Universidade de Aveiro Departamento de Ambiente e Ordenamento

2017

Maria dos Anjos de Jesus Barros Monteiro Lopes

BIOVALORIZAÇÃO DE RESÍDUOS ALIMENTARES POR PROCESSOS DE ACIDIFICAÇÃO ANAERÓBIA

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Ciências e Engenharia do Ambiente, realizada sob a orientação científica da Doutora Maria Isabel Aparício Paulo Fernandes Capela, Professora Associada do Departamento de Ambiente e Ordenamento da Universidade de Aveiro, e do Doutor Luís Manuel Guerreiro Alves Arroja, Professor Associado com Agregação, aposentado, do Departamento de Ambiente e Ordenamento da Universidade de Aveiro.

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Dedico este trabalho:

à minha amada filha Emma Luísa,
ao meu marido Florentino Neves,
à minha mãe (in memória),
ao meu pai e irmãos.

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o júri

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palavras-chave Biovalorização de resíduos alimentares, digestão anaeróbica, acidificação anaeróbia, ácidos orgânicos voláteis (AOV), polihidroxialcanoatos (PHA)

resumo

A biodegradação constitui uma opção ecológica e sustentável para a eliminação e valorização de resíduos orgânicos, nomeadamente de origem alimentar. Estes resíduos podem ser tratados e valorizados através de processos de digestão anaeróbia, reduzindo assim o seu teor poluente e ao mesmo tempo produzir compostos intermediários valorizáveis, como os ácidos orgânicos voláteis (AOV). Estes compostos resultantes da fermentação acidogénica são fontes de carbono preferenciais para a obtenção de produtos de valor acrescentado, nomeadamente polihidroxialcanoatos (PHA) ou bioenergia, sob a forma de metano ou hidrogénio.

Neste trabalho estudou-se a fermentação acidogénica de vários resíduos orgânicos, em mono ou co-digestão, nomeadamente a fração orgânica de resíduos sólidos urbanos (FORSU), resíduos da indústria de processamento de tomate (RT), borras de café (BC) e lamas ativadas prevenientes de uma estação de tratamento de águas residuais domésticas (LA), em reatores descontínuos e semi-contínuos, a fim de avaliar o seu potencial de produção de AOV.

Nestes ensaios foram estudados os efeitos de vários parâmetros, nomeadamente a 1) concentração de sólidos totais (ST) no interior do reator, 2) a alcalinidade, 3) a carga orgânica (CO) e 4) a relação carbono-azoto (C:N).

Nos ensaios de mono-digestão, verificou-se um comportamento muito distinto nos quatro substratos estudados, com um grau de acidificação substancialmente superior para o RT (49 %) e FORSU (41 %) do que para as borras de café (10 %) e lamas ativadas (6 %). Observou-se também que nos ensaios de co-digestão, o ensaio com a percentagem mais elevada de RT (75 %) e BC (25 %) apresentaram o mais elevado grau de acidificação (57 %), confirmando a sinergia que ocorreu com esta mistura.

Dos três modelos cinéticos utilizados para estudar o desempenho da co-digestão entre os dois resíduos maioritários em Cabo Verde (FORSU e LA), o modelo que apresentou a melhor correlação para obtenção do potencial metanogénico foi o modelo de exponencial (com *Curve factor*). Os valores para a constante de velocidade metanogénica aumentaram com o aumento de FORSU na mistura, com o valor experimental máximo de k_M (0.27 d^{-1}) obtido no ensaio com 75% de FORSU. Verificou-se também que apesar da baixa biodegradabilidade das LA, este substrato promoveu a estabilidade do processo de digestão da FORSU neste ensaio, evitando assim a inibição da produção de metano devido a valores baixos de pH e a concentrações elevadas de AOV, conforme verificado experimentalmente no ensaio de mono-digestão da FORSU. Assim, o estudo cinético forneceu uma ferramenta simples e útil para prever o desempenho do reator no que diz respeito à produção de metano, tendo em conta as proporções de cada um dos co-substratos nas condições aplicadas.

Recorrendo à modelação dos resultados obtidos na digestão da FORSU, através de superfícies de resposta, demonstrou-se que o aumento do teor de ST no digestor induziu uma diminuição do grau de acidificação, enquanto que o aumento da concentração de alcalinidade adicionada conduziu ao aumento do grau de acidificação. Por conseguinte, o maior grau de acidificação obtido foi de 78% com a combinação de ST mais baixo estudado (5 %) e a alcalinidade adicionada mais elevada (50 $gCaCO_3.L^{-1}$). No entanto, e dependendo da utilização final dos AOV que são produzidos, as condições que apresentaram elevado teor de AOV (99 %), com uma concentração elevada de ácido propiónico na sua composição (mais adequado para a produção de PHA de elevada qualidade), foram os teores de ST intermédios (8 %). A partir das superfícies de resposta obtidas observou-se também que todas as variáveis de resposta estudadas (produção de AOV, grau de acidificação e qualidade do efluente) apresentaram uma dependência maior do teor em ST do que da adição de alcalinidade.

O processo de fermentação acidogénica da FORSU foi posteriormente desenvolvido em modo semi-contínuo num reator CSTR, que operou a longo prazo. De todas as condições testadas (carga orgânica entre 3.0–6.5 g COD L^{-1} e alcalinidade entre 2.0-5.0 g $CaCO_3$ L^{-1}), a condição onde se obteve o maior grau de acidificação (59 %), a melhor qualidade de efluente em termos de AOV (66 %), e uma boa razão impar-par em AOV (0.44), foi o ensaio com carga orgânica de 6,0 g CQO $L^{-1}d^{-1}$ e alcalinidade de 2,5 g $CaCO_3$ L^{-1}. O aumento da carga orgânica levou ao aumento de AOV, sendo os ácidos acético, propiónico e butírico as espécies predominantes em todas as fases do processo.

O efluente acidificado no processo anaeróbio foi então usado como substrato em reatores SBR operados para seleção de culturas microbianas mistas com capacidade para acumular PHA, nos quais foi aplicado um regime de alimentação dinâmica (fartura/fome) em condições aeróbias. Foram estudadas três cargas orgânicas e duas razões C:N para avaliar o potencial de enriquecimento da cultura. Durante o processo, todas as condições testadas apresentaram uma eficiência de remoção de CQO superior a 80 %, com uma acumulação de PHA entre 17 % e 53 %. Em estudos de acumulação de PHA efetuados em reatores semi-contínuos foram estudados três valores de pH, entre 7 e 8.5, em que a acumulação de PHA foi mais favorável a pH neutro, resultando num teor de PHA de 25% (w/w). O monómero HB foi o principal composto do polímero sintetizado a partir de FORSU acidificada.

Com base nestes resultados, pode concluir-se que os resíduos orgânicos de origem alimentar podem ser tratados por processos biológicos, com tratamento convencional de resíduos, e ao mesmo tempo podem ser convertidos em materiais de valor acrescentado.

keywords Biovalorization of food waste, anaerobic acidification, volatile fatty acids (VFA), polyhydroxyalkanoates (PHA).

abstract

Biodegradation is an eco-friendly option for the disposal and recovery of organic waste, including food waste (FW). These residues can be treated and recovered through anaerobic digestion processes, thereby reducing their pollutant content and, at the same time, producing high value-products such as volatile fatty acids (VFA). These compounds resulting from acidogenic fermentation are the preferred carbon sources for the production of added-value products, namely polyhydroxyalkanoates (PHA) or bioenergy, in the form of methane or hydrogen.

In this work, it was studied the acidogenic fermentation of several organic residues, such as the organic fraction of municipal solid waste (OFMSW), waste from the tomato processing industry (TW), coffee grounds waste (CG) and waste activated sludge (WAS) from a wastewater treatment plant. The assays were performed in batch and semi-continuous reactors, either in mono- or co-digestion assays, in order to assess and optimize its potential for VFA production. In these tests, the effects of various parameters, such as 1) total solids (TS) content in the reactor, 2) alkalinity addition, 3) organic loading rate (OLR) applied and 4) carbon-nitrogen ratio (C:N) were studied.

In the mono-digestion assays, a very distinct behavior was observed in the four substrates studied, with a substantially higher acidification rate for TW (49 %) and OFMSW (41 %) than for CG (10 %) and WAS (6 %). It was also observed that in the co-digestion assays, the assay with the highest percentage of TW (75 %) and GC (25 %) showed the highest acidification degree (57 %), confirming the synergy that occurred with this mixture.

Out of the three kinetic models used to study the co-digestion performance between the two major residues in Cape Verde (OFMSW and WAS), the model that presented the best correlation to obtain the methanogenic potential was exponential Curve factor model. The values for the methanogenic rate constant increased with the increase of OFMSW in the mixture, with the maximum experimental value of k_M (0.27 d^{-1}) obtained in the 75 % OFMSW assay. It was also found that, despite the low biodegradability of WAS, this substrate promoted the stability of the OFMSW digestion process in this assay, thus avoiding the inhibition of methane production due to low pH values and high concentrations of VFA, as verified experimentally in the OFMSW mono-digestion test. Thus, the kinetic study provided a simple and useful tool to predict reactor performance with respect to methane production, taking into account the proportions of each of the co-substrates, and under the conditions applied.

Analyzing the results of the response surfaces obtained for the OFMSW digestion assays, it has been demonstrated that the increase in the TS reactor content led to a decrease in the acidification degree whereas the increase in the alkalinity addition led to the increase of the degree of acidification. Therefore, the highest degree of acidification (78 %) was obtained at the lowest TS reactor content (5 %) and the highest alkalinity addition (50 $gCaCO_3.L^{-1}$). However, depending on the ultimate use of the produced VFA mixture, the conditions presenting the highest VFA content (99 %) with high propionic acid concentration (VFA mixture more suitable for the production of high quality PHA), were the intermediate TS reactor content (8 %). From the response surfaces obtained, it was also observed that all response variables under study (VFA production, degree of acidification and effluent quality) presented a higher dependency on TS reactor content than on initial alkalinity addition.

The FORSU acidogenic fermentation process was further developed in a semi-continuous CSTR reactor, which was operated under long-term and several operational conditions (organic load between 3.0 - 6.5 g COD L^{-1} and alkalinity between 2.0 - 5.0 g $CaCO_3L^{-1}$).The operational condition correspondent to of 6.0 g COD $L^{-1}d^{-1}$ and the alkalinity of 2.5 g $CaCO_3$ L^{-1}, was the condition where the highest degree of acidification (59 %), the best effluent quality In terms of VFA (66 %), and a good odd-to-even ratio in VFA (0.44) were achieved. In general, the increase on the organic load applied led to the increase of VFA, with acetic, propionic and butyric acids being always the predominant species in all experimental stages.

The acidified effluent in the anaerobic process was then used as substrate in SBR reactors operated for the selection of mixed microbial cultures with high capacity for PHA accumulation, where it was applied a regime of dynamic feeding (feast/famine) under aerobic conditions. Three organic loads and two C:N ratios were studied, in order to evaluate the enrichment potential of the microbial mixed culture. During the process, all tested conditions showed a COD removal efficiency higher than 80 % with a PHA accumulation capacity between 17 % and 53 %. In PHA accumulation studies carried out in fed-batch reactors, three different pH values, between 7 and 8.5 were studied, where PHA accumulation was more favorable at neutral pH, , resulting in a PHA content of 25 % (w/w). The HB monomer was the main compound of the polymer synthesized from acidified OFMSW.

Based on these results, it can be concluded that organic waste from food sources can be treated by biological processes, as a conventional waste treatment, and at the same time can be converted into value-added materials.

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AD - Anaerobic digestion

AcoD – Anaerobic co-digestion

Alk – Alkalinity

CG – Coffee grounds

CH_4 – Methane

C:N – Carbon-nitrogen ratio

CO_2 – Carbon oxygen

COD – Chemical oxygen demand

CSTR – Continues stirred tank reactor

DA – degree of acidification

DAF – Dynamic aerobic feed

DCP – Dichlorophenols

DS – Degree solubilization

EC – European Commission

EU – European Union

FAS – Ferrous ammonium sulphate

F:F – Feast and famine ratio

FW – Food waste

H_2 – Hydrogen

H-Ac – Acetic acid

H-Prop – Propionic acid

H-Bu – Butiric acid

H-Val – Valeric acid

H-Cap – Caprionic acid

HRT – Hydraulic retention time

k_H – Hydrolysis rate constant

k_M – Methanogenic rate constant

MFC – Microbial fuel cell

MH – Maize husk

MMC – Mixed microbial culture

MPB – Methane producing bacteria

MSW – Municipal solid waste

MSWM – Municipal solid waste management

MT – Mesophilic temperature

O_2 – Oxygen

OD – Oxygen dissolved

OFMSW – Organic fraction of municipal solid waste

OW – Organic Waste

PCP – Pentachlorophenol

PE – Polyethylene

PHA – Polyhydroxyalkanoates

PHB – Polyhydroxybutyrate

PHV – Polyhydroxyvalerate

$^{-q}_{VFA}$ – Specific substrate

$^{q}_{PHA}$ – Specific PHA production

R^2 – Coefficient of Correlation

S – Substrate concentration

S_0 – Substrate concentration initial

SRT – Solid retention time

STR – Sequencing Batch Reactors

SW – Solid waste

t – Time

TCOD- Total chemical oxygen demand

TOC- Total organic carbon

TN- Total nitrogen

TKN- Total kjeldhal nitrogen

TS – Total Solids

TSS – Total suspended solids

TT – Thermophilic temperature

TP – total phosphorous

TW – Tomato waste

TVFA – Total volatile fatty acid

WAS – Waste activated sludge

WWTP – Wastewater treatment plants

VS – Volatile Solids

VFA – Volatile fatty acid

1. Introduction

1.1 General introduction

The energy crisis and the environmental degradation are currently two of the vital issues for global sustainable development. It is now accepted that consumption of fossil fuels is over 80 % of the total energy consumption and that it contributes not only to climate change and the global warming, but also to a rapid exhaustion of natural energy resources (Mao et al., 2015; Abudi et al., 2016).

In a similar trend, it is observed nowadays a rapid increasing of the amount of waste generated annually, due to an increase of population, a rapid increase on urbanization and a growth of industrialization, constituting one of the most serious problems of contemporary societies (Forster-Carneiro et al., 2008). As result of these growing and development trends, millions of tons of wastes are produced per year worldwide, including food wastes, agricultural residues, or sewage sludge from wastewater treatment processes, which can be considered harmful for the environment, if not effectively treated and efficiently of disposed (Barrantes et al., 2014). The environmental effects of incorrect management of wastes can include surface water pollution and eutrophication, residues accumulation and odor pollution (Nielsen et al., 2009; Beyene et al., 2011), creating several risks to public health.

Therefore, proper waste management is crucial to minimize further environmental degradation and simultaneously foster the transition to a sustainable society. The most common waste management approach is treatment oriented, which mainly focuses on meeting environmental regulations (Lee et al., 2014). This approach omits the potential of using the waste as a feedstock for the production of value added products, reducing the amounts of waste produced and creating value. Then, a more embracing waste management approach is needed, where resources recovery is the focus, allowing a simultaneous minimization of waste and a generation of value added products.

According to Lebiocka and Piotrowicz (2012), other strategies in waste management include the change in the practice of landfilling, taking into account a waste-to-energy approach. For this purpose, several countries worldwide are interested in the search for new and economical process, which can be used to treat biodegradable waste and, simultaneously, reduce the volume of waste generated (Monnet, 2003).

