25809	2,34949	0,87355	0,05	0	-26,07806	73,45306
175°C 1.9 85°C 1.9	168,0625	14,25809	16,66957	2,78664E-8	0,05	1	118,29694	217,82806
175°C 1.9 115°C 1.9	127,89625	14,25809	12,68561	3,92847E-8	0,05	1	78,13069	177,66181
175°C 1.9 145°C 1.9	25,375	14,25809	2,51686	0,81802	0,05	0	-24,39056	75,14056

Sig equals 1 indicates that the difference of the means is significant at the 0,05 level.
Sig equals 0 indicates that the difference of the means is not significant at the 0,05 level.

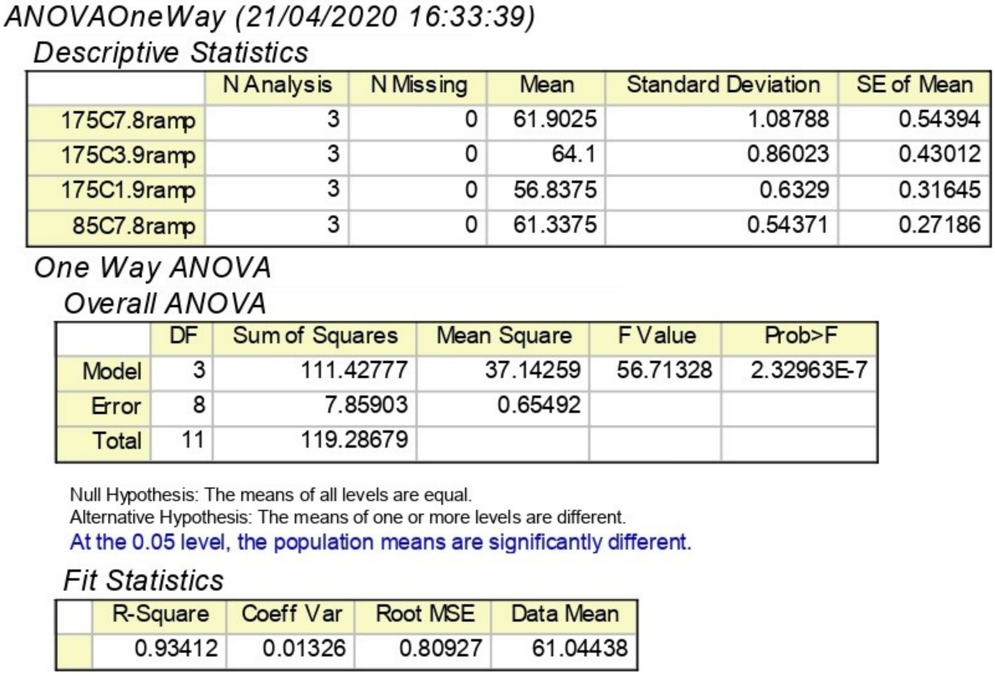
Homogeneity of Variance Test

Levene's Test(Absolute Deviations)

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	11	3910,47141	355,4974	3,70012	0,00141
Error	24	3458,7792	96,0772		

At the 0.05 level, the population variances are significantly different.

E.



ANOVAOneWay (21/04/2020 16:33:39)

Descriptive Statistics

	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean
175C7.8ramp	3	0	61.9025	1.08788	0.54394
175C3.9ramp	3	0	64.1	0.86023	0.43012
175C1.9ramp	3	0	56.8375	0.6329	0.31645
85C7.8ramp	3	0	61.3375	0.54371	0.27186

One Way ANOVA

Overall ANOVA

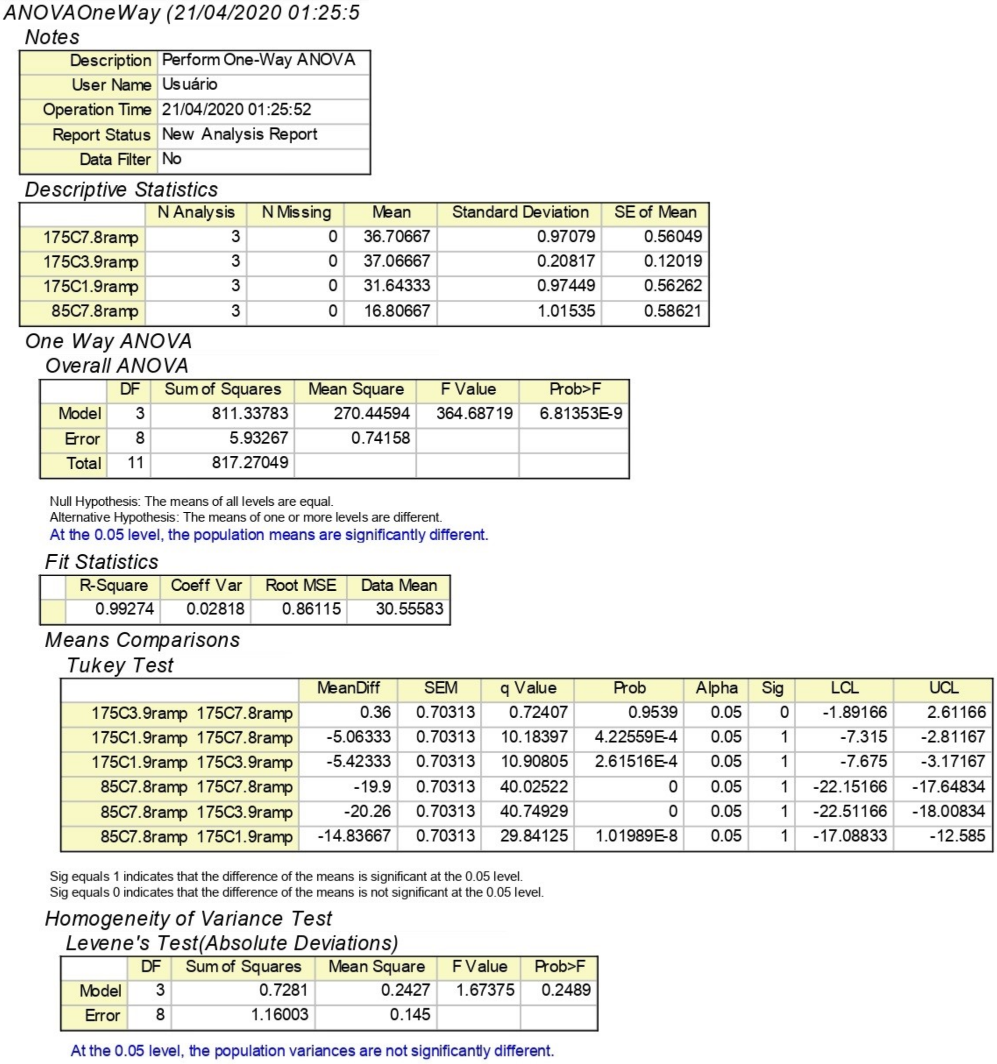
	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	3	111.42777	37.14259	56.71328	2.32963E-7
Error	8	7.85903	0.65492		
Total	11	119.28679			