The biological treatment (anaerobic and aerobic) is known as one of the most beneficial methods for maximizing the recycling and the recovering of several components present in the waste or wastewater to be treated, and has been demonstrated as the most effective waste management technique for bioconversion and the most cost-effective technology for different high strength biowaste. Several types of wastes were effectively treated and valorized using biological process, as wastewater sludge, organic fraction of municipal solids waste, agricultural residues, food industries residues and other organic wastes.

There are multiple characteristics that make this technology applicable to treatment for the most organics solid wastes and wastewaters are well known (Abouelenien et al., 2014; Bacenetti et al., 2015; Gohil and Nakhla, 2006; Hernández et al., 2014; Jabłoński et al., 2015; Jang et al., 2016; Kalyuzhnyi et al., 1997; Khan and Martin, 2016; Lee et al., 2014; Li et al., 2015a; Molino et al., 2013). Currently, there is an increasing number of small-scale digestion plants, which include the development of high rate reactor systems for the treatment of organic waste (Kinyua et al., 2016). On the other hand, there is a growing scientific and commercial interest in developed and under developing countries in using these technologies, in order to improve the treatability as well as the additional recovery of bioproducts (Arroja et al., 2012; Kinyua et al., 2016; Madsen et al., 2011; Mata-Alvarez et al., 2000; Molino et al., 2013; Monnet, 2003).

Among biological treatments, anaerobic digestion (AD) is an economically viable process to treat high-strength organic waste, due to the associated low operational costs, which allows high energy recovery linked to the process (Bonk et al., 2015). Besides contributing to the biological treatment of organic waste, AD is also used to obtain intermediate materials, which can be incorporated into new added-values products. Some of these intermediates are used for biopolymers synthesis like polyhydroxyalkanoates (PHA) (Coats et al., 2011; Elain et al., 2016; Korkakaki et al., 2016) or for energy production such as bioethanol, biohydrogen or biomethane (Singh and Harvey, 2010). In addition, biomass from AD contains primary nutrients (nitrogen, phosphorus, potassium), that have agronomic benefits if used as a soil amendment to improve plant growth and at the same time reduce the environmental impact from agricultural activities (Capela et al., 2008; Zhang et al., 2014).

AD is recognized as a practical technology for the rapid stabilization of organic waste prior to final disposal in landfill (Shao et al., 2013), contributing to the reduction of the organic

content, to the minimization of odors and pathogens. This technology is commonly applied to treat waste activated sludge (Metcalf, 2003), or to remove nitrogen compounds from effluents (Ahn, 2006; Jokela et al., 2003), but can be applied to a wide range of substrates. In a previous study, Murthy and Novak (1999) reported that aerobic digestion caused poor decomposition of organic substances and increased biopolymer content. Thus, in recent years, aerobic treatment process has engaged increased attention into this particular aspect and in the simultaneous use of anaerobic and aerobic treatment strategies (Akizuki et al., 2016; Bahar and Ciggin, 2016; Di Maria and Micale, 2015a; Jin et al., 2016; Z. Zhang et al., 2015).

However, several factors can affect the AD process performance and stability (Di Maria and Micale, 2015a, 2015b; Moñino et al., 2016). To overcome the above problems, anaerobic co-digestion (AcoD) has been reported as an optimal solution to the treatment of mixture of various organic wastes (Ağdağ and Sponza, 2007, 2005; Agyeman and Tao, 2014; Capela et al., 2008; Fonoll et al., 2015a).

In this context, various organic wastes have been treated by biological treatments, both anaerobic and aerobic process, in this present work, in order to maximize the VFA production for further use in the PHA and energy production, with a perspective to contribute in the process of waste management and recovery, mainly in developing countries as it is the case of Cabo Verde.

1.2 Aim of the work

The general aim of this work was to investigate the performance of the bioreactors treating various organic wastes from different sources. This thesis reports several studies that were undertaken in order to improve VFA and methane production. For that, food waste and waste activated sludge were used, by either mono-digestion or co-digestion and investigating the maximum organic loading rate to increase the quality of effluents in terms of VFA, for further use in the biodegradable polymer-PHA production.

In order to reach the aim, this study comprises several specific objectives:

- ✓ to performs the comparison of mono and co-digestion potential of different organic solid wastes which are the potential substrates and the interaction (synergisms) between substrates was investigated, as well as the stability of the system,
- ✓ to compare the kinetic constants for hydrolytic and methanogenic steps of anaerobic process for the proposed substrate mixtures, in order to provide a simple basis to obtain a stable digestion process,
- ✓ to optimize the production of a VFA mixture from organic fraction of municipal solid waste (OFMSW) by hydrolytic/acidogenic fermentation, taking into account the influence of two main operational parameters: the initial addition of external alkalinity and the TS content inside of reactor,
- ✓ to determine the maximum loading rate for continuous stirred tank reactor (CSTR) treating organic waste in order to increase and optimize the generation effluent rich in VFA, from the point of view of their used as substrate for PHA production,
- ✓ to investigate the potential of OFMSW for PHA production in Sequencing Batch Reactors (SBR) system in feeding regime dynamics, in order to select mixed cultures for a high PHA storage capacity.

1.3 Structure of the thesis

This thesis is organized in nine chapters, as follows:

Chapter 1 presents a general description and contextualizes the scientific relevance and the global and specific objectives of the thesis.

Chapter 2 includes the literature review about the objectives of the thesis, wastes generation and management, problematic of the production of organic solids wastes in Cape Verde, a brief description of the anaerobic digestion process and some examples of the valorization of anaerobic digestion.

Chapter 3 describes the experimental facilities used in the different experimental tests, the characteristics of the raw materials and the wastes tested, including the different calculations used in the treatment and discussion of results.

Chapter 4 describes the comparative study of biochemical acidogenic co-fermentation of different food wastes as co-substrates (coffee grounds, tomato waste, OFMSW) and waste activated sludge was added in this study in order to comparative the biochemical acidogenic co-fermentation in anaerobic co-digestion. The VFA production and the composition of the acids produced from these substrates operated in anaerobic batch reactor were comparatively evaluated, as well as the performed the comparison of mono and co-digestion potential of selected substrates and the interaction (synergisms), stability between substrates was investigated.

Chapter 5 focuses on the anaerobic co-fermentation process of simulated OFMSW produced in Cape Verde with waste activated sludge (WAS) at different percentages of each substrate, determining the influence of the substrate mixture in either hydrolyzed COD, VFA concentration or methane production, without pH control during the operation assays. Based on experimental data, kinetics parameter was used in order to compare the kinetic constants for hydrolytic and methanogenic steps of anaerobic process for the proposed substrate mixtures.

Chapter 6 details evaluation of the anaerobic fermentation of OFMSW and the influence of both TS content inside the reactor and alkalinity addition. In this chapter it is described the influence of operational parameters on the process performance and VFA production, using a discontinuous pilot-scale reactor under ambient temperature conditions (25+ 2°C). Response surface methodology was used to explore the relationships between the two predictors (TS content and initial alkalinity added) and the response variables (total VFA concentration, degree of acidification and effluent quality in terms of VFA).

Chapter 7 studies the acidogenic potential of long-term of OFMSW carried out in CSTR reactor in semi - continuous mode. The influence of operational parameters (organic load rate and alkalinity added) in the performance of acidogenic system for the VFA production and composition was monitored in order to achieved the effluent rich in quantity and quality of VFA to be used as a feedstock for the culture selection and PHA batch accumulation.

Chapter 8 studies the aerobic processes for the selection of microbial culture under regime "Feast:Famine" (F:F) for a PHA accumulation. Thus, as an organic substrate was used OFMSW fermented effluent from Chapter 7 was using as feedstock for batch PHA accumulation, in order to evaluate the profile on PHA production.

Chapter 9 refers to the general discussion, as well as the suggestions for future works particularly in defining suitable waste management operations in order to achieve sustainable solutions.

2. State of the art

2.1 Wastes generation

Human activities always produce waste, and the generation rates increase with the population expansion and the economic development. The trends in intensification of urbanization and improved living standards in cities led to an increase of the amount of solid wastes (SW) and wastewater sludge generated throughout the world (Karak, 2012).

According to the European Council Directive 2008/98/CE, wastes are heterogeneous materials mixtures resulting from human activities and nature. Among the diversity of solid wastes, it can be highlighted the SW which contain a recyclable fraction (as paper), biodegradable organic wastes or organic fraction municipal solid waste (containing fruit and vegetal peels), food wastes, plastic, glass and metals, toxic substances (as paints, pesticides, used batteries or medicines) and hospital wastes. The combination of household and commercial residues is designated as municipal solid waste (Rajkumar et al., 2010). Municipal solid waste (MSW) is one of the most abundant by-products resulting from urban lifestyle with faster growth than the rate of urbanization (Wang and Nie, 2001). The MSW generated in cities around the world exceeds 1.3 billion tons per year. On average the developed countries typically generate 521.95 – 759.2 kg per person per year (kpc) and the developing countries generate 109.5 – 525.6 kpc (Global Waste Management, 2007) with estimated growth for 2015 of 4.3 billion tones.

According to Late and Mule (2013), the amount and the characteristics of MSW vary with geography, social behavior and education. Table 2-1 summarizes the amounts of MSW generated by urban population, for the year 2012 and the estimates for the year 2025 (census estimated data), in seven different regions in the words, considering data from 161 countries (Hannan et al., 2015).

It can be seen from Table 2-1 that, the MSW generation rate per capita is high in the Organization for Economic Co-operation and Development (OECD) countries. The high amount of MSW can be explained by the development level of these countries. However, in the countries designated as “underdeveloped or middle development” (as East Asia and Pacific, South Asia and Africa), the per capita generation rate of MSW is relatively low. Medina (1997) reported that many developed countries are the major sources of MSW due to the high consumption of industrialized products, which results in an increase in the amount of waste generated and this increment could add challenges to waste treatment and disposal.

Therefore, MSW management (MSWM) is becoming an emerging problem for the successful planning of efficient waste management systems and environmental sustainability.

Table 2-1: Urban population and MSW generation rate of different regions of the globe for 2012 and 2025 (adapted from Hannan et al. (2015)).

Name of the region	Number of countries covered	2012		2025 (estimated)	
		Urban population (millions)	Urban MSW generation (kg/capita/day)	Urban population (millions)	MSW generation (kg/capita/day)
Latin America and the Caribbean	33	400	1.09	466	1.56
Europe and Central Asia	19	227	1.12	240	1.48
East Asia and Pacific	17	777	0.95	1,230	1.52
South Asia	7	426	0.45	734	0.77
Africa	42	261	0.65	518	0.85
Middle East and North Africa	16	162	1.07	257	1.43
OECD	27	729	2.15	842	2.07

OECD - Organization for Economic Co-operation and Development.

MSWM has been reported by several researchers in different countries all over the world (Mohanty et al., 2014; Das and Bhattacharya, 2013; Noorjahan et al., 2012; Jafari et al., 2010; Chatterjee, 2010), and the focus in some key aspects of MSWM, such as recycling, landfilling, incineration and pollutant emissions are illustrated. According to the European Landfill Directive, one of the possibilities in waste management is to change the practice of landfilling at the same time, increasing the alternatives for waste recycling and reusing (Costuleanu et al., 2015; Gaba et al., 2014; Ghinea et al., 2014).

For Vergara and Tchobanoglous (2012), efficient MSWM is one of the most important and challenging issues throughout the world, and wastes recycling can represent a significant opportunity along with major challenges, contributing to renewable natural resources and for a plethora of inexpensive eco-friendly and sustainable materials. Some wastes, such as food waste, sludge from wastewater treatment and agricultural and industrial wastes, can be valuable sources for renewable energy production. Normally, these wastes are referred to as

organic solid wastes or organic biodegradable wastes due to high contents of carbohydrates, cellulose, lipids and proteins in their composition, and present moisture contents below 85-90% (Mata-Alvarez et al., 2000). Thus, "*The increasing volumes of waste being generated would not be a problem if waste was viewed as a resource and managed properly"* (UNEP, 2001).

2.1.1 Organic solid wastes production

The production of organic solid wastes (OSW) as the organic fraction of municipal solid wastes (OFMSW), agricultural wastes, sewage sludge or other food wastes, is a major problem in almost all cities in the world. OFMSW is considered as the most relevant organic wastes largely produced from MSW. OFMSW is a common name for the heterogeneous mixture of wastes from houses, hospitals and commercial activities such hotels, supermarkets, restaurants, canteens, companies of food production and processing in urban areas (Gupta et al., 2015; Karak, 2012; Mao et al., 2015; Miezah et al., 2015). It consists of different organic and inorganic mixed fractions of food waste, encompassing vegetables, carbohydrates, paper, wood, garden waste and other inert materials. Despite the variability in its composition, the OFMSW has a higher fraction of biodegradable organic matter and high pollution loads (Angelidaki et al., 2003).

According to What a Waste (2012), OFMSW represents about 46 ± 2 % of the world's total amount or volume of waste generated. Due to a rapid growth of global productivity from food processing industry, every day it is generated a large variety of OSW (Gupta et al., 2015; Khan et al., 2016; Kolekar et al., 2016; Miezah et al., 2015; Troschinetz and Mihelcic, 2009). According to FAOUN (2011), approximately 1/3 of the food cultivated for consumption is transformed in OSW by human activity with an annual production in the world of about 1.3 billion tones. In Portugal the wastes generation tended to increase over the years, being produced almost $4.57x10^6$ ton of MSW in 2013, with a fermentable fraction from 20 to 65 %. The majority of these wastes are deposited in landfills (51 %) and only a small fraction is used for organic valorization (13 %) (INE 2014). With regard to the fast OW growth, the European Parliament (European Parliament, 2015) adopted recently that the separate collection of the organic fraction of wastes will become mandatory in Europe by 2020. In East Asia and in Africa, the production of OFMSW is the highest percentage (62%),

compared with the OECD countries, which have the lowest percentage (27 %). Table.2-2 presents the global organic waste generation relatively with the total annual waste mass or volume (in percentage), by income, and compared between current values and values projected for 2025 (What a Waste, 2012).

Table 2-2: Global MSW generation and OW produced in % by income level for 2012 and projections for 2025 (adapted from What a Waste (2012)).

	Available Data (2012-2013) Urban MSW Generation		Projections for 2025 Urban MSW Generation	
Income Level	Total (tonnes/day)	OW produced (%)	Total (tonnes/day)	OW produced (%)
Lower Income	204,802	64	584,272	62
Lower Middle Income	1,012,321	59	2,618,804	55
Upper Middle Income	665,586	54	987,039	50
High Income	1,649,546	28	1,879,590	28

Owing to the high growth of OSW production (Table 2-2), the management strategies for these wastes have been raising a series of environmental concerns in recent years, due to the large environmental impact of landfills (Khan et al., 2016; Kolekar et al., 2016; Ma and Hipel, 2016; Mao et al., 2015; Troschinetz and Mihelcic, 2009). Nowadays, high amounts of OSW contain high concentration of organic and easily biodegradable matter and low calorific values, so traditional management practices such as landfilling, incineration, composting and animal feed are less satisfactory in terms of environmental sustainability (Zhang and Jahng, 2012).

According to EPA (2013), OFMSW, agricultural residues, waste activated sludge and some industrial wastes have a high potential for biomethane production, thus these wastes, when landfilled, produces greenhouse gases (rich in methane and carbon dioxide), odors and other gases with harmful consequences for climate and human health. With high costs associated with waste disposal, and its negative impact on environment, and with the European Union legislations for the reduction of the landfilling of OW by 75 % until 2020, it is necessary to put forward new strategies for the management and treatment of OSW (Capela et al., 2008).