Null Hypothesis: The means of all levels are equal.
Alternative Hypothesis: The means of one or more levels are different.
At the 0.05 level, the population means are significantly different.

Fit Statistics

	R-Square	Coeff Var	Root MSE	Data Mean
	0.93412	0.01326	0.80927	61.04438

F.



ANOVAOneWay (21/04/2020 01:25:5

Notes

Description	Perform One-Way ANOVA
User Name	Usuário
Operation Time	21/04/2020 01:25:52
Report Status	New Analysis Report
Data Filter	No

Descriptive Statistics

	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean
175C7.8ramp	3	0	36.70667	0.97079	0.56049
175C3.9ramp	3	0	37.06667	0.20817	0.12019
175C1.9ramp	3	0	31.64333	0.97449	0.56262
85C7.8ramp	3	0	16.80667	1.01535	0.58621

One Way ANOVA

Overall ANOVA

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	3	811.33783	270.44594	364.68719	6.81353E-9
Error	8	5.93267	0.74158		
Total	11	817.27049			

Null Hypothesis: The means of all levels are equal.
Alternative Hypothesis: The means of one or more levels are different.
At the 0.05 level, the population means are significantly different.

Fit Statistics

	R-Square	Coeff Var	Root MSE	Data Mean
	0.99274	0.02818	0.86115	30.55583

Means Comparisons

Tukey Test

	MeanDiff	SEM	q Value	Prob	Alpha	Sig	LCL	UCL
175C3.9ramp 175C7.8ramp	0.36	0.70313	0.72407	0.9539	0.05	0	-1.89166	2.61166
175C1.9ramp 175C7.8ramp	-5.06333	0.70313	10.18397	4.22559E-4	0.05	1	-7.315	-2.81167
175C1.9ramp 175C3.9ramp	-5.42333	0.70313	10.90805	2.61516E-4	0.05	1	-7.675	-3.17167
85C7.8ramp 175C7.8ramp	-19.9	0.70313	40.02522	0	0.05	1	-22.15166	-17.64834
85C7.8ramp 175C3.9ramp	-20.26	0.70313	40.74929	0	0.05	1	-22.51166	-18.00834
85C7.8ramp 175C1.9ramp	-14.83667	0.70313	29.84125	1.01989E-8	0.05	1	-17.08833	-12.585

Sig equals 1 indicates that the difference of the means is significant at the 0.05 level.
Sig equals 0 indicates that the difference of the means is not significant at the 0.05 level.

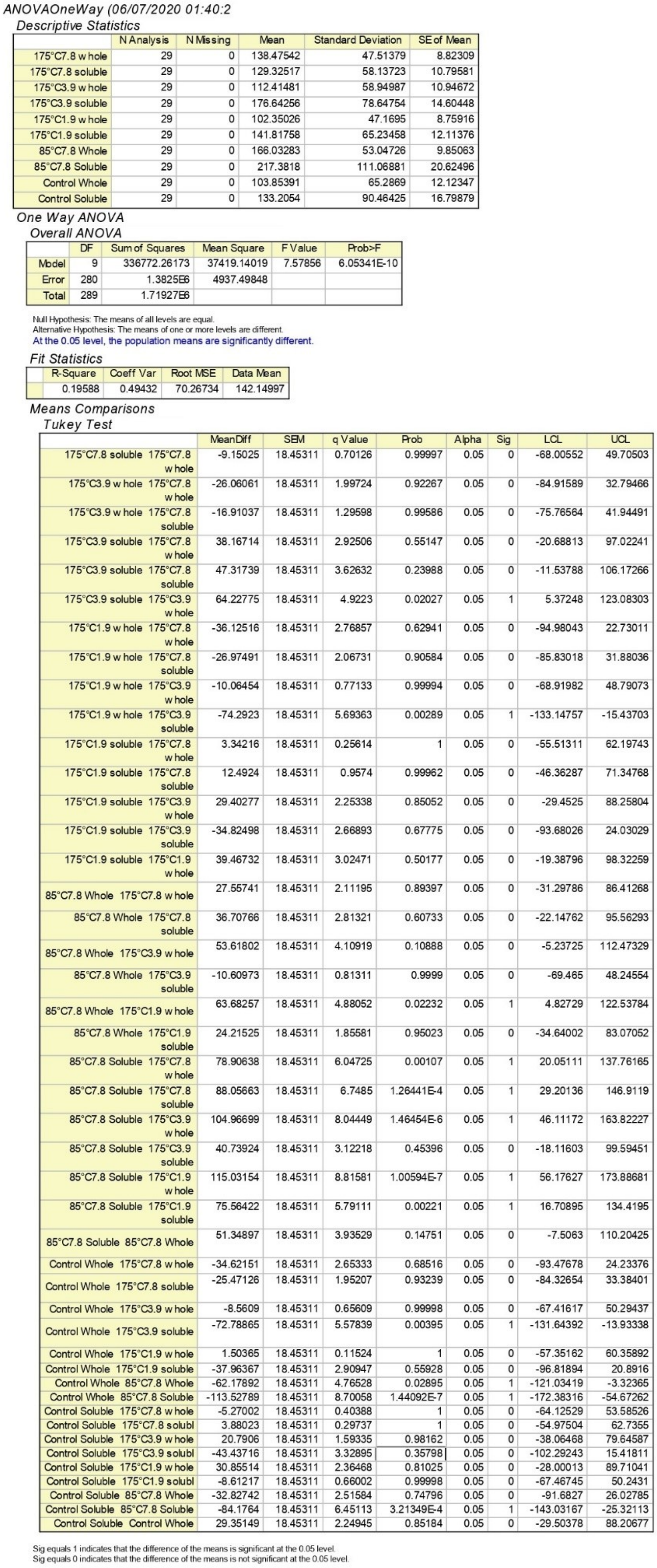
Homogeneity of Variance Test

Levene's Test(Absolute Deviations)

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	3	0.7281	0.2427	1.67375	0.2489
Error	8	1.16003	0.145		

At the 0.05 level, the population variances are not significantly different.