Thus, the treatment of OSW through the use of technologies as anaerobic digestion (AD) or aerobic process present two of the most suitable methods for the valorization of these wastes owing to valuable products, being environmental friendly process with high economic feasibility (Álvarez et al., 2010; Ariunbaatar et al., 2015; Bonk et al., 2015; Dhamodharan et al., 2015; Grimberg et al., 2015; Lee et al., 2014; Molino et al., 2013).

2.1.2 Organic wastes produced worldwide

2.1.2.1 Coffee grounds

Coffee is one of the most popular drinks in the world and second largest traded commodity in the world after petroleum and occupies the top of the business ranking in the export of raw materials with an average annual production of 5.9 Mtons (Battista et al., 2016). It is cultivated in tropical areas where the temperature oscillates between 16 and 32 °C, and in altitudes between 500 and 5000 meters above the level of the sea.

In the last century, coffee cultivation has experienced steady growth worldwide. Globally, about 80 countries across the globe are coffee products. Despite the vast production, only ten producing countries account for 70% of world producers. Table 2-3 report the Top 10 of largest coffee producing and exporting countries in the World (data consulted on www.ico.org). Brazil and Vietnam are the highest global producers and exporters of coffee beans worldwide.

Table 2-3: The Top 10 coffee producing and exporting countries in 2015/2016 (adapted from: *www. ico.org* (2016)).

	Production		Exportation		
Rank	Country	Coffee Production (kilograms)	Rank	Country	Coffee exports (60 kg sacs)
1	Brazil	2,594,100,000	1	Brazil	45,420,000
2	Vietnam	1,650,000,000	2	Vietnam	27,500,000
3	Columbia	810,000,000	3	Columbia	11,600,000
4	Indonesia	739,020,000	4	Indonesia	6,850,000
5	Ethiopia	384,000,000	5	Ethiopia	6,500,000
6	India	349,980,000	6	India	5,005,000
7	Honduras	345,000,000	7	México	4,500,000
8	Uganda	285,300,000	8	Guatemala	4,000,000
9	México	234,000,000	9	Honduras	2,700,000
10	Guatemala	204,000,000	10	Uganda	2,500,000

Coffee exports slightly decrease to 9.13 million bags in 2016-2017 compared with 9.31 million bags in October 2015. Europe, the United States and Japan are the main consumers with annual importation of 61 %, 25 % and 7 % respectively.

According to consolidated data in (http://www.ico.org/trade_statistics.asp), world coffee consumption presented an average annual growth rate of 2.4 % since 2011 to reach approximately 9 million tons in 2014/15. In the year 2015 – 2017, it has estimated 151.3 million of 60 kg bags of coffee. Fig. 2-1 shows the worldwide production and consumption of coffee between the periods 2012/13 - 2015/16.

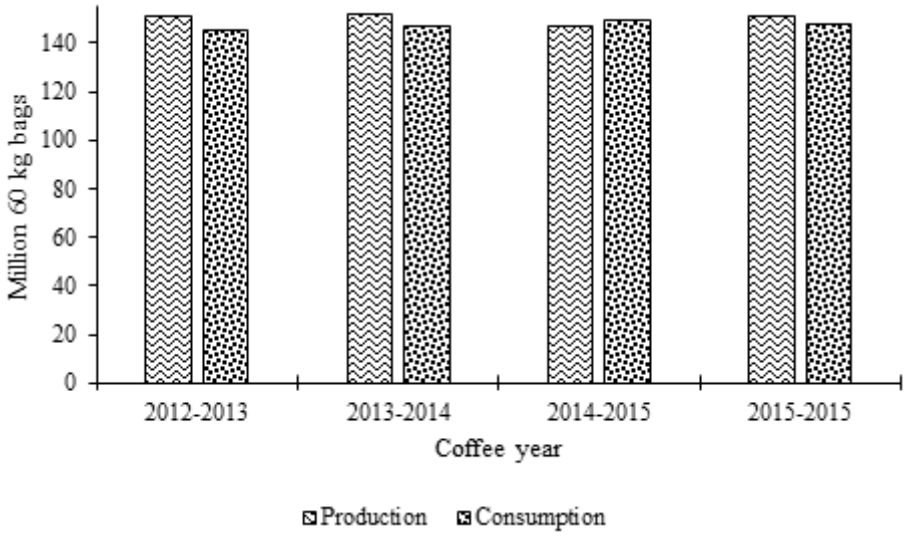

Figure 2-1: World production and consumption of coffee between 2012/13 and 2015/16 (adapted from www. ico.org, 2016).

Due to the great demand and consumption of this product, large amounts of by products and residues are generated during the entire processing of coffee beans (Li et al., 2015b; Mussatto et al., 2011), being coffee silver skin and coffee grounds the main products from coffee industry. According to Qiao et al. (2013), every year it is produced about 6 million tons of coffee grounds in the coffee beverage industry. Coffee grounds (CG) waste is a mixture of fine particles and high organic matter content, with proteins and hemicelluloses as the main carbon sources. Due to the putrefaction and the toxic characteristic of this waste, the improperly management is becoming an emerging environmental problem, polluting soil, water and air (Abouelenien et al., 2014). Hence, the use and management of coffee grounds waste in large-scale still remains a challenge worldwide, due not only to the generation of earlier gases, but also to their high contents of caffeine, free phenols and tannins, which are known to be toxic agents for many biological processes used for their treatment (Fan et al., 2003). The deposition of this waste in the environment and sanitary landfill are disposal strategies still performed nowadays. To reduce the negative environmental impact, all developed and underdeveloped countries are trying to adapt to this reality by modifying their management and treatment processes in order to maximize the recycling of a wide range of materials, lowering the emission of secondary pollutants and converting the organic matter into valuable products (Solange et al., 2011; Fernández et al., 2013; Vintiloiu et al., 2013).

Several studies have confirmed that the toxic materials in CG can be minimized by microbial degradation (Selvamurugan et al., 2010; Shofie et al., 2015). In this point of view, anaerobic

digestion (AD) is an attractive process for GC treatment to maximize recycling, helping the reduction of environmental impact and emissions of secondary pollutants, converting the organic matter present in wastes into valuable products (Fernández et al., 2013; Vintiloiu et al., 2013). The generation of bioproducts by AD such as biogas, has not been fully used so far, due to the main problems associated with inhibition by accumulation of ammonia and VFA during the anaerobic process (Shofie et al., 2015). Despite this, the use of CG for VFA production is limited and there is a need of evaluation of its functional potential.

This study focuses on the evaluation of the feasibility of the CG anaerobic acidification, in mono and co-digestion processes, to evaluate the potential acidification for this waste.

2.1.2.2 Tomato wastes

Tomato (*Lycopersicon esculentum L.*) is one of the most important crops in the industrialized countries and the second most produced vegetable in the world, next to potato, with an annual production of 171 million tons (Faostat, 2014), being Italy and Spain the main producers. Only in Portugal, about 1.4 million tons of tomato was generated annually. And, significant amounts of tomatoes are consumed either as fresh fruit or as processed product.

According with the Word Process Tomato Council (WPTC, 2014), considering about 95 % of world producers of tomatoes, every year more than 35 million tons of tomatoes are somehow processed globally and it usually represents an environmental source of pollution, during the production and processing phases, mainly due to energy and natural resources usage and associated greenhouse gas (GHG) emissions. To González-González et al. (2013), the tomato processing industry generates abundant wastes containing carbohydrates, with an average value of 80 % of total fiber, being insoluble fibers the major component.

To Del Valle Cámara and Torija (2006), tomato waste (TW) from vegetables processing activities generally contains large amounts of nutrients and bioactive compounds, such as sugars, organic acids, pigments, fibers, proteins, oils, antioxidants and vitamins, with a high potential for VFA and biogas production applying anaerobic digestion. Despite that, the most relevant agro-food industrial or processing wastes such as tomato residues and other food processing residues, are often not valorized or in some cases even wasted, or can be used for animal feeding (Bacenetti et al., 2015).

Some studies have been carried out on the potential use of several vegetable-origin by-products, including TW to reduce GHG emissions from agricultural and food processing activities (Aboudi et al., 2016; Abouelenien et al., 2014; Bacenetti et al., 2015; Fiore et al., 2016; Gohil and Nakhla, 2006).

Tommonaro et al. (2007) studied the production of biopolymers by TW via anaerobic digestion. Other authors focused on the production of energetic valorization as a suitable and effective solution to produce renewable energy (Ingrao et al., 2015; Rossini et al., 2013). Gonzalez-Gonzalez et al. (2013) used TW for biomethane production and has been achieved yields that range from 199 to 384 mL CH_4 g^{-1}VS. However, due to tomato physical-chemical characteristics as low pH (around 4.5), low buffering capacity and imbalanced nutrients (carbon and nitrogen), there are some limitations for its use as a mono-substrate in anaerobic digestion. However, it can be considered an excellent co-substrate when added to the substrate of low biodegradability (Ye et al., 2013), but there is limited information in the literature on the effect studies available the co-digestion of TW with other organic substrates. Recently, Li et al. (2016) studied anaerobic co-digestion of tomato residues with dairy manure and corn stover for biogas production and conclude that the mixture of tomato residues with dairy manure and corn stover improved methane yields, in comparison to mono-feedstock.

In Cape Verde Islands, due to the intensification of the agricultural activity, about 15,611 tons of tomato were produced in 2014 (Faostat, 2014), causing large amounts of agro-food wastes, and the amount has increased rapidly in recent years. Many of these wastes are discarded as a valueless waste. As alternative scenario, AD of TW and other organic wastes is evaluated as a strategy to reduce the environmental load of agro-food wastes and at the same time to generate high value-added products such as VFA and biogas. In addition, the digestate can be used as soil conditioner or organic fertilizer.

2.1.2.3 Wasted activated sludge

Waste activated sludge (WAS) is the main byproduct of the physical, chemical and biological processes used in wastewater treatment plants (WWTP) and represent up to 50% of the current operating costs of a WWTP (Baeyens et al., 1997). Municipal WWTP are increasing significantly year by year in the world, due to rapid development of population and urbanization. In Cape Verde Islands, it has been observed the increases of wastewater

generation, which resulted in an increase on the amount of sewage sludge derived from the high amount of wastewater being treated. Most of the WAS generated is currently deposited in the city dumps, causing serious pollution problems because of the poor treatment, although some fractions of WAS are already used as agricultural soil conditioning. Therefore, is urgent to develop strategies for treating the generated WAS, in order to improve its characteristics and to reduce the associated health problems that these type of wastes can cause.

Currently, many conventional ways have been tried to treat WAS, including the landfilling, combustion and composting for further use on agricultural crops. Considering the waste stabilization and energy recovery, the interest has gradually focused on AD, which is considered the major and an essential technique to treat WAS. Applying AD it is possible to reduce the sludge volume, to generate methane-containing biogas, which is an important future contributor to the energy supply, and to obtain a nutrient-rich final product (Appels et al., 2008; Appels et al., 2011). However, due to a generally slow hydrolysis step, the biogas production from WAS in mono-digestion has a relatively low gas yield and the AD performance is largely limited, taking into account that WAS is mainly composed of microbial cells within extracellular polymeric substances and cell walls, which are physical barriers that is difficult the degradation in anaerobic conditions (Toreci et al., 2011).

The co-digestion process of WAS with other organic-rich wastes seems to be an attractive economically viable method, and it has been used to overcome its low digestibility in several studies (Di Maria et al., 2016; Gao et al., 2016; Silvestre et al., 2011; Zahedi et al., 2016). Some studies have been conducted on the optimization of AD for treating the WAS using co-substrates using a variety of organic wastes such as OFMSW, due to its low concentrations of inhibitors and alkalinity (Lin et al., 2011). The addition of municipal biowaste to improve WAS digestion proved that the small-size particulate organics present significant influence on the biodegradation rate during the co-digestion process (Gao et al., 2016, Zahedi et al., 2016).

Jang et al. (2015), in their study about thermophilic anaerobic co-digestion system between a mixture of manure, WAS and food wastewater (FWW), observed that the increasing of FWW from 0 % to 100 % of mixing ratio, increased the biogas production and the organic matter removal. The highest organic matter removal and biogas production (VS removal of

77 % and 1422.50 mL CH_4 (L d)$^{-1}$, respectively) were achieved when FWW mixing ratio was 75 %.

Naran et al. (2016), studied the effect of pretreatment and the anaerobic co- digestion of WAS and food wastes (FW) both in mesophilic conditions. Results showed that co-digestion of substrates conferred superior result than mono digestion with FW or WAS. Estimated parameters (methane production potential and the rate) with Gompertz equation also inferred that co-digestion increased methane production significantly.

Bolzonella et al. (2006) reported that the co-digestion of WAS and OFMSW in a full-scale facility of 90,000 population equivalent led to an increase in biogas production from about 600 to 950 m^3 per day. Methane concentration ranged from about 66% v/v to 68% v/v, whereas the organic loading rate (OLR) of the existing digester went from 1.02 kg VS/m^3 day to 1.21 kg VS/m^3 day.

Similar performances were also reported by several authors highlighting the advantages of co-digestion using WAS and OFMSW (Di Maria et al., 2015; Garcia-Pena et al., 2011). Thus, OFMSW is an excellent co-substrate, supplies the nutrients required for bacterial growth, and dilutes the feedstock as a result of its low hydrolyses.

2.1.3 Production and characterization of MSW in Cape Verde

Cape Verde archipelago is located between parallels 17º 12' and 14º 48' north latitude and meridians 22º 44 'and 25 22' west longitude, at a distance of about 500 km from the western coast of Africa, with an average annual temperature from 20 to 26 ºC, and an absolute maximum temperature that can exceed 32 ºC (De Vit and Parry, 2012).

Municipal solid waste generation is rapidly increasing in Cape Verde, mainly in the urban areas because of the dynamic development of the archipelago, boosted by the demographic growth and the increase on tourism, which started to create massive problems in MSW management (Tavares et al, 2011). In addition, as a result of this development, there was an increase in the amount of municipal wastewater generated, which in turn increased the accumulation of WAS produced in wastewater treatment plants, hence contributing to the problem of waste disposal and management (Appels et al., 2008).

The production of MSW in Cape Verde had increased from about 100 thousand tons in 2003 to c.a. 140 thousand tons in 2012, leading to an average increase of 3.5 % per year (Rio, 2012). The OFMSW corresponds to 30 – 47 % of the total weight of MSW produced results from residential waste and commercial activities in urban areas.

In Cape Verde, the system for MSW disposal and treatment is still underdeveloped. A significant part of MSW is deposited in landfills, but nowadays, there is still a lot of daily MSW produced in Cape Verde, particularly in Praia city (this case of study), are deposited in called "dumps" without any prior treatment, making the waste management of OW still incipient. There is no selective separation of organic fraction at the production source and nearly 63 % of it end in landfills. The other portion of these wastes is used for family animal feed or little domestic composting.

Due to absence of published data that thoroughly explain the composition and the characteristics of MSW in major cities of Cape Verde Islands, it was performed during the last two years (2013 to 2015), there been conducted a intense study of 100 families selected in different districts of the Praia city (Santiago) and also in municipal dump, in order to qualify and quantify the organic wastes. Thus, the residues were separated by category using specific bags in family houses, measured using a digital scale in wet weight basis. Fig. 2-2 shows the composition of MSW produced in Praia city in year 2013 to 2015. It can be seen that organic wastes are the highest fraction of total MSW (47 %), followed by plastic (15 %) and glass (11%). Others, including hazardous waste and not identified wastes account for less than 5 % of production. Construction residues were not included in this study.