G.



ANOVAOneWay (06/07/2020 01:40:2

Descriptive Statistics

	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean
175°C7.8 whole	29	0	138.47542	47.51379	8.82309
175°C7.8 soluble	29	0	129.32517	58.13723	10.79581
175°C3.9 whole	29	0	112.41481	58.94987	10.94672
175°C3.9 soluble	29	0	176.64256	78.64754	14.60448
175°C1.9 whole	29	0	102.35026	47.1695	8.75916
175°C1.9 soluble	29	0	141.81758	65.23458	12.11376
85°C7.8 Whole	29	0	166.03283	53.04726	9.85063
85°C7.8 Soluble	29	0	217.3818	111.06881	20.62496
Control Whole	29	0	103.85391	65.2869	12.12347
Control Soluble	29	0	133.2054	90.46425	16.79879

One Way ANOVA

Overall ANOVA

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	9	336772.26173	37419.14019	7.57856	6.05341E-10
Error	280	1.3825E6	4937.49848		
Total	289	1.71927E6			

Null Hypothesis: The means of all levels are equal.
Alternative Hypothesis: The means of one or more levels are different.
At the 0.05 level, the population means are significantly different.

Fit Statistics

	R-Square	Coeff Var	Root MSE	Data Mean
	0.19588	0.49432	70.26734	142.14997

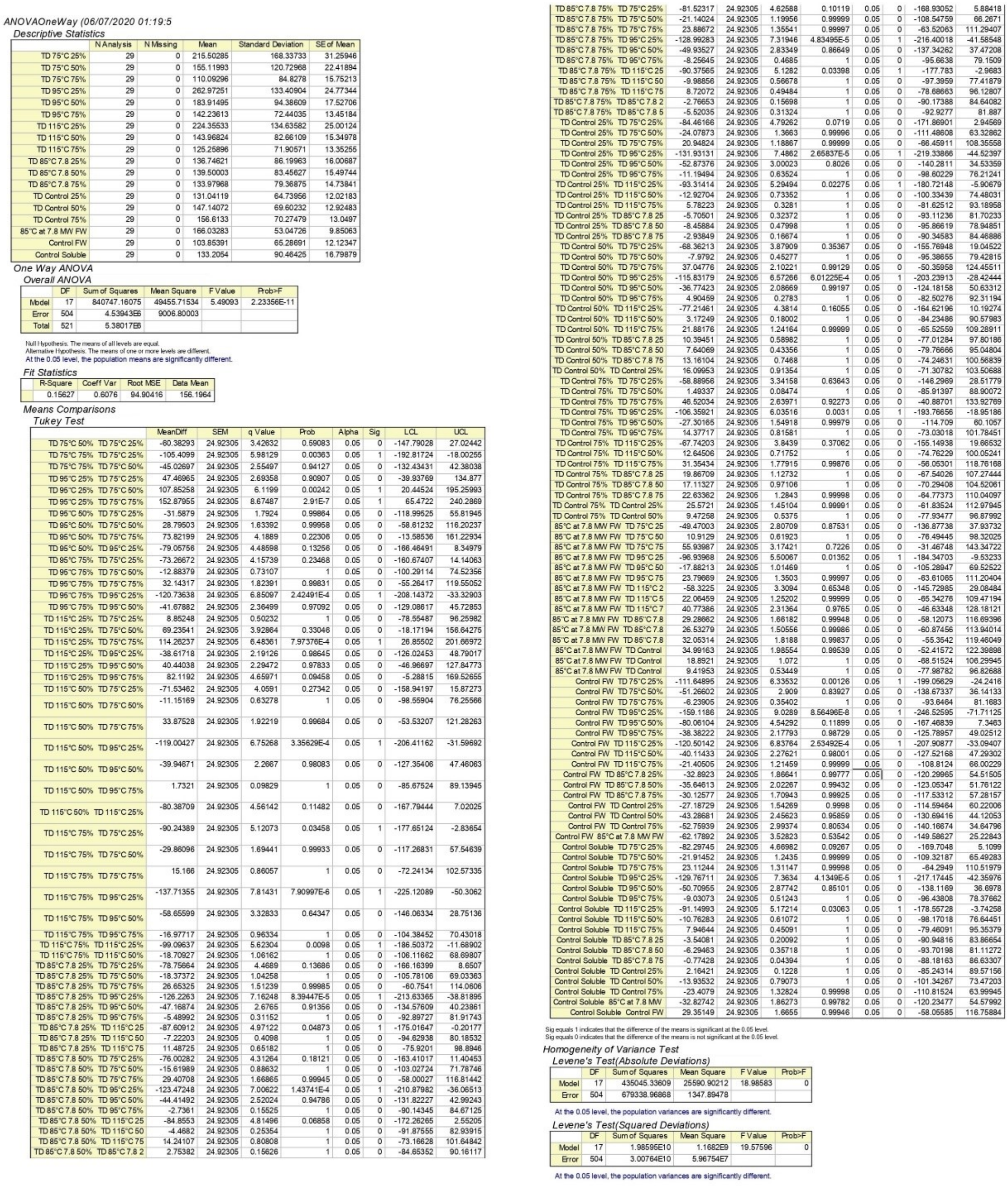
Means Comparisons

Tukey Test

	MeanDiff	SEM	q Value	Prob	Alpha	Sig	LCL	UCL
175°C7.8 soluble 175°C7.8 whole	-9.15025	18.45311	0.70126	0.99997	0.05	0	-68.00552	49.70503
175°C3.9 whole 175°C7.8 whole	-26.06061	18.45311	1.99724	0.92267	0.05	0	-84.91589	32.79466
175°C3.9 whole 175°C7.8 soluble	-16.91037	18.45311	1.29598	0.99586	0.05	0	-75.76564	41.94491
175°C3.9 soluble 175°C7.8 whole	38.16714	18.45311	2.92506	0.55147	0.05	0	-20.68813	97.02241
175°C3.9 soluble 175°C7.8 soluble	47.31739	18.45311	3.62632	0.23988	0.05	0	-11.53788	106.17266
175°C3.9 soluble 175°C3.9 whole	64.22775	18.45311	4.9223	0.02027	0.05	1	5.37248	123.08303
175°C1.9 whole 175°C7.8 whole	-36.12516	18.45311	2.76857	0.62941	0.05	0	-94.98043	22.73011
175°C1.9 whole 175°C7.8 soluble	-26.97491	18.45311	2.06731	0.90584	0.05	0	-85.83018	31.88036
175°C1.9 whole 175°C3.9 whole	-10.06454	18.45311	0.77133	0.99994	0.05	0	-68.91982	48.79073
175°C1.9 whole 175°C3.9 soluble	-74.2923	18.45311	5.69363	0.00289	0.05	1	-133.14757	-15.43703
175°C1.9 soluble 175°C7.8 whole	3.34216	18.45311	0.25614	1	0.05	0	-55.51311	62.19743
175°C1.9 soluble 175°C7.8 soluble	12.4924	18.45311	0.9574	0.99962	0.05	0	-46.36287	71.34768
175°C1.9 soluble 175°C3.9 whole	29.40277	18.45311	2.25338	0.85052	0.05	0	-29.4525	88.25804
175°C1.9 soluble 175°C3.9 soluble	-34.82498	18.45311	2.66893	0.67775	0.05	0	-93.68026	24.03029
175°C1.9 soluble 175°C1.9 whole	39.46732	18.45311	3.02471	0.50177	0.05	0	-19.38796	98.32259
85°C7.8 Whole 175°C7.8 whole	27.55741	18.45311	2.11195	0.89397	0.05	0	-31.29786	86.41268
85°C7.8 Whole 175°C7.8 soluble	36.70766	18.45311	2.81321	0.60733	0.05	0	-22.14762	95.56293
85°C7.8 Whole 175°C3.9 whole	53.61802	18.45311	4.10919	0.10888	0.05	0	-5.23725	112.47329
85°C7.8 Whole 175°C3.9 soluble	-10.60973	18.45311	0.81311	0.9999	0.05	0	-69.465	48.24554
85°C7.8 Whole 175°C1.9 whole	63.68257	18.45311	4.88052	0.02232	0.05	1	4.82729	122.53784
85°C7.8 Whole 175°C1.9 soluble	24.21525	18.45311	1.85581	0.95023	0.05	0	-34.64002	83.07052
85°C7.8 Soluble 175°C7.8 whole	78.90638	18.45311	6.04725	0.00107	0.05	1	20.05111	137.76165
85°C7.8 Soluble 175°C7.8 soluble	88.05663	18.45311	6.7485	1.26441E-4	0.05	1	29.20136	146.9119
85°C7.8 Soluble 175°C3.9 whole	104.96699	18.45311	8.04449	1.46454E-6	0.05	1	46.11172	163.82227
85°C7.8 Soluble 175°C3.9 soluble	40.73924	18.45311	3.12218	0.45396	0.05	0	-18.11603	99.59451
85°C7.8 Soluble 175°C1.9 whole	115.03154	18.45311	8.81581	1.00594E-7	0.05	1	56.17627	173.88681
85°C7.8 Soluble 175°C1.9 soluble	75.56422	18.45311	5.79111	0.00221	0.05	1	16.70895	134.4195
85°C7.8 Soluble 85°C7.8 Whole	51.34897	18.45311	3.93529	0.14751	0.05	0	-7.5063	110.20425
Control Whole 175°C7.8 whole	-34.62151	18.45311	2.65333	0.68516	0.05	0	-93.47678	24.23376
Control Whole 175°C7.8 soluble	-25.47126	18.45311	1.95207	0.93239	0.05	0	-84.32654	33.38401
Control Whole 175°C3.9 whole	-8.5609	18.45311	0.65609	0.99998	0.05	0	-67.41617	50.29437
Control Whole 175°C3.9 soluble	-72.78865	18.45311	5.57839	0.00395	0.05	1	-131.64392	-13.93338
Control Whole 175°C1.9 whole	1.50365	18.45311	0.11524	1	0.05	0	-57.35162	60.35892
Control Whole 175°C1.9 soluble	-37.96367	18.45311	2.90947	0.55928	0.05	0	-96.81894	20.8916
Control Whole 85°C7.8 Whole	-62.17892	18.45311	4.76528	0.02895	0.05	1	-121.03419	-3.32365
Control Whole 85°C7.8 Soluble	-113.52789	18.45311	8.70058	1.44092E-7	0.05	1	-172.38316	-54.67262
Control Soluble 175°C7.8 whole	-5.27002	18.45311	0.40388	1	0.05	0	-64.12529	53.58526
Control Soluble 175°C7.8 solubl	3.88023	18.45311	0.29737	1	0.05	0	-54.97504	62.7355
Control Soluble 175°C3.9 whole	20.7906	18.45311	1.59335	0.98162	0.05	0	-38.06468	79.64587
Control Soluble 175°C3.9 solubl	-43.43716	18.45311	3.32895	0.35798	0.05	0	-102.29243	15.41811
Control Soluble 175°C1.9 whole	30.85514	18.45311	2.36468	0.81025	0.05	0	-28.00013	89.71041
Control Soluble 175°C1.9 solubl	-8.61217	18.45311	0.66002	0.99998	0.05	0	-67.46745	50.2431
Control Soluble 85°C7.8 Whole	-32.82742	18.45311	2.51584	0.74796	0.05	0	-91.6827	26.02785
Control Soluble 85°C7.8 Soluble	-84.1764	18.45311	6.45113	3.21349E-4	0.05	1	-143.03167	-25.32113
Control Soluble Control Whole	29.35149	18.45311	2.24945	0.85184	0.05	0	-29.50378	88.20677

Sig equals 1 indicates that the difference of the means is significant at the 0.05 level.
Sig equals 0 indicates that the difference of the means is not significant at the 0.05 level.