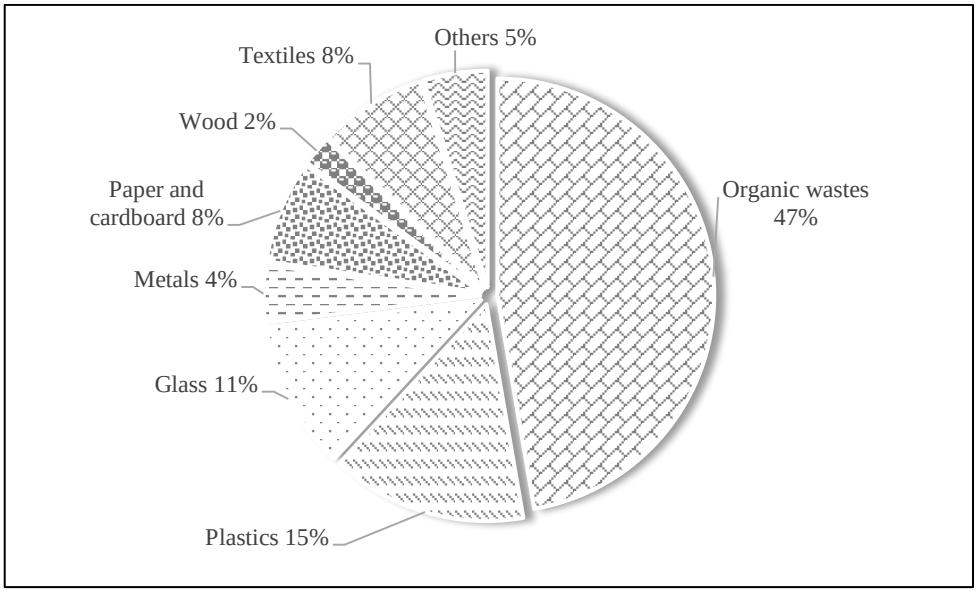

Figure 2-2: MSW composition and composition for the Praia city (data from 2013 – 2015).

2.2 Anaerobic digestion process

2.2.1 Historical perspective

Anaerobic digestion (AD) is one of the main processes used for organic wastes stabilization and is considered as the most effective method for the treatment of MSW. AD is a natural process that occurs with organic waste in confined space such as landfills and agricultural soils. Scientifically, AD is defined as the method to converts organic matter by a variety of anaerobic microorganisms into carbon dioxide and methane in environments without oxygen (Gijzen, 2002). This engineering method was verified by Robert Boyle and Stephen Hale, when they uncovered natural decomposition of organic matter in the sediment of streams (Fergusson and Mah, 1987). Nevertheless, this method gained attention when Alessandro Volta discovered methane in 1776, collecting gas from marshes (Moletta, 2008).

After a century, in 1859, India has installed the first digestion plant in the Build-Bombay (Meynell, 1976). In 1997 AD was recognized by the United Nations as one of the promising sources of energy supply, and then was considered key to combat poverty and the energy crisis in developing countries (Rio, 1997). Nowadays, AD is still one of the most successful alternative and innovative treatment technology for MSW such as OFMSW, agricultural wastes and industrial wastewater treatments applied during the last 20 years, due to its capability of reduction the biological oxygen demand (BOD) of wastes streams and

producing renewable energy (Bharathiraja et al., 2016; Cabbai et al., 2015; Chen et al., 2015; Dahiya et al., 2015).

The use of AD results in a number of benefits including social and public health because biogas combustion results in very low air emissions and decreased air pollution. Socially, this technology provides energy to a wide range of human survival activities. In addition, the effluent from anaerobic digestion, is rich in primary nutrients (nitrogen, phosphorus, potassium), which has agronomic benefits if used as a soil amendment to improve plant growth (Kinyua et al., 2016). Figure 2-3 shows the interrelationships between substrate characteristics, operating parameters and biochemical conditions within the digesters and these conditions translate the benefit of AD and Table 2-4 summarizes the main advantages of AD over other forms of waste treatment applied worldwide nowadays.

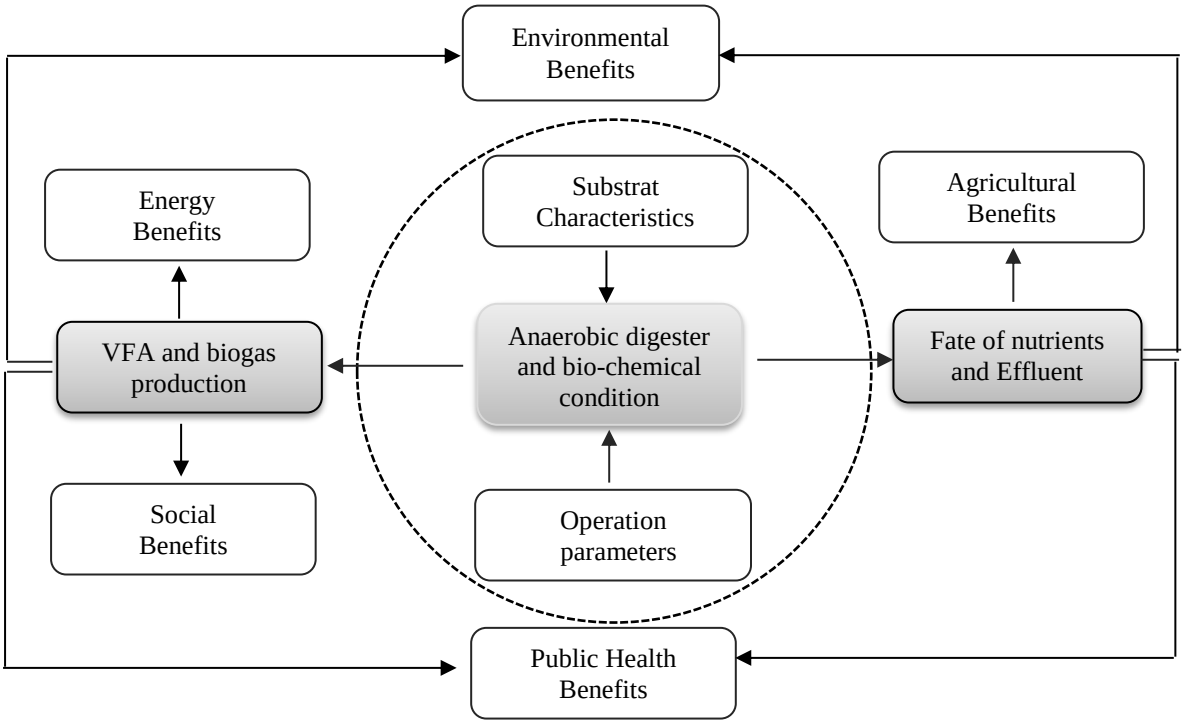

Figure 2-3: Schematic example relationship between digester operation and benefits (adapted from: Kinyua et al., 2016).

Table 2-4: Main advantages of AD of the organic wastes treatment.

Advantages	Reference
→ Allows low emission of secondary pollutants, and is high economically feasible	Zhang et al. (2015)
→ The possibility of nutrient recycling and reduction of waste volumes	Monnet (2003)
→ A direct positive effect on greenhouse gas reduction	EPA (2013)
→ Production of sanitized compost	Álvarez et al. (2010)
→ Effective pathogen removal	Cabbai et al. (2015)
→ Less biomass sludge is produced in comparison to aerobic treatment technologies	Ward et al. (2008)
→ Treat high organic loading rates with low sludge production	Dhar et al. (2015)
→ Recycling possibility and waste-to-energy approach	Matthew and Themelis (2007)
→ Efficient method of bio hydrogen production	Bharathiraja et al. (2016)
→ A biogas facility generates high-quality renewable fuel	Mao et al. (2015)

Comparing AD and other alternative treatment technologies (gasification, pyrolysis, incineration, biological drying and others), AD have large advantages, since it requires less investment cost than the thermal conversion technologies (Murphy and McKeogh, 2002) and also have limited environmental impact. Because of this, several studies of the above points have been related to practical industrial applications (Arroja et al., 2012; Batstone et al., 2002; Bonk et al., 2015; Holm-Nielsen et al., 2009; Kinyua et al., 2016; Mata-álvarez, 2015).

Due to their advantages, only in Europe more than 36,000 anaerobic digesters are operating to deal with the organic fraction of MSW as a significant portion of the feedstock (De Baere and Mattheeuws, 2010). A good example of implementation of AD technology for energy production is described by DiStefano and Belenky (2009). Results show that the 127 million tons of MSW annual produced and deposited in landfills in the United States could be biologically converted to (theoretical) 5.9 billion m^3 of methane. Others countries with a successful example of AD application are Germany, with about 2 million tons of annual capacity, Spain with 1.6 million tons and the Netherlands and Switzerland become the highest in installed annual capacity, with about 52,400 tons and 49,000 tons per million people respectively (Baere and Mattheeuws, 2012; Mata-álvarez, 2015).

Other studies showed the potential of the AD of organic wastes for the generation of other added-value products such as volatile fatty acids (VFA). VFA are produced as intermediates in the AD and could subsequently be extracted and used as raw material for the production

of other high-value fuels and chemical-basis products as liquid hydrocarbon (Yin et al., 2016a, 2016b) or for PHA production (Elain et al., 2016; Korkakaki et al., 2016; Morgan-Sagastume et al., 2015; Queirós et al., 2014). Furthermore, these processes could be integrated into a possible strategy for the reduction of municipal solid wastes and value generation (Kumi et al., 2016; Kuruti et al., 2015; Lee et al., 2014; Michele et al., 2015).

Acidogenic fermentation process is an important step in AD of organic compounds. In acidogenic fermentation, monomers are formed from hydrolysis of organic compounds by hydrolytic microorganisms and consequently the bio-products such H_2, VFA and CO_2 are habitually formed (Arroja et al., 2012; Venkata Mohan, 2009). Thus, the production of byproducts from acidogenic fermentation become an attractive alternative to override products derived from fossil fuels (Mohan et al., 2009).

However, AD process also has disadvantages such as high initial time adapting of to adapt the biomass, high sensitivity to toxic compounds, need for addition of alkali and sometimes high-energy consumption (Metcalf and Eddy, 2004).

2.2.2 Microbiology and biochemistry

Under anaerobic conditions, complex organic compounds present in the organic wastes are catabolized through a series of steps by complex consortia of microorganisms in the digester, in order to convert a variety of intermediates products into CH_4 gas and CO_2 (Lozano et al., 2009). This process is divided in four microbial steps, which can be classified as hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Figure. 2-4 illustrates this AD process, with some details about each step of this process.

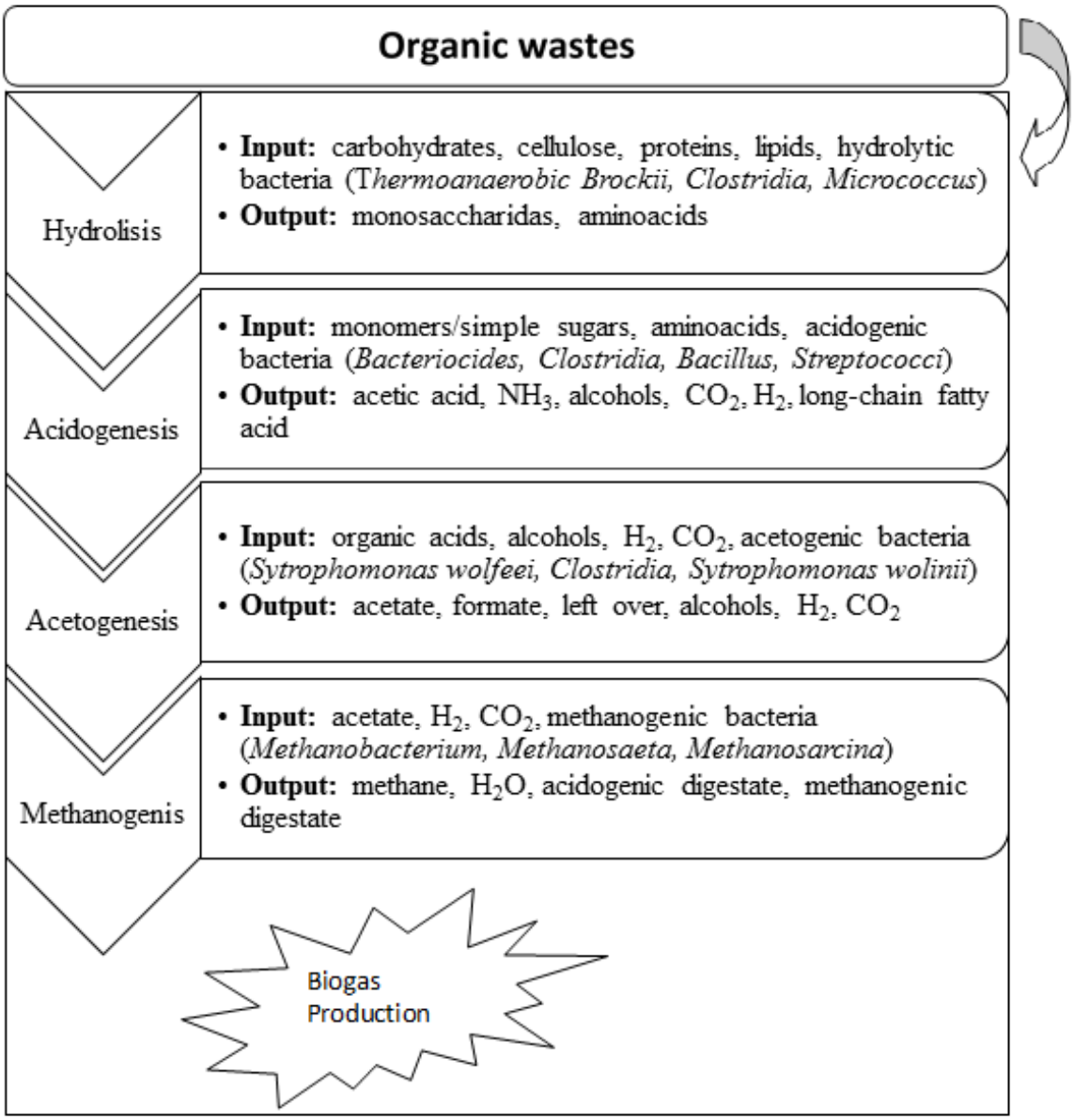

Figure 2-4: Process Diagram for Anaerobic Digestion of Organic Municipal Solid Waste (Modified from Naik and Wung (2013) and Stronach et al., 1986)

2.2.2.1 Hydrolysis

This is the first step of the AD process. In this step, complex particulate matter (e.g. proteins, cellulose, lignin, and lipids) is converted into dissolved compounds such as amino acids, simple sugars, glycerol, and fatty acids with a lower molecular weight, by extracellular enzymes. Generally, most of the particulate soluble products need to be converted into smaller molecules, in order to be transported through the cell membrane of the hydrolytic

microorganisms. Li et al. (2011) refereed some of these hydrolytic microorganisms, namely *Thermoaerobic Brockii, Ruminococcus, Clostridium, Erwinia, Micrococus and Streptomyces.* The process requires the mediation of exo-enzymes that are excreted by fermentative bacteria. Proteins are degraded via (poly) peptides to amino acids, carbohydrates are transformed into soluble sugars (mono and disaccharides) and lipids are converted to long chain fatty acids and glycerin (Batstone and Jensen, 2011).