H.



ANOVAOneWay (06/07/2020 01:19:5

Descriptive Statistics

	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean
TD 75°C 25%	29	0	215.50285	168.33733	31.25946
TD 75°C 50%	29	0	155.11993	120.72968	22.41894
TD 75°C 75%	29	0	110.09296	84.8278	15.75213
TD 95°C 25%	29	0	262.97251	133.40904	24.77344
TD 95°C 50%	29	0	183.91495	94.38609	17.52706
TD 95°C 75%	29	0	142.23613	72.44035	13.45184
TD 115°C 25%	29	0	224.35533	134.63582	25.00124
TD 115°C 50%	29	0	143.96824	82.66109	15.34978
TD 115°C 75%	29	0	125.25896	71.90571	13.35255
TD 85°C 7.8 25%	29	0	136.74621	86.19963	16.00687
TD 85°C 7.8 50%	29	0	139.50003	83.45627	15.49744
TD 85°C 7.8 75%	29	0	133.97968	79.36875	14.73841
TD Control 25%	29	0	131.04119	64.73956	12.02183
TD Control 50%	29	0	147.14072	69.60232	12.92483
TD Control 75%	29	0	156.6133	70.27479	13.0497
85°C at 7.8 MW FW	29	0	166.03283	53.04726	9.85063
Control FW	29	0	103.85391	65.28691	12.12347
Control Soluble	29	0	133.2054	90.46425	16.79879

One Way ANOVA

Overall ANOVA

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	17	840747.16075	49455.71534	5.49093	2.23356E-11
Error	504	4.53943E6	9006.80003		
Total	521	5.38017E6			

Null Hypothesis: The means of all levels are equal.
Alternative Hypothesis: The means of one or more levels are different.
At the 0.05 level, the population means are significantly different.

Fit Statistics

	R-Square	Coeff Var	Root MSE	Data Mean
	0.15627	0.6076	94.90416	156.1964

Means Comparisons

Tukey Test

	MeanDiff	SEM	q Value	Prob	Alpha	Sig	LCL	UCL
TD 75°C 50% TD 75°C 25%	-60.38293	24.92305	3.42632	0.59083	0.05	0	-147.79028	27.02442
TD 75°C 75% TD 75°C 25%	-105.4099	24.92305	5.98129	0.00363	0.05	1	-192.81724	-18.00255
TD 75°C 75% TD 75°C 50%	-45.02697	24.92305	2.55497	0.94127	0.05	0	-132.43431	42.38038
TD 95°C 25% TD 75°C 25%	47.46965	24.92305	2.69358	0.90907	0.05	0	-39.93769	134.877
TD 95°C 25% TD 75°C 50%	107.85258	24.92305	6.1199	0.00242	0.05	1	20.44524	195.25993
TD 95°C 25% TD 75°C 75%	152.87955	24.92305	8.67487	2.91E-7	0.05	1	65.4722	240.2869
TD 95°C 50% TD 75°C 25%	-31.5879	24.92305	1.7924	0.99864	0.05	0	-118.99525	55.81945
TD 95°C 50% TD 75°C 50%	28.79503	24.92305	1.63392	0.99958	0.05	0	-58.61232	116.20237
TD 95°C 50% TD 75°C 75%	73.82199	24.92305	4.1889	0.22306	0.05	0	-13.58536	161.22934
TD 95°C 50% TD 95°C 25%	-79.05756	24.92305	4.48598	0.13256	0.05	0	-166.46491	8.34979
TD 95°C 75% TD 75°C 25%	-73.26672	24.92305	4.15739	0.23468	0.05	0	-160.67407	14.14063
TD 95°C 75% TD 75°C 50%	-12.88379	24.92305	0.73107	1	0.05	0	-100.29114	74.52356
TD 95°C 75% TD 75°C 75%	32.14317	24.92305	1.82391	0.99831	0.05	0	-55.26417	119.55052
TD 95°C 75% TD 95°C 25%	-120.73638	24.92305	6.85097	2.42491E-4	0.05	1	-208.14372	-33.32903
TD 95°C 75% TD 95°C 50%	-41.67882	24.92305	2.36499	0.97092	0.05	0	-129.08617	45.72853
TD 115°C 25% TD 75°C 25%	8.85248	24.92305	0.50232	1	0.05	0	-78.55487	96.25982
TD 115°C 25% TD 75°C 50%	69.23541	24.92305	3.92864	0.33046	0.05	0	-18.17194	156.64275
TD 115°C 25% TD 75°C 75%	114.26237	24.92305	6.48361	7.97376E-4	0.05	1	26.85502	201.66972
TD 115°C 25% TD 95°C 25%	-38.61718	24.92305	2.19126	0.98645	0.05	0	-126.02453	48.79017
TD 115°C 25% TD 95°C 50%	40.44038	24.92305	2.29472	0.97833	0.05	0	-46.96697	127.84773
TD 115°C 25% TD 95°C 75%	82.1192	24.92305	4.65971	0.09458	0.05	0	-5.28815	169.52655
TD 115°C 50% TD 75°C 25%	-71.53462	24.92305	4.0591	0.27342	0.05	0	-158.94197	15.87273
TD 115°C 50% TD 75°C 50%	-11.15169	24.92305	0.63278	1	0.05	0	-98.55904	76.25566
TD 115°C 50% TD 75°C 75%	33.87528	24.92305	1.92219	0.99684	0.05	0	-53.53207	121.28263
TD 115°C 50% TD 95°C 25%	-119.00427	24.92305	6.75268	3.35629E-4	0.05	1	-206.41162	-31.59692
TD 115°C 50% TD 95°C 50%	-39.94671	24.92305	2.2667	0.98083	0.05	0	-127.35406	47.46063
TD 115°C 50% TD 95°C 75%	1.7321	24.92305	0.09829	1	0.05	0	-85.67524	89.13945
TD 115°C 50% TD 115°C 25%	-80.38709	24.92305	4.56142	0.11482	0.05	0	-167.79444	7.02025
TD 115°C 75% TD 75°C 25%	-90.24389	24.92305	5.12073	0.03458	0.05	1	-177.65124	-2.83654
TD 115°C 75% TD 75°C 50%	-29.86096	24.92305	1.69441	0.99933	0.05	0	-117.26831	57.54639
TD 115°C 75% TD 75°C 75%	15.166	24.92305	0.86057	1	0.05	0	-72.24134	102.57335
TD 115°C 75% TD 95°C 25%	-137.71355	24.92305	7.81431	7.90997E-6	0.05	1	-225.12089	-50.3062
TD 115°C 75% TD 95°C 50%	-58.65599	24.92305	3.32833	0.64347	0.05	0	-146.06334	28.75136
TD 115°C 75% TD 95°C 75%	-16.97717	24.92305	0.96334	1	0.05	0	-104.38452	70.43018
TD 115°C 75% TD 115°C 25%	-99.09637	24.92305	5.62304	0.0098	0.05	1	-186.50372	-11.68902
TD 115°C 75% TD 115°C 50%	-18.70927	24.92305	1.06162	1	0.05	0	-106.11662	68.69807
TD 85°C 7.8 25% TD 75°C 25%	-78.75664	24.92305	4.4689	0.13686	0.05	0	-166.16399	8.6507
TD 85°C 7.8 25% TD 75°C 50%	-18.37372	24.92305	1.04258	1	0.05	0	-105.78106	69.03363
TD 85°C 7.8 25% TD 75°C 75%	26.65325	24.92305	1.51239	0.99985	0.05	0	-60.7541	114.0606
TD 85°C 7.8 25% TD 95°C 25%	-126.2263	24.92305	7.16248	8.39447E-5	0.05	1	-213.63365	-38.81895
TD 85°C 7.8 25% TD 95°C 50%	-47.16874	24.92305	2.6765	0.91356	0.05	0	-134.57609	40.23861
TD 85°C 7.8 25% TD 95°C 75%	-5.48992	24.92305	0.31152	1	0.05	0	-92.89727	81.91743
TD 85°C 7.8 25% TD 115°C 25	-87.60912	24.92305	4.97122	0.04873	0.05	1	-175.01647	-0.20177
TD 85°C 7.8 25% TD 115°C 50	-7.22203	24.92305	0.4098	1	0.05	0	-94.62938	80.18532
TD 85°C 7.8 25% TD 115°C 75	11.48725	24.92305	0.65182	1	0.05	0	-75.9201	98.8946
TD 85°C 7.8 50% TD 75°C 25%	-76.00282	24.92305	4.31264	0.18121	0.05	0	-163.41017	11.40453
TD 85°C 7.8 50% TD 75°C 50%	-15.61989	24.92305	0.88632	1	0.05	0	-103.02724	71.78746
TD 85°C 7.8 50% TD 75°C 75%	29.40708	24.92305	1.66865	0.99945	0.05	0	-58.00027	116.81442
TD 85°C 7.8 50% TD 95°C 25%	-123.47248	24.92305	7.00622	1.43741E-4	0.05	1	-210.87982	-36.06513
TD 85°C 7.8 50% TD 95°C 50%	-44.41492	24.92305	2.52024	0.94786	0.05	0	-131.82227	42.99243
TD 85°C 7.8 50% TD 95°C 75%	-2.7361	24.92305	0.15525	1	0.05	0	-90.14345	84.67125
TD 85°C 7.8 50% TD 115°C 25	-84.8553	24.92305	4.81496	0.06858	0.05	0	-172.26265	2.55205
TD 85°C 7.8 50% TD 115°C 50	-4.4682	24.92305	0.25354	1	0.05	0	-91.87555	82.93915