The hydrolysis or solubilization is relatively a slow process with significant importance in the high organic content wastes and may become the rate-limiting step in all AD process that determines the conversion efficiency of the biomass feedstock (Lee et al., 2011). In consideration of this process, much attention was being paid to the hydrolysis of carbohydrates and it is recommended the control of parameters as temperature and alkalinity to obtain at good hydrolysis/solubilization yield in the digesters (Liu et al., 2008; Tang et al., 2014). In addition, the kinetics of hydrolysis process is also dependent on the type and the characteristic of waste used (Lee et al., 2014).

The single first-order kinetics or Monod kinetics is usually used in this step to expressed concepts of process solubilization, biodegradation or hydrolysis, since the enzymatic activity is not directly coupled to the bacterial growth (Beevi et al., 2015). A wide range of hydrolysis rate constants concerning the hydrolysis of carbohydrates, proteins and lipids have been reported assuming first-order hydrolysis (k_H) and it is very dependent on the initial experimental conditions such as particle size, pH, stirring conditions, inoculum/substrate ratio and solubility of the proteins (Fernández-Rodríguez et al., 2013).

2.2.2.2 Acidogenesis

Acidogenesis or acidification step is driven by a very diverse group of bacteria, the majority of which are strictly anaerobic. These heterogeneous microbial population, which could be composed by *Bacteriocides*, *Clostridium, Bacillus* or *Streptococcis*, among others are responsible for consuming the soluble organic matter obtained in the hydrolysis reaction (sugars, amino acids, and fatty acids) and convert these products into various intermediate such as short chain fatty acids (e.g., acetic acid (C_2), propionic acid (C_3), butyric acid (C_4) and valeric acid (C_5)), carbon dioxide (CO_2), hydrogen gas (H_2) and ammonia (NH_3) at very low pH (Chen et al., 2014; Gao et al., 2015). In addition, short chain carboxylic acids can be

further used because they are sources of production of many organic compounds, including alcohols, aldehydes, ketones, esters, olefins, and the others (Singhania et al., 2013).

In turn, the higher chain fatty acids (C3 and above) are further oxidized to acetic acid through the action of syntrophic bacteria (H_2-producing acetogenic bacteria). Acetic acid can also be formed by homo-acetogens using H_2 and CO_2 (Dahiya et al., 2015).

Recently, much attention has been paid to the acidogenic system, due to the multiplicity of industrial applications of its by-products. In this perspective, several studies also exist on the production stoichiometry of the various products coming from carbohydrates and/or proteins metabolism (Van Haandel et al., 2006). Generally, food waste (FW) with high biodegradability is considered as an excellent substrate for acidogenic fermentation.

This step of AD process has been mainly used for optimization of VFA production and some environmental conditions parameters such as short solid retention times (SRT), hydraulic retention time (HRT), mesophilic temperatures and acidic values of pH (ranged 4.5–5.5) are essential to avoid the growth of specific microorganism populations and consequentially perform a variety of oxidation-reduction reactions (Lee et al., 2014). Most studies on fermentative VFA production have been based on mesophilic temperature. The addition of the chemical compound 2-bromoethanosulfophate (BES) to inhibit methanogenesis activity is other of the strategies used for enhance VFA production (Grootscholten et al., 2013).

In general, acidogenic fermentation can be considered a way of pretreating a substrate because biological degradation occurs and acidic conditions are produced. VFA and H_2 are two important added value products derived from acidogenic fermentation (Lee et al., 2014), which are also subsequently used by methanogens to produce methane. In terms of further valorization, H_2 has been widely recognized as an ideal alternative energy source to substitute fossil fuels, which if it is harvested properly in an integrated approach, it will make the whole process environmentally sustainable and economically viable (Bharathiraja et al., 2016; Dahiya et al., 2015).

2.2.2.3 Acetogenesis

In the third step, called acetogenesis, the higher organic acids and alcohols produced by acidogenic bacteria are further digested and converted into acetic acid, as well as CO_2 and H_2, by acetogenesis *Archaea* microorganisms. This conversion is controlled largely by the

partial pressure of H_2 in the mixture. Acetogenic bacteria as a *Syntrophomonas wolfei*, *Clostridium*, and *Syntrophomonas wolinii*, further breaks down the H_2 and CO_2 to produce mainly acetic acid and organic acids and alcohols are converted into acetate. The acetate produced in the acidogenesis serves as a substrate for methane forming bacteria whereas the alcohols are oxidized by the bacterial population claimed as *acetogens*, to produce H_2 and CO_2 (Naik and Wung, 2013). In the presence of hydrogen oxidizing acetogenic bacteria referred as *homoacetogens*, H_2 and CO_2 are further converted to acetate, in a process claimed as *homoacetogenesis* via the acetyl-CoA pathway (Khanal, 2008; Ye et al., 2014). The first three steps of AD are often grouped together and are called as acid fermentation, where no organic material is removed from the liquid phase.

Several studies report that acetogenesis is the most important step in the metabolism of propionic acid, due to the importance of this compound in the stability of anaerobic systems at real scale (Nielsen et al., 2007; Gallert e Winter, 2008). In addition, other products such as alcohols and long chain volatile acids (from 4 to 7 carbon atoms) may be obtained from the metabolization of propionate by anaerobic bacteria (Lens et al., 1996).

2.2.2.4 Metanogenesis

The final step of AD process is the formation of methane, also called methanogenesis. Methanogenic microorganisms convert the previously formed acetic acid into methane, CO_2 and water under strict anaerobic conditions. These microorganisms are also classified as *Archaea*, composed of both gram-positive and gram-negative microorganisms with a wide variety of shapes, e.g., *coccoid* and *bacilli* (Michael and Constantinos, 2006). Around 66 % of the methane is formed from acetate by an acetate decarboxylation process, where methanogenic microorganisms as *Methanococcales, Methanobacteriales, Methanomicrobiales and Methanosaeta sp.* are involved. The remaining 34 % of the methane is formed from carbon dioxide reduction using hydrogen (Chandra et al., 2012). The methanogenic microorganisms are considered to cover the majority of the methanogens encountered in anaerobic digesters. From the referred microorganisms, the *Methanosaeta sp*. has the unique characteristic of relying on acetate as the sole energy source; the other three are H_2-utilizing methanogens (Lee et al., 2009). For this efficiency, methanogenesis is regarded as the key step in anaerobic digestion (Appels et al., 2008).

Methanogenic microorganisms are very sensitive to temperature, loading rate, pH value and type of substrate. The optimal conditions if pH is in the 7 – 7.5 range, although the optimal value varies with substrate and digestion process (Liu et al., 2012). In the case of temperature, three ranges of temperature are usually suggested: thermophilic range (45 – 60 °C), mesophilic range (30 – 40 °C) or psychrophilic conditions (about 20 ºC). Laboratory scale assays are usually performed at 37 °C or 55 °C (Molino et al., 2013).

Another important parameter is chemical oxygen demand (COD) of biowastes during anaerobic degradation process, frequently carried out in batch mode. According to Álvarez et al. (2010), CH_4 production can be evaluated from the COD balance in the system, based on the COD removed.

In general, the biochemical methane production is the optimal operation process to combine the synergies between the substrates and inoculum concentration to finally obtained biomethane production (Ariunbaatar et al., 2015).

2.2.3 Anaerobic digestion of organic solid waste

The introduction of AD in to treatment of OSW is the major progress and innovative technology in the last two decades and an even preferred method for the intensive biodegradation phase of OSW (De Baere and Mattheeuws, 2010). Therefore, several studies of AD using OSW as carbon source to produce biogas and a stable solid compost were published in recent years. According to the Micolucci et al. (2016) at present some 8 million tons of OSW are anaerobically digested within EU Countries. Many of OSW are usually comprised of carbohydrates, lignocellulosic, cellulose, hemicellulose, lignin, lipids and proteins, and they are suitable for use in producing renewable natural resources through anaerobic digestion (Bharathiraja et al., 2016; Fantozzi and Buratti, 2011; Lee et al., 2014; Michele et al., 2015; Yin et al., 2016a, 2016b). Neves et al. (2008) reported that organic wastes with high lipids content are excellent substrates for biogas production, but in terms of the hydrolysis rate, it was found the lowest kinetic constant in AD assays fed with kitchen waste with excess of lipids.

Owing to variability in its composition and quality in terms of low level of impurities (<10%) OFMSW is considered as one of the main wastes for AD processes due to a high volatile

solids contain (85 – 95%) and 75 – 85% moisture, favoring the microbial development (Zahn et al., 2014).

AD of organic wastes could be classified, taking into account the amount of solids as wet (between 4 % and 10 % of Total Solids – TS) or dry (between 20 % and 30 % of TS). The TS content inside the reactor also influence the bioprocess efficiency, in terms of VFA production and, ultimately, in methane generation (Liotta et al. 2015). Same authors refer that high TS content decreases the methane formation due to limitations in mass transfer, decreasing the performance of the system (Le Hyaric et al. 2012). As a result, the biogas production is usually higher at low TS content than the observed at dry processes (Vaz et al., 2008).

Due to several limitations in achieving a stable reactor performance and high biogas production, this technology is not always economically viable when applied to readily biodegradable wastes, such as OFMSW (Fernández-Rodríguez et al., 2015, 2013, Fonoll et al., 2015a, 2015b). According to Murto et al. (2004), the AD performance is much dependent on substrates composition. Recent researches indicate that OFMSW as an easily biodegradable substrate for AD with high carbohydrate, lipid, and protein content, is responsible for rapid decrease of pH due to a acidification (Angeriz-Campoy et al., 2015; Gameiro et al., 2016; Núñez Fernández et al., 2013). Because of that, OFMSW is widely used for VFA production (Dahiya et al., 2015; Gameiro et al., 2016; Silva et al., 2013). Up to the present, numerous studies had been devoted to maximize the production of VFA from various organic wastes.

It is known that, AD is a proven and established technology for the treatment of organic solid wastes (Capela et al., 2008). Currently studies are being developed in order to achieve a better stability of AD. Thus, it is urgent to solve the constraint concerning the effects of changing the input of a digester and how the waste composition influences the overall stability of the process.

2.2.4 Anaerobic co-digestion of organic solid waste

Anaerobic co-digestion (AcoD) consists on the biological degradation of different co-substrates (two or more) that are mixed and treated together (Fernández et al., 2005), in order to improve the performances of AD, aiming to optimize the biological treatment of these

wastes and also to increase the generation of products with economic value (Di Maria et al., 2016; Gao et al., 2015; Maria and Micale, 2016). This methodology is common in WWTP, as it some intrinsic advantages when compared the digestion of a single substrate. As advantages are highlighted the synergistic effect of nutrients, the increase of biodegradable organic waste load to the reactor, the dilution of inhibitory compounds, the stabilization of the digester ecosystem, and the improvement of biostimulation due to the excess of nutrients provided by its enrichment in substrate composition and higher biogas yield (Cavinato et al., 2013; Nielfa et al., 2015; Zhang et al., 2014).

Due to the advantages of the AcoD process, several studies have been conducted on the optimization of AD for treating OFMSW with low biodegradable substrates, such as WAS, Coffee ground and other (Callaghan et al., 1999; Sosnowski et al., 2008). Hamzawi et al. (1998) and Sosnowski et al. (2003) referred that the AcoD of OFMSW with WAS created value through the biogas production.

A detailed knowledge of OFMSW as a co-substrate in the co-digestion process is reported in several studies: the co-digestion of OFMSW with fats of animal and vegetable origin (Fernández et al., 2005), kitchen wastewaters (Tawfik and El-Qelish, 2014), sewage sludge (Borowski, 2015), biological sludge (Nielfa et al., 2015), and other. These approaches allow achieve a successful and efficient biodegradation of the referred wastes, and also enable the use of digestate obtained in agriculture (Di Maria et al., 2016).

In terms of biogas generation, Jang et al. (2015) achieved a maximum biogas production about seven times higher in the co-digestion process when compared to the single digestion of WAS. The performance of different types of reactors and the efficiency of OFMSW bioconversion used as co-substrate have been widely studied (Hartmann et al., 2003). In this respect, kinetic models may be very useful tools to understand the biodegradation of different substrates (Mottet et al., 2013; Sánchez et al., 1996) and the efficiency of the anaerobic process (Li et al., 2009), providing some key parameters for the optimization of the overall anaerobic process (Fernández-Rodríguez et al., 2013).

The temperature is also one of the key parameters to improve the performance of bioreactors operated in co-digestion (Micolucci et al., 2016), especially in what concerns to bacterial growth and to speed up the hydrolysis by the biomass. The temperature changes during the co-digestion of WAS with OFMSW, in order to avoid an overload of organic substrate

during the microbial shift from mesophilic (35 ºC) to the thermophilic temperature (45 ºC), it was concluded that thermophilic range can be considered as the best condition in a co-digestion process in terms of bacterial growth and biogas production.

Kim et al., (2003) referred to the feasibility of food waste as a co-substrate in anaerobic digestion of sewage sludge, using batch tests. These authors observed that the mixed food waste led to an increase in CH_4 production, both at mesophilic and thermophilic conditions.

Based on mixing and homogenization, the choice of co-substrates and the percentage of total solids (TS) inside the reactor are also requirements of high importance for the performance of the co-digestion. Nielfa et al.(2015) and (Sosnowski et al.(2003) found that the optimal mixing ratios of sewage sludge (75% vol.) and OFMSW (25% vol.) increased biogas production. Similar results were found by Lebiocka and Piotrowicz (2012), using comparable biowastes.

Processes with high TS content (dry processes) have less sensitivity to the input of the untreatable materials to the reactor because there is no synergistic effect of particles inside the reactor, as it is observed in wet systems (Capela et al., 2008).

In general, AcoD is known as an effective technique for bioconversion of different high-strength biowastes and energy recovering. However, this technique can be used in the optimization of OSW management in developing countries, where the volume of waste is relatively low (Gao et al., 2015). Table 2-5 shows some examples of AcoD of different types of OSW, with the operating temperature and products (VFA and methane) generated.

It should be noted in the Table 2-5 that the sewage sludge and OFMSW are the main co-substrate used in AcoD for bioenergy production. According to Cavinato et al. (2013), waste activated sludge (WAS) has a relatively low biodegradability and carbon to nitrogen ratio (from 6 to 16), which makes it hard to digest, with limited volatile solids reduction. Thus, mixing WAS with OFMSW with high carbon to nitrogen ratio (25 to 30) provides an improved carbon to nitrogen ratio balance and access to essential micro and macronutrients (Di Maria and Micale 2016; Hong and Haiyun 2010). Among the benefits of mixing organic wastes in AD, it is highlighted the increase in biogas production (Cavinato et al. 2010; Chen and Chen 2010) and concomitant organic matter degradation, which can be considered responsible for increasing the economic feasibility (Martínez et al., 2012).

Table 2-5: Different types of solid organic waste from the co-digestion.

Substrate	Co-substrate	T ºC	VFA mgCODL^{-1}	Methane (L kg^{-1} VS)	Comments	Reference
Cattle manure	Agricultural waste	35	N/d	620	Significant increase in biogas production from the AcoD.	Cavinato et al. (2010)
Sewage sludge	MSW	35	N/d	532	Biogas production increased with increasing proportions of the MSW.	Sosnowski et al. (2003)
Food wastes	Sewage sludge	35	N/d	2.1	Optimization of the process efficiency in terms of biogas and biomethane yield was achieved by increasing the organic loading rate.	Di Maria and Micale (2016)
OFMSW	WAS	37 to 55	52.6	0.57	Thermophilic option can be considered as the best condition in a co-digestion process in terms of biogas yields	Cavinato et al. (2013)
Food wastes	sludge	35	29,100	N.d	Process efficiency in terms of VFA production was achieved by increasing the organic loading rate semi-continuous reactor	Hong and Haiyun (2010)
Primary sludge	WAS	21	118.21	N.d	Mixing ratio 1:1 (on VSS basis) on batch reactor, VFA production increased with maximum organic load content (22,25mg COD L^{-1})	Chen and Chen (2010)

N.d: not determined

2.3 Key parameters in anaerobic digestion of solid waste

The anaerobic digestion process represents an integrated system of a biological process of microbial community and energy metabolism. Several parameters can affect the balance and the synergies of microorganisms during the process, thus, most of the microorganisms are sensitive to variations in the operating conditions applied (Ye et al., 2007). The most relevant conditions that affect AD are the temperature, the pH and alkalinity, the retention time, the organic loading rate, the nutrient species and the presence of inhibitory compounds. Each of the key parameters are detailed below.

2.3.1 Temperature

Temperature is an important environmental factor, which directly affects the dynamic situation of microorganism yields, and speed up hydrolysis of biomass (Micolucci et al., 2016). Fernández-Rodríguez et al. (2015) classified the biological process in accordance to the range of temperature applied: psychrophilic (0 – 20 °C), mesophilic (30 – 37 °C) and thermophilic (45 – 60 °C). Generally, thermophilic (in the range of 45 – 55 ºC) and mesophilic (in the range of 33 – 35 ºC) conditions are the most common in AD processes. Besides that, thermophilic digestion is reported to be the more efficient method for microorganism metabolism growth, for the hydrolysis step and sometimes to enhance the acidification process, inhibiting the biogas production (Li and Fang, 2007). The kinetics of hydrolysis is also increased with the thermophilic range, although many of the microorganisms (such mesophilic bacteria) are sensitive to high temperatures (Jang et al., 2014).

Other studies demonstrated that the thermophilic condition is efficient in terms of organic matter removal and biogas production in AD process. Cavinato et al. (2013) reported that the biogas yield of anaerobic digestion of OFMSW at thermophilic conditions is much higher than in mesophilic conditions. According to Mackie et al. (1998), thermophilic digestion processes support higher loads with reduced hydraulic retention time, and it is observed much higher conversion efficiency of organic matter and pathogen disinfection.

However, at industrial scale, most studies on anaerobic digestion process based on mesophilic temperature conditions (Ağdağ and Sponza, 2005; Agyeman and Tao, 2014; Ariunbaatar et al., 2014; Astals et al., 2011), because the process has low energy

requirements, is more stable and also inhibits the ammonia formation (Beevi et al., 2015). On the other hand, in developing countries most digesters operate at ambient temperatures (in range of 20 – 30 °C), allowing to save in the extra heat supplying, but in terms of methane production it is observed lower yield, comparing with mesophilic process (Amani et al., 2010). For this reason, several authors recommend the increasing on the temperature within the ambient and mesophilic temperature ranges for the enhancement of hydrolysis and acidogenic steps, increasing the concentration of VFA produced (Mao et al., 2015; Yuan et al., 2011; Zhang et al., 2009).

2.3.2 pH and alkalinity

The pH values in biological systems have a significant effect on the growth rate of microorganisms and their dynamics, mainly AD, due to the regulation of all AD steps (Jie et al., 2014). The metabolic pathways of the microorganisms in anaerobic conditions is dependents greatly on the changes in pH value. In the acidogenic process, when pH is around 6.5 or less the accumulation of VFA species is favored and the inhibition of the methanogenic step occurred (Ruggeri et al., 2015). The pH ranges between 6.5 and 8.0 is the most advantageous for methanogenesis phase and for the methanogenic bacteria activity, being the neutral pH (at 7.0) considered as the optimal value (Zonta et el., 2013). However, several authors have shown that the specific ranges of pH in AD are dependent on the type of waste material used and on the bioreactor configuration (Khan et al., 2016; Wang et al., 2016). For example, Wang et al (2016) found the optimal pH values for acidogenic digestion of kitchen waste around 7.0 allowing the highest solubilization percentage of the complex compounds as well as the highest VFA accumulation in the system. On other hand, Bengtsson et al. (2008) reported in their study that the optimum pH conditions for the VFA production from wastewaters ranges from 5.25 to 6.0.

The pH value depends also on the alkalinity of the substrate (Lee et al., 2014). Alkalinity is the capacity to neutralize acids during the process and therefore to mitigate pH changes, expressed as a concentration of $CaCO_3$. According to some authors, alkaline condition enhances the solubilization of organic material and improves the efficiency of the anaerobic treatment. Low alkalinity causes an acidification of the digester and an increased VFA concentration inside of the rector (Zhang et al., 2009). The optimal alkalinity values for

anaerobic digestion process are mainly in the range from 2000 to 18000 mg $CaCO_3$ L^{-1}, but the wide range of alkalinity is dependent on the substrate used (Cuetos et al., 2008; Lee et al., 2014; Murto et al., 2004). Nevertheless, a very low pH (extremely acidic) or high alkalinity in the system causes inhibition of the acetogenic bacteria and do not promote the methanogenesis (Liu et al., 2012). In addition, pH can also affect the profile of VFA produced during the acidogenic fermentation, mainly acetic, propionic and butyric acids (Wang et al., 2014). According to Gameiro et al. (2016), the optimal pH values for propionic acid production were in the range of 4 – 5.5. For the acetic and butyric acids, it was observed a range of pH from 6.0 to 6.5.

2.3.3 Retention time

The retention time is an important parameters in AD and it is dependent on the type of substrates and temperature. Retention time of the waste is known as the residence time needed for a complete degradation or as the limit of anaerobic microbes to degrade substrates inside the digester (Li et al., 2010). This period includes parameters such as hydraulic retention time (HRT), solid retention time (SRT), volume of the reactor and substrate composition (Kim et al., 2013). The research investigation from Bengtsson et al. (2008), reveals that the production of both VFA and CH_4 depends greatly on the HRT. For instance, HRT from 2 to 4 days, in general has a positive effect on the CH_4 production, but an HRT less than 2 to 4 days, tend to inhibit the activity of methanogenic bacteria and also results in a higher concentration of VFA in the reactor (Cysneiros et al., 2012). Romero Aguilar et al. (2013) reported similar observation, where it was found best performance of acidogenic digester at low HRT (1.9 days). Lee et al. (2009), also reported that VFA increased with HRT in a range of 2 to 6 days during of digestion of OFMSW.

On one hand, if the average HRT is longer than 15 to 30 days, mesophilic conditions are the best options to treat organic wastes and are more efficient for degradation of then. On the other hand, a long HRT causes an increase of microorganisms that favoring the maximum and constant methane production (Nges and Liu, 2010).

Another important factor in consideration is the SRT. During the digestion, the HRT and SRT must be controlled to provide stability in the process. Constant variations in SRT promote destabilization of microbial species in the reactor and sometimes, reduce the

efficiency of anaerobic process (Bolzonella et al., 2005; Mao et al., 2015). Shorting SRT in the digester is an optimal strategy for biogas production. Nges and Liu (2010), during the digestion of dewatered-sewage sludge found three times higher of biogas production at a SRT of 9 to 12 days comparing with biogas production observed for a 35-day SRT. In addition to this, the VFA accumulation was observed at a SRT of 9 day.

2.3.4 Organic loading rate

The Organic loading rate (OLR) is the amount of organic waste, expressed as COD (grams of volatile solids (VS) or total solids (TS)), of the feed into digester per day and per unit of volume reactor. This parameter is related directly with HRT. Several studies in the literature, related the influence of OLR on VFA production in the acidogenic fermentation and in the performance of digestion process. Optimal OLR mostly depends on the nature, viscosity and composition of the substrate, HRT and fermentation pH employed (Lee et al., 2014).

The increase on OLR at low HRT increases the biogas yield, but the equilibrium and the efficiency of the digestion process can also be effected (Yu, 2001). This could occur because bacterial inhibition take place, due to an increase on OLR and thus increasing the VFA production. During the acidogenic process, the VFA concentration increased due to the increases of OLR. For instance, Lim et al. (2008), when studied the anaerobic digestion of food waste, found an increase of VFA concentration with rises of OLR from 5 g L^{-1} d^{-1} under semi-continuous conditions.

2.3.5 Nutritional additives

Nutritional additives such as macro/micro nutrients or the carbon-to-nitrogen (C:N) ratio are essential in anaerobic or aerobic process to improve the growth of microorganisms and the effectiveness of digestion process. Carbon is an essential source of energy for bacteria, while nitrogen is used for building the cell structure (Jain et al., 2015). Therefore, these nutrients must be in desired concentrations, according to the preferred digestion process, due to the variety of nutrients composition in different organics wastes (Gunaseelan, 2004).

According to Salminen and Rintala (2002), the use of organic wastes with low C:N ratio can affects the performance of the digester and can lead to ammonia release, which consequently

inhibits the methanogenic microorganisms and favor the VFA accumulation in the digester. Additionally, the inhibition of methanogenic bacteria activity can also occurs when the C:N ratio is very high due to deficiency of nitrogen or organic acid accumulation caused by excess of carbon (Li et al., 2011; Lin et al., 2011). According to the same authors, the optimum C:N ratio for the formation of methane ranges between 20:1 to 30:1.

For acidogenic process, Zhang et al. (2008) recommended an C:N ratio from 16:1 to 20:1. Additionally, high VFA concentration was found by Jiang et al. (2009) in the acidogenic reactor treating organic waste in which the C:N ratio was in the range of 16:1 to 20:1. In this sense, for digestion performance, substrates with high C:N ratio must be balanced with other organics wastes with low carbon-to-nitrogen quantity, in order to achieve methane generation (Giuliano et al., 2013).

Considering the studies of culture selection for PHA production, C:N ratio is an important parameter, however it is not decisive for PHA accumulation. Johnson et al. (2010) studied the influence of the C:N ratio in PHB production using mixed cultures and reported that the low nitrogen content found in the assays enhanced the PHA accumulation stage but also caused some negative effects regarding the selection of culture. Additionally, Albuquerque et al. (2007) also confirmed that even when using fermented effluent in PHA production, the nitrogen limitation leads to a decrease capacity of PHA storage in the enrichment phase.

In terms of macro/micro-nutrient balance, they are critical for microbial growth metabolism, which often require significant energy inputs. This chemical stimulatory effect is significant, namely, for example, in the anaerobic digestion of energy crops, agricultural residues and OFMSW, which lack some of these essential elements (Mao et al., 2015). Zhang et al. (2011), reported that both macronutrients and micronutrients have positive effects as additives during biogas digestion. For example, nitrogen and phosphorus are good additives for anaerobic process and thus, the addition of nitrogen and phosphorus to the substrates enhances the solubilization of the extracellular polymeric substances and allows to obtain the maximum biogas production. Calcium, sodium, magnesium and others are essential for the growth of some microorganisms and for the formation of microbial aggregates (Murray and Zinder, 1985). But the nutrient requirements can be tough and it depends on characteristics of the waste, on the nutrients availability, on the reactor design and on other parameters (Van Lier et al., 1997).

2.3.6 Toxicants inhibitory

One of the main disadvantages of anaerobic digestion in comparing with the traditional aerobic digestion, it is highly susceptibility to toxic compounds. Some chemical compounds (organic and inorganic substances) can be toxic and inhibit the reactor performance, particularly the methanogenic process. This topic it will be summarized some recent examples of organic and also some inorganic toxic compounds that can inhibit the anaerobic digestion process.

2.3.6.1 Toxic Organic compounds

Several toxic organic compounds are usually stable and tend to resist biological treatment. Some of these compounds can be found in agrochemicals such as pesticides, herbicides, antiseptics and fungicides, as well as preservatives for wood, agricultural residues and also in WWTP and waste sludge (Yang et al., 2013). For instance, the chlorophenols and other chlorinated organics are highly persistent in aquatic environments and are also considered highly toxic to anaerobic systems (Puyol et al., 2012; Wang et al., 1991). The toxicity of organic compounds depends on their concentration in organic wastes and on their chemical position in the structure of the substrates.

Chen et al. (2008), studied the level of inhibition some organic compounds such as dichlorophenols (DCP) and pentachlorophenol (PCP), used in pesticide, under acidogenic and methanogenic conditions. It was found that the studied compounds promote significant inhibition on the digester, being PCP considered as a more toxic compound than DCP, at low concentrations (0.5–10 mgL^{-1}). Most of the organic compounds found in WWTP are strong methanogenic inhibitors, but with positive effect in the acidogenic step (Cappelletti et al., 2012), because they can be easily removed at low values of pH and, consequently, can cause the increasing of the VFA concentration in the influents (Oh and Martin, 2010).

2.3.6.2 Toxic Inorganic compounds

Ammonia, sulfide, heavy metals and light metal ions are the main inorganic compounds potentially toxic to anaerobic digestion process. The variation in the inhibition/toxicity levels of these compounds are reported on several studies (Angelidaki and Ahring, 1994; Bayr et al., 2012; Cai et al., 2008; Kieu et al., 2011).

2.3.6.2.1 Ammonia

Ammonia is the intermediate from the acidogenic biodegradation of organic waste, namely the biological degradation of the nitrogenous matter in the form of proteins and urea and it is also an important nutrient for the growth of anaerobic microorganisms. The fermentation of proteins-rich organic wastes releases ammonia nitrogen, which exists largely as the ionized form (NH_4^+) highly depending on pH (Jin et al., 2012).

The free ammonia (FA) and NH_4^+ are the main forms of inorganic ammonia nitrogen in influent, hence the FA has been widely known to inhibit methanogenic microorganisms in a greater extent than ionized ammonium (NH_4^+), since the former is freely membrane permeable and cause imbalances in protons and/or potassium (K^+) deficiency (Lin et al., 2014). Sprott et al. (1984) reported that when FA progressively diffuses into methanogens, the difference in intracellular pH origins some of them to convert to ammonium (NH_4^+), absorbing protons (H^+) in the process, while by using a potassium anti-porter, the cells then expend energy on proton balance. According to Garcia and Angenent (2009), the toxicity of the FA concentration (CNH_3) in anaerobic digestion depends primarily on the temperature, the pH and the characteristics of the organic waste.

2.3.6.2.2 Sulfide

Sulfides are substances frequently found in many industrial wastewaters treated by anaerobic treatment, where sulfite is reduced to sulfide by the sulfate reducing bacteria (SRB), having this chemical conversion an important role in the anaerobic digestion of complex organic waste (Hilton and Oleszkiewicz, 1988). In their studied McCartney and Oleszkiewicz (1991) described three major groups of SRB: i) inhibition and/or toxic to SRB and methane producing bacteria is due to competition for common organic and inorganic substrates from SRB, ii) inhibition from the methanogen producing bacteria groups and iii) reduction of amount of methane produced due to the competition for carbon and hydrogen. On the other hand, Karhadkar et al. (1987) proposed two stages of inhibition as a result of sulfate reduction. Primary methanogenic inhibition owing to competition for substrate from the SRB and secondary inhibition results from the toxicity of sulfide to many microorganisms' consortiums leading to a decline methanogenic population. The competition between SRB and methane producing bacteria (MPB) for acetate during the anaerobic treatment of sulfate

rich wastewater affects the treatment efficiency, maintaining a low oxidation–reduction potential in the reactors (Paula and Foresti, 2009). Furthermore, it showed that sulfide ions could reduce the formation of methane suggesting non-competitive inhibition of methanogenesis, due to the resulting sulfide SRB activity, which can led to the failure of the process.

2.3.6.2.3 Heavy metals

The heavy metals such as copper (Cu), zinc (Zn), lead (Pb), mercury (Hg), chromium (Cr), cadmium(Cd), iron (Fe), nickel (Ni), cobalt (Co) and molybdenum (Mo) are the most common compounds that can be found in the anaerobic digesters and they are present in significant high concentrations in MSW and in several industrial wastewaters (Altas, 2009). However, many metals as Ni, Co and Mo, at low concentrations, are essential for the activation or functioning of many enzymes and coenzymes of anaerobic microorganisms (Takashima and Speece, 1989). On other hand, these heavy metals compounds can be inhibitory to anaerobic microorganisms at excessive concentration (Li and Fang, 2007). Zayed and Winter (2000) thought that acidogenic bacteria are less sensible to heavy metal toxicity than methanogens. However, Hickey et al. (1989) have reported that some trophic or organisms within the anaerobic group in digesters might be more severely inhibited by a pulsed addition of heavy metals than the methanogenic populations.

Several studies have been conducted on the effect of heavy metals on a variety of microbial species such as acetogens (Li and Fang, 2007), acidogens (Yu and Fang, 2001a; Zayed and Winter, 2000), methanogens (Karri et al., 2006; Mori et al., 2000) and SRB (Utgikar et al., 2003).

In general, the potential toxicity of heavy metals in anaerobic digestion is related with various chemical arrangements, concentrations, and low solubility under anaerobic conditions (Leighton and Forster, 1997).

2.3.6.2.4 Salts

Most of the salts generally used in AD are mainly for stimulating the biogas production. Some metal ions such as sodium (Na^+), calcium (Ca^{2+}), potassium (K^+) and magnesium

(Mg^{2+}) are frequently found in influent of AD, and present low toxicity, being the degree of toxicity of one ion dependent on the presence of other ions (Braun et al., 1981; Hendriksen and Ahring, 1991). However, excessive amounts of inorganic salts can lead to inhibition or toxicity. The toxicity effect of salts on the anaerobic microorganisms is mostly determined by cation, once these, compounds are present in significant concentrations in organic waste (Fernandes, 1986). For example, the inhibition caused by Na^{+} is directly related to the Mg^{2+} concentration. When the Mg^{2+} was 0.05 mM or less, 0.35 M Na^{+} completely inhibited the microbial growth (Ahring et al., 1991). This result was in agreement with the findings of McCarty (1964 b), who found that the Na^{+} toxicity increased in the order of $Na^{+} < K^{+} < Ca^{2+} < Mg^{2+}$.

According with the work of Caetano (1989), the optimum concentrations of some salts for acidogenic stimulating metabolism elements lie in the range of 75 to 4000 mg L^{-1}, causing a moderate inhibition at concentrations of 1000 to 5500 mg L^{-1} and severe inhibition at concentrations between 3000 and 12000 mg L^{-1}.

2.4 Biovalorization of anaerobic digestion byproducts

The overall pollution prevention targets and the dependency on fossil fuels have led scientists all over the world to look for alternative sources of energy. One of the objectives of the 21st century is the employment of biological processes to produce bioenergy and other added-value products. The recycling of organic wastes for bioenergy production by anaerobic digestion and commodities from organic materials has been proposed (Yin et al., 2016). Attention has also been given to the production of bio hydrogen and production of biodegradable polymers to chance the conventional petrochemical polymers origin, being one of the greatest challenges of the 21st century (Levis et al., 2010). Bio-products and bioenergy can be produced through anaerobic digestion by the biodegradation of complex organic materials, using complex groups of microorganisms, as mentioned previously. This sub-chapter some of the AD by-products potential will be briefly present.

2.4.1 Volatile fatty acids

The soluble acid metabolites or volatile fatty acids (VFA) as acetic acid, butyric acid, propionic acid or valeric acid, consist of six or less carbon atoms and are produced in the acidogenesis and acetogenesis steps of anaerobic digestion. The VFA produced as intermediates in the AD could be subsequently extracted and used as raw materials for the production of high-value products (Lee et al., 2015). Currently, commercial production of VFA is mostly accomplished by chemical routes. In general, biological VFA production, the use of pure sugars such as glucose and sucrose is very common as main carbon sources (Kondo and Kondo, 1999). However, the use of pure carbon sources is not environmentally sustainable. To overcome this point, the use of renewable carbon sources is preferable from the viewpoint of sustainable development (Akaraonye et al., 2010). Thus, the VFA production from wastes rich in organic matter can solve the majority of sustainable issues with in a low cost biological process.

In the last decades, numerous strategies had been developed for biological treatment of waste such as food waste, OFMSW, municipal and industrial wastewaters in order to maximize the VFA production. It is possible to obtain high VFA quantity and quality in recovering by regulating the operating conditions of the anaerobic reactor, such as pH, temperature, retention time and organic loading rate. In addition to these parameters, the use of additives (macro and micronutrients) for enhancing VFA production has been recommended, as referred in previous sections. Beyond these parameters, the optimization of VFA generation is also performed based on types of bioreactors (Grady et al., 2011).

Several bioreactor designs have provided promising results in terms of VFA production, namely: packed bed biofilm column reactor (operated based on attached growth technology in which the biomass grows and attaches on porous packing material (Beccari et al., 2009)), fluidized bed reactor, upflow anaerobic sludge blanket (operated based on suspended growth technology) and continuous stirred-tank reactor (involves complete mixing of waste and biomass) (Eddy, 1991). These types of bioreactors are commonly operated in continuous mode, at long retention times if the inoculum is not yet granulated (Poh and Chong, 2009) and some of these reactors can be converted into batch and semi-continuous reactors, when it require slow and short reaction period (Lee et al., 2016). Batch reactors are ideal to solid waste with high presence of suspended solids in the waste (Zhang et al., 2005). Table 2-6

shows the different types of organic wastes and bioreactors used for VFA production and the values of the maximum VFA production performance for several studies.

Table 2-6: Different types of organic wastes used for production of VFA (adapted from Lee et al. (2014)).

Type of wastes	Organic content (mg COD L^{-1})	Reactor type and operation conditions	Max.VFA production performance	References
Solid waste				
Food waste	91,900	Batch reactor, 37 ºC, initial pH 5.5	8950 mg COD L^{-1}	Elbeshbishy et al. (2011)
Food waste	n.a	Semi-continuous reactor, pH 6, 35 ºC	25.000 mg COD L^{-1}	Lim et al. (2008)
Food waste	146,100	Batch reactor, 35 ºC, Pretreated food waste	5610 mg COD L^{-1}	Kim et al. (2006)
OFMSW	347,000	Batch reactor, ST = 8%, 25 ºC, 50 g $CaCO_3$ L^{-1}	34,460 mg COD L^{-1}	Gameiro et al. (2016)
Kitchen waste	166,180	Batch reactor, pH 7, 35 ºC, 4 d	36,000 mg COD L^{-1}	Zhang et al. (2005)
OFMSW	196,700	Plug flow reactor, pH 5.7-6.1, 37 ºC, HRT 6 d	23,110 mg COD L^{-1}	Sans et al. (1995)
WAS	5470	Batch reactor, pH 11, 60 ºC, 7 d, 0,002 g VSS	2561 mg TOC L^{-1}	Cai et al. (2009)
Liquid waste				
Palm oil mill effluent	88,000	Semi-continuous reactor, pH 6.5, 30 ºC	15,300 mg L^{-1}	Hong et al. (2009)
Olive oil mill effluent	70,400	Batch reactor, initial pH 6.5, 25 ºC, 45d	15,600 mg COD L^{-1}	Dionisi et al. (2005)
Wood mill effluent	11,100	Continuous strirred-tank reactor, 30 ºC	0.42	Ben et al. (2011)
Paper mill effluent	7740	Continuous strirred-tank reactor, 30 ºC	0.75	Bengtsson et al. (2008)
Cheese whey	4590	Continuous strirred-tank reactor, 37 ºC	0.84	Bengtsson et al. (2008)
Dairy wastewater	4420	Continous reactor, 35 ºC	3100 mg L^{-1} d^{-1}	Demiriel et al. (2004)
Pharmaceutical wastewater	40,000-60,000	Continuous reactor 35 ºC	44 %[d]	Horiuchi et al. (2002)
Mixture wastes				
Primary sludge + starch-rich wastewater	22,256	Batch reactor, 21 ºC, 6 d	118 mg COD VSS^{-1}	Ji et al. (2011)
Food waste + sludge	n.a	Semi-continuous reactor, pH 6.99, 35 ºC	29,100 mg COD L^{-1}	Hong and Haiyum (2010)
Sugar industry wastewater + pressed beet pulp	6621	Semi-continuous reactor, pH 5.6-6.2 35 ºC	3635 mg H-Ac L^{-1}	Alkaya et al. (2011)

n.a – not available; d – (mg VFA-COD in the effluent/mg COD in the influent) x 100%

As it can be seen on Table 2-6, it was clearly that organic solid waste such as food waste, OFMSW and sludge waste are the main substrates for VFA production and are the most investigated organic wastes. Liquid wastes generated from the agricultural activities are potential substrates for VFA production compared with dairy wastewater or pulp and paper

industrial wastewaters. Co-digestion of substrates is also investigated to improve the production of VFA. Mixture between food waste and sludge are commonly used for VFA production, due to a high organic matter content and a better synergistic effect on the hydrolysis of the substrates.

VFA produced by anaerobic process can be converted into alcohols (Uyar et al., 2009), biohydrogen (Srikanth et al., 2009) bioplastics and bioenergy (Mohanakrishna et al., 2010). VFA acids can be produced at low cost in the WWTP (Katsou et al., 2015; Longo et al., 2015), using fermented organic wastes as OFMSW (Gameiro et al., 2016; Silva et al., 2013), or industrial wastes from pulp industry (Queirós et al., 2014), and also in co-digestion of various substrates (Lee et al., 2015). Figure 2-5 shows a summary of the main production routs/applications of VFA in industrial services.

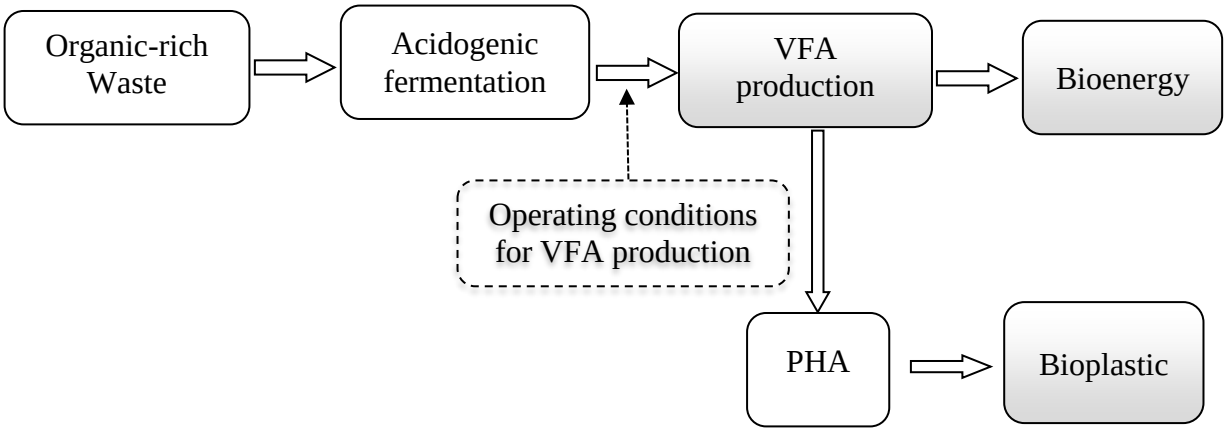

Figure 2-5: Production of waste derived VFA and their applications (adapted from Lee et al. (2014)).

2.4.2 Bioenergy

VFA from organic wastes treatment is an inexpensive energy source that can be used for the generation of several forms of energy such as electricity generation, biogas and biodiesel, described below.

2.4.2.1 Electric energy

Electric energy is produced by using microbial cells in which the microorganisms take advantage of the chemical properties of the organic substrate. In many cases, the operation of these systems does not require pre-treated waste and can be used in its raw state, making

the process very cost-effective (Nam et al., 2010). Acetic, propionic, *n*-butiric and valeric acids used in microbial fuel cell (MFC) have considerable influence on the performance of electricity generation performance. According to Freguia et al. (2010), the amount of electricity produced from acetate-fed MFC was about two times higher than the other higher molecular weight VFA, while achieving the highest coulombic efficiency (CE) of 93%. Propionate and butyrate-fed MFC, in terms of CE and power density, present high performance similar to acetate-fed MFC (Chae et al., 2009). MFC is a bioelectrochemical system that consisting of an anaerobic anodic cavity and an aerobic cathodic cavity which are separated by a proton exchange membrane (Du and Gu, 2007) and that uses microorganisms to harness the chemical energy of the organic substrate as a source of electricity. For better electricity generation it is important to increase the fraction of VFA from the organic waste. Higher electricity power could be attained when acetate was the main VFA in a mixture of acetate, propionate and butyrate (Teng et al., 2010).

2.4.2.2 Biogas

Regarding the production of biogas, it can be obtained during AD using the intermediate VFA as precursors VFA, particularly acetic acid, is the main source for methane generation and contributed more than 73% for methane production. There are two processes for biogas production under anaerobic condition: one stage AD, using VFA as the intermediate products, and two-stage AD, where two separate digesters are operated with the two main groups of microorganisms physically separated (Grady et al., 2011).

Comparing the two processes for biogas production, the second process is more feasible, for it ensures good conversion of VFA into biogas and achieves better biogas productivity, when compared to one-stage anaerobic digestion (Dimirer and Chen, 2005).

2.4.2.3 Hydrogen

Relatively to hydrogen (H_2), H_2 is a sustainable and ideal alternative energy source to substitute fossil fuels because it does not contribute to the greenhouse effect (Venkata Mohan et al., 2013). Hydrogen has the highest energy content and can be easily transported for domestic and industrial use (Das and Verziroglu, 2008), being recognized as one of the most potential and clean fuels for the future. Recently, most of the H_2 is produced from non-

renewable sources such as natural gas, oil, and petroleum (Cheong and Conly, 2007). Due to the trend of use of renewable energy resources, many studies have been carried out on H_2 production from industrial wastewater, food waste or municipal waste, using natural populations of microorganisms without sterilization (Bengtsson et al., 2008; Lee et al., 2015). Among the hydrogen production methods, the dark fermentation from organic wastes is the most promissing and environmental friendly method (Özgür et al., 2010), with the advantage of production of both hydrogen and VFA.

Hydrogen is commercially produced in almost a dozen processes. Most of them involve its extraction from hydrocarbons. The most widely used and least costly process is steam reforming, in which natural gas is forced to react with steam, releasing hydrogen. The production of hydrogen by water electrolysis, in which water is broken down into hydrogen and oxygen by running an electrical current through it, is used where electricity is cheap and where high purity is required (Hoffmann, 2001). More recently, biological routes for H2 production have been assuming high importance by employing biophotolysis by green algae, indirect water biophotolysis by cyanobacteria, photofermentation by photosynthetic bacteria, and dark fermentation by strict or facultative anaerobic bacteria (Ren et al., 2009).

2.4.2.4 Biodiesel

Biodiesel is a methyl ester of long chain fatty acids can be produced from lipids by transesterification process. Although biodiesel is a renewable energy source, its commercialization has been impeded by the high cost of production attributed to the use of expensive raw material that is 70 – 75% of the total cost (Xue et al., 2008). The synthesis of lipids from microbial VFA appears as a good alternative for the synthesized lipid levels have similar fatty acids of soybean oil, making it suitable for the production of biodiesel (Gui et al., 2008). Thus, the production lipid at low cost for biodiesel production is being interest in the academic community. Biodiesel produced from OW can contribute to the mitigation of greenhouse gas emissions, providing a clean and therefore sustainable energy source.

2.4.3 Added-value compounds

2.4.3.1 Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHA) are the environmentally friendly bioplastics with zero toxic effects, representing class of polyesters consisting of hydroxyl acid monomers associated by an ester bond (Fig. 2-6). This biopolymer represents a class of microbial polyesters accumulated as intracellular granules of energy reserve materials composed, mostly, by 3-hydroxy fatty acid monomers varying from one carbon to over 14 carbons and although approximately 150 different constituents of PHAs have been identified (Suriyamongkol et al., 2007).

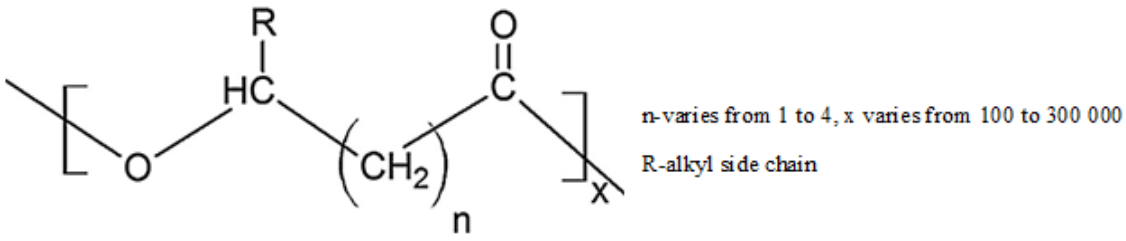

Figure 2-6: General structure of PHA.

PHA was observed for the first time in 1888 by Beijerincka. During long time, PHA had been connected of biopolymers produced only in the cytoplasm of bacterial cells, but Sabirova et al. (2006) shown interesting information that PHA can be also obtained by extracellularly accumulation from genetically modified *Alcanivorax borkumensis SK2* with similar characteristic. Due to this similarity, PHA are observed as potential substitutes for the traditional petro-chemically produced plastics or synthetic plastic (Valentino et al., 2014).

Synthetic plastic is non-biodegradable polymers with comprehensive characteristics of strength, lightness and durability. Due to these characteristics, the global demand and production of synthetic plastics is still growing and widely used are around the globe for a wide variety of applications (European Commission, 2013).

The continuous demand for synthetic plastics lead to the accumulation of plastic wastes that are responsible to remain in the environment for hundreds of years. According to Plastics Europe (2015), global plastics production amounted from 299 million Mg in 2013 to 311 million Mg in 2014. Only in Europe 59 million Mg of plastics were produced with annual

average utilization of 47.8 million Mg. In general, the recycled of plastic wastes are still underprovided. In Europe a small amount of plastic waste is recycled, about 29.7% (Plastic Europe, 2015). The disposal of the plastic waste present a serious environmental problem, being the landfill is the main system for the disposal of municipal solid wastes.

To face this, recently extensive efforts had been made to develop alternative processes to produce biologically derived polymers in order to found suitable alternatives for most applications with low environmental impact. As a result, the global production of bioplastics has increased to 5.1 million tons in 2013 and is expected to reach 6.2 million tons in 2017. The previsions by 2020 indicate that the annual production of bioplastics should reach almost 17 million tons (Aeschelmann and Carus, 2015). Europe production bioplastics have increased 1.7 million tons in 2013, and is expected to reach at 6.7 million tons in 2018 (European Bioplastics, 2015). Regarding this, the use of organic wastes for the PHA production is a favorable approach, which provides dual benefits of the waste treatment with simultaneous value addition a low cost production (Venkata Mohan et al., 2010).

Different types of organic wastes (solid and liquid) represent a promising carbon source for the PHA production. PHA production with acidogenic fermentation has recently been demonstrated using organic wastes, such as paper mill effluent (Bengtsson et al., 2009), sugar cane molasses (Albuquerque et al., 2007), sugar cane industry waste (Chandel et al., 2012), agro-industrial wastes (Albuquerque et al., 2011), food waste (Reddy and Venkata Mohan, 2012), fruit pomace and waste frying oil (Follonier et al., 2014), and other organic wastes.

According to Możejko-Ciesielska and Kiewisz (2016), PHA can be classified into two distinct groups: group I, short chain length PHA (scl-PHA), when PHA contains of 3–5 carbon atoms and are synthesized by a wide range of bacteria such as *Cupriavidus necator* and group II, medium chain length PHA (mcl-PHA), consisted of monomers having 6 –14 carbon atoms and are accumulated mainly by *Pseudomonas* species. In addition, PHA can be produced using microbial mixed culture as carbon sources. In this case, bacteria convert the carbon sources into scl-copolymers such as poly(3-hydroxybutyrate-co-3-hydroxyvalerate (P(3HB-co-3HV)) or poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P(3HB-co-4HB)) and mcl-copolymers such as poly(3-hydroxyhexanoate-co-3hydroxyoctanoate) (P(3HHx-co-3HO)) (Takabatake et al., 2000). In turn, PHA diversity

and structure is dependent on the synthesis metabolic pathway, and it can produced by two basic synthesis: first, β-oxidation cycles when the microorganism is grown on related carbon sources, such as fatty acids and second, fatty acid de novo biosynthesis in the presence of glucose, acetate, or ethanol as carbon source (Ciesielski et al., 2010; Huijberts et al., 1992; Magdouli et al., 2015). In the first case, Fatty acids are rapidly metabolized acetyl-CoA to produce 3-hydroxyacyl-CoA via β-oxidation cycle. Subsequently, 3-hydroxyacyl-CoA hydratase is transformed to R-3-hydroxyacyl-CoA, and finally polymerized by to PHA synthase (Pha C) (Fiedler et al., 2002). Second case, via de novo synthesis, 3-hydroxyacyl-ACP intermediates generated from glucose, fatty acids, or acetate are successively converted to R-3-hydroxyacyl-CoA reductase and by transacylase (Pha G). This reaction (R)-3-hydroxyacyl (ACP to CoA) transferase contain a scl PHA synthase (Pha C) specific for C3–C5 substrates and illustrates the classical PHB (Magdouli et al., 2015). Fig. 2-7 shows the linkages between fatty acid metabolism and PHA biosynthesis and metabolic pathways to overproduce PHA.

During the past few decades, the scl-PHAs biosynthesis pathway has been studied extensively. The resulting PHA structure depends on the carbon source compound supplied as the growth substrate. Carbon course such as valeric and butyric acids were recommended to added bacterial culture for scl-PHA production such as P(3HB-co-3HV) or P(3HB) (Lizarraga-Valderrama et al.,2015). In addition, this intermediated products from fermentation acidogenic is intensively used as carbon source for PHA production. Thurs, it is source for P(3HB) production. Therefore, when the carbon source rich in propionic acid promote the production of 3HV, acetoacetyl-CoA (Hermann-Krausset al. (2013). Currently, large scale application of PHA has been observed due to advantages and benefits, such as biodegradability, thermoplasticity, biocompatibility, non-toxicity, with high-value applications, especially, in pharmaceutical sector, industry and other sector services. Figure 2-8 summarize the main potential applications of PHA.

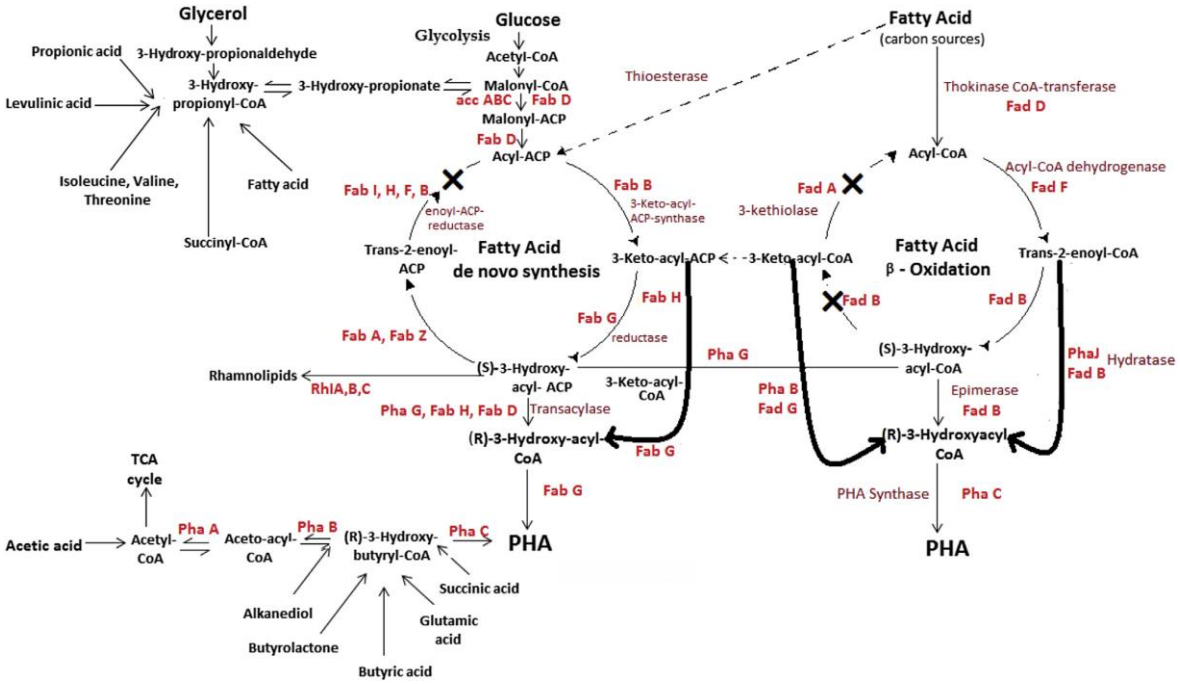

Figure 2-7: PHAs biosynthesis pathways and metabolic pathways to overproduce PHA. From (Magdouli et al., 2015).

Where: FabA:3-hydroxyacyl-ACP dehydrase, FabB, 3-ketoacyl-ACP synthase, FabD: malonyl-CoA-ACP transacylase, FabG: 3-ketoacyl-ACP reductase, Fab H: 3-ketoacyl-ACP synthase III, Fab I: enoyl-ACP reductase, Fad A: 3-ketoacyl-CoA thiolase, Fad B: multiple function enzyme with activity of (S)-specific enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase, Fad D acyl-CoA synthetase, FadF: acyl-CoA dehydrogenase, PhaA: b-ketothiolase, PhaB: NADPH-dependent acetoacetyl-CoA reductase, PhaC: PHA synthase, PhaG: 3-hydroxyacyl-ACP CoA transferase, PhaJ: (R)-specific enoyl-CoA hydratase, PhaZ: PHA depolymerase, RhlA: HAA synthetase, RhlB: rhamnosyl transferase I, RhlC: rhamnosyl transferase II, 3-(R)-HAA: 3-(R)-hydroxyalkanoyloxy) alkanoic acids

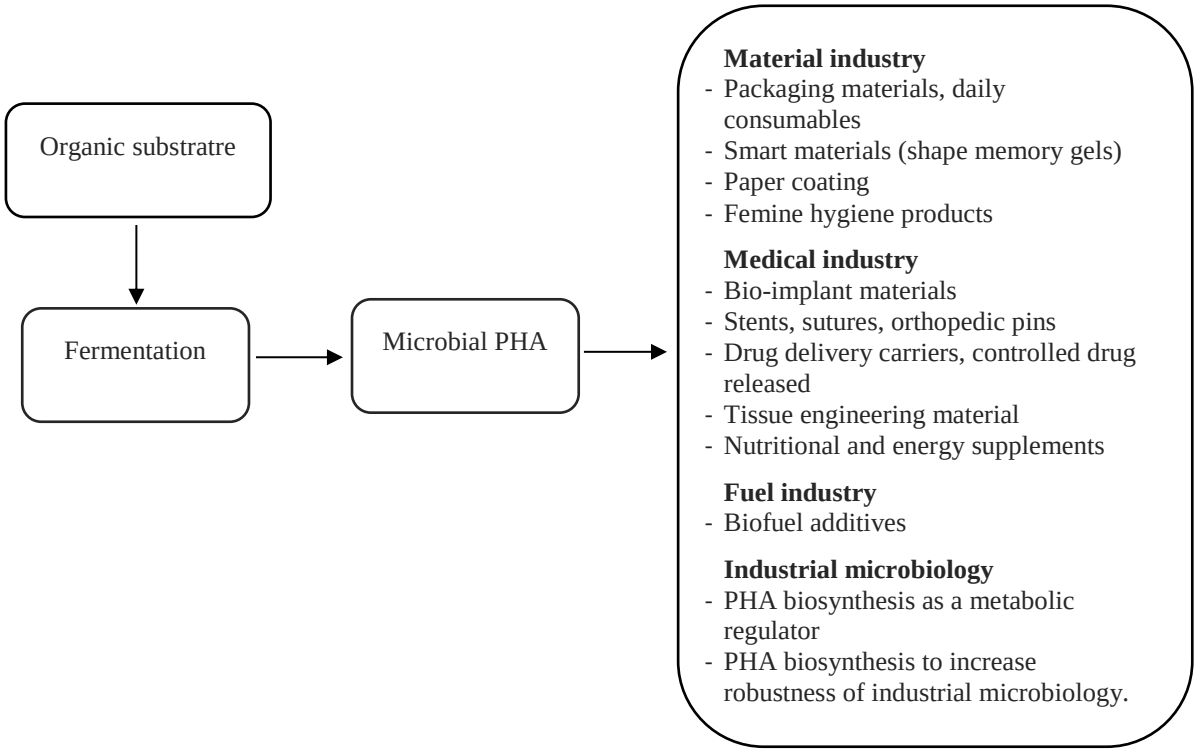

Figure 2-8: Potential applications of PHAs in sector services (adapted from Możejko-Ciesielska and Kiewisz (2016)).

2.5 Kinetic model of anaerobic digestion

Kinetic model of the anaerobic digestion describes the biochemical kinetic reactions behavior of the solids, liquids and gases to biogas production. In AD process, kinetic model are useful both to predict the performance of digesters treating complex wastes and to design appropriate digesters. In addition, some factors such as total solids content inside of reactor, the presence of an inhibitor and inadequate temperature control can affect the performance of anaerobic digesters (Fdez-Güelfo and Álvarez-Gallego, 2011). To solve this problem, appropriate mathematical models have been developed in order to understand and increases efficiently the anaerobic systems.

It is very difficult to describe a mathematical model while that shows clearly the enzymatic