TD 85°C 7.8 50% TD 115°C 75	14.24107	24.92305	0.80808	1	0.05	0	-73.16628	101.64842
TD 85°C 7.8 50% TD 85°C 7.8 2	2.75382	24.92305	0.15626	1	0.05	0	-84.65352	90.16117
TD 85°C 7.8 75% TD 75°C 25%	-81.52317	24.92305	4.62588	0.10119	0.05	0	-168.93052	5.88418
TD 85°C 7.8 75% TD 75°C 50%	-21.14024	24.92305	1.19956	0.99999	0.05	0	-108.54759	66.2671
TD 85°C 7.8 75% TD 75°C 75%	23.88672	24.92305	1.35541	0.99997	0.05	0	-63.52063	111.29407
TD 85°C 7.8 75% TD 95°C 25%	-128.99283	24.92305	7.31946	4.83495E-5	0.05	1	-216.40018	-41.58548
TD 85°C 7.8 75% TD 95°C 50%	-49.93527	24.92305	2.83349	0.86649	0.05	0	-137.34262	37.47208
TD 85°C 7.8 75% TD 95°C 75%	-8.25645	24.92305	0.4685	1	0.05	0	-95.6638	79.1509
TD 85°C 7.8 75% TD 115°C 25	-90.37565	24.92305	5.1282	0.03398	0.05	1	-177.783	-2.9683
TD 85°C 7.8 75% TD 115°C 50	-9.98856	24.92305	0.56678	1	0.05	0	-97.3959	77.41879
TD 85°C 7.8 75% TD 115°C 75	8.72072	24.92305	0.49484	1	0.05	0	-78.68663	96.12807
TD 85°C 7.8 75% TD 85°C 7.8 2	-2.76653	24.92305	0.15698	1	0.05	0	-90.17388	84.64082
TD 85°C 7.8 75% TD 85°C 7.8 5	-5.52035	24.92305	0.31324	1	0.05	0	-92.9277	81.887
TD Control 25% TD 75°C 25%	-84.46166	24.92305	4.79262	0.0719	0.05	0	-171.86901	2.94569
TD Control 25% TD 75°C 50%	-24.07873	24.92305	1.3663	0.99996	0.05	0	-111.48608	63.32862
TD Control 25% TD 75°C 75%	20.94824	24.92305	1.18867	0.99999	0.05	0	-66.45911	108.35558
TD Control 25% TD 95°C 25%	-131.93131	24.92305	7.4862	2.65837E-5	0.05	1	-219.33866	-44.52397
TD Control 25% TD 95°C 50%	-52.87376	24.92305	3.00023	0.8026	0.05	0	-140.2811	34.53359
TD Control 25% TD 95°C 75%	-11.19494	24.92305	0.63524	1	0.05	0	-98.60229	76.21241
TD Control 25% TD 115°C 25%	-93.31414	24.92305	5.29494	0.02275	0.05	1	-180.72148	-5.90679
TD Control 25% TD 115°C 50%	-12.92704	24.92305	0.73352	1	0.05	0	-100.33439	74.48031
TD Control 25% TD 115°C 75%	5.78223	24.92305	0.3281	1	0.05	0	-81.62512	93.18958
TD Control 25% TD 85°C 7.8 25	-5.70501	24.92305	0.32372	1	0.05	0	-93.11236	81.70233
TD Control 25% TD 85°C 7.8 50	-8.45884	24.92305	0.47998	1	0.05	0	-95.86619	78.94851
TD Control 25% TD 85°C 7.8 75	-2.93849	24.92305	0.16674	1	0.05	0	-90.34583	84.46886
TD Control 50% TD 75°C 25%	-68.36213	24.92305	3.87909	0.35367	0.05	0	-155.76948	19.04522
TD Control 50% TD 75°C 50%	-7.9792	24.92305	0.45277	1	0.05	0	-95.38655	79.42815
TD Control 50% TD 75°C 75%	37.04776	24.92305	2.10221	0.99129	0.05	0	-50.35958	124.45511
TD Control 50% TD 95°C 25%	-115.83179	24.92305	6.57266	6.01225E-4	0.05	1	-203.23913	-28.42444
TD Control 50% TD 95°C 50%	-36.77423	24.92305	2.08669	0.99197	0.05	0	-124.18158	50.63312
TD Control 50% TD 95°C 75%	4.90459	24.92305	0.2783	1	0.05	0	-82.50276	92.31194
TD Control 50% TD 115°C 25%	-77.21461	24.92305	4.3814	0.16055	0.05	0	-164.62196	10.19274
TD Control 50% TD 115°C 50%	3.17249	24.92305	0.18002	1	0.05	0	-84.23486	90.57983
TD Control 50% TD 115°C 75%	21.88176	24.92305	1.24164	0.99999	0.05	0	-65.52559	109.28911
TD Control 50% TD 85°C 7.8 25	10.39451	24.92305	0.58982	1	0.05	0	-77.01284	97.80186
TD Control 50% TD 85°C 7.8 50	7.64069	24.92305	0.43356	1	0.05	0	-79.76666	95.04804
TD Control 50% TD 85°C 7.8 75	13.16104	24.92305	0.7468	1	0.05	0	-74.24631	100.56839
TD Control 50% TD Control 25%	16.09953	24.92305	0.91354	1	0.05	0	-71.30782	103.50688
TD Control 75% TD 75°C 25%	-58.88956	24.92305	3.34158	0.63643	0.05	0	-146.2969	28.51779
TD Control 75% TD 75°C 50%	1.49337	24.92305	0.08474	1	0.05	0	-85.91397	88.90072
TD Control 75% TD 75°C 75%	46.52034	24.92305	2.63971	0.92273	0.05	0	-40.88701	133.92769
TD Control 75% TD 95°C 25%	-106.35921	24.92305	6.03516	0.0031	0.05	1	-193.76656	-18.95186
TD Control 75% TD 95°C 50%	-27.30165	24.92305	1.54918	0.99979	0.05	0	-114.709	60.1057
TD Control 75% TD 95°C 75%	14.37717	24.92305	0.81581	1	0.05	0	-73.03018	101.78451
TD Control 75% TD 115°C 25%	-67.74203	24.92305	3.8439	0.37062	0.05	0	-155.14938	19.66532
TD Control 75% TD 115°C 50%	12.64506	24.92305	0.71752	1	0.05	0	-74.76229	100.05241
TD Control 75% TD 115°C 75%	31.35434	24.92305	1.77915	0.99876	0.05	0	-56.05301	118.76168
TD Control 75% TD 85°C 7.8 25	19.86709	24.92305	1.12732	1	0.05	0	-67.54026	107.27444
TD Control 75% TD 85°C 7.8 50	17.11327	24.92305	0.97106	1	0.05	0	-70.29408	104.52061
TD Control 75% TD 85°C 7.8 75	22.63362	24.92305	1.2843	0.99998	0.05	0	-64.77373	110.04097
TD Control 75% TD Control 25%	25.5721	24.92305	1.45104	0.99991	0.05	0	-61.83524	112.97945
TD Control 75% TD Control 50%	9.47258	24.92305	0.5375	1	0.05	0	-77.93477	96.87992
85°C at 7.8 MW FW TD 75°C 25	-49.47003	24.92305	2.80709	0.87531	0.05	0	-136.87738	37.93732
85°C at 7.8 MW FW TD 75°C 50	10.9129	24.92305	0.61923	1	0.05	0	-76.49445	98.32025
85°C at 7.8 MW FW TD 75°C 75	55.93987	24.92305	3.17421	0.7226	0.05	0	-31.46748	143.34722
85°C at 7.8 MW FW TD 95°C 25	-96.93968	24.92305	5.50067	0.01352	0.05	1	-184.34703	-9.53233
85°C at 7.8 MW FW TD 95°C 50	-17.88213	24.92305	1.01469	1	0.05	0	-105.28947	69.52522
85°C at 7.8 MW FW TD 95°C 75	23.79669	24.92305	1.3503	0.99997	0.05	0	-63.61065	111.20404
85°C at 7.8 MW FW TD 115°C 2	-58.3225	24.92305	3.3094	0.65348	0.05	0	-145.72985	29.08484
85°C at 7.8 MW FW TD 115°C 5	22.06459	24.92305	1.25202	0.99999	0.05	0	-65.34276	109.47194
85°C at 7.8 MW FW TD 115°C 7	40.77386	24.92305	2.31364	0.9765	0.05	0	-46.63348	128.18121
85°C at 7.8 MW FW TD 85°C 7.8	29.28662	24.92305	1.66182	0.99948	0.05	0	-58.12073	116.69396
85°C at 7.8 MW FW TD 85°C 7.8	26.53279	24.92305	1.50556	0.99986	0.05	0	-60.87456	113.94014
85°C at 7.8 MW FW TD 85°C 7.8	32.05314	24.92305	1.8188	0.99837	0.05	0	-55.3542	119.46049
85°C at 7.8 MW FW TD Control	34.99163	24.92305	1.98554	0.99539	0.05	0	-52.41572	122.39898
85°C at 7.8 MW FW TD Control	18.8921	24.92305	1.072	1	0.05	0	-68.51524	106.29945
85°C at 7.8 MW FW TD Control	9.41953	24.92305	0.53449	1	0.05	0	-77.98782	96.82688
Control FW TD 75°C 25%	-111.64895	24.92305	6.33532	0.00126	0.05	1	-199.05629	-24.2416
Control FW TD 75°C 50%	-51.26602	24.92305	2.909	0.83927	0.05	0	-138.67337	36.14133
Control FW TD 75°C 75%	-6.23905	24.92305	0.35402	1	0.05	0	-93.6464	81.1683
Control FW TD 95°C 25%	-159.1186	24.92305	9.0289	8.56496E-8	0.05	1	-246.52595	-71.71125
Control FW TD 95°C 50%	-80.06104	24.92305	4.54292	0.11899	0.05	0	-167.46839	7.3463
Control FW TD 95°C 75%	-38.38222	24.92305	2.17793	0.98729	0.05	0	-125.78957	49.02512
Control FW TD 115°C 25%	-120.50142	24.92305	6.83764	2.53492E-4	0.05	1	-207.90877	-33.09407
Control FW TD 115°C 50%	-40.11433	24.92305	2.27621	0.98001	0.05	0	-127.52168	47.29302
Control FW TD 115°C 75%	-21.40505	24.92305	1.21459	0.99999	0.05	0	-108.8124	66.00229
Control FW TD 85°C 7.8 25%	-32.8923	24.92305	1.86641	0.99777	0.05	0	-120.29965	54.51505
Control FW TD 85°C 7.8 50%	-35.64613	24.92305	2.02267	0.99432	0.05	0	-123.05347	51.76122
Control FW TD 85°C 7.8 75%	-30.12577	24.92305	1.70943	0.99925	0.05	0	-117.53312	57.28157
Control FW TD Control 25%	-27.18729	24.92305	1.54269	0.9998	0.05	0	-114.59464	60.22006
Control FW TD Control 50%	-43.28681	24.92305	2.45623	0.95859	0.05	0	-130.69416	44.12053
Control FW TD Control 75%	-52.75939	24.92305	2.99374	0.80534	0.05	0	-140.16674	34.64796
Control FW 85°C at 7.8 MW FW	-62.17892	24.92305	3.52823	0.53542	0.05	0	-149.58627	25.22843
Control Soluble TD 75°C 25%	-82.29745	24.92305	4.66982	0.09267	0.05	0	-169.7048	5.1099
Control Soluble TD 75°C 50%	-21.91452	24.92305	1.2435	0.99999	0.05	0	-109.32187	65.49283
Control Soluble TD 75°C 75%	23.11244	24.92305	1.31147	0.99998	0.05	0	-64.2949	110.51979
Control Soluble TD 95°C 25%	-129.76711	24.92305	7.3634	4.1349E-5	0.05	1	-217.17445	-42.35976
Control Soluble TD 95°C 50%	-50.70955	24.92305	2.87742	0.85101	0.05	0	-138.1169	36.6978
Control Soluble TD 95°C 75%	-9.03073	24.92305	0.51243	1	0.05	0	-96.43808	78.37662
Control Soluble TD 115°C 25%	-91.14993	24.92305	5.17214	0.03063	0.05	1	-178.55728	-3.74258
Control Soluble TD 115°C 50%	-10.76283	24.92305	0.61072	1	0.05	0	-98.17018	76.64451
Control Soluble TD 115°C 75%	7.94644	24.92305	0.45091	1	0.05	0	-79.46091	95.35379
Control Soluble TD 85°C 7.8 25	-3.54081	24.92305	0.20092	1	0.05	0	-90.94816	83.86654
Control Soluble TD 85°C 7.8 50	-6.29463	24.92305	0.35718	1	0.05	0	-93.70198	81.11272
Control Soluble TD 85°C 7.8 75	-0.77428	24.92305	0.04394	1	0.05	0	-88.18163	86.63307
Control Soluble TD Control 25%	2.16421	24.92305	0.1228	1	0.05	0	-85.24314	89.57156
Control Soluble TD Control 50%	-13.93532	24.92305	0.79073	1	0.05	0	-101.34267	73.47203
Control Soluble TD Control 75%	-23.4079	24.92305	1.32824	0.99998	0.05	0	-110.81524	63.99945
Control Soluble 85°C at 7.8 MW	-32.82742	24.92305	1.86273	0.99782	0.05	0	-120.23477	54.57992
Control Soluble Control FW	29.35149	24.92305	1.6655	0.99946	0.05	0	-58.05585	116.75884

Sig equals 1 indicates that the difference of the means is significant at the 0.05 level.
Sig equals 0 indicates that the difference of the means is not significant at the 0.05 level.

Homogeneity of Variance Test

Levene's Test(Absolute Deviations)

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	17	435045.33609	25590.90212	18.98583	0
Error	504	679338.96868	1347.89478		

At the 0.05 level, the population variances are significantly different.

Levene's Test(Squared Deviations)

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	17	1.98595E10	1.1682E9	19.57596	0
Error	504	3.00764E10	5.96754E7		

At the 0.05 level, the population variances are significantly different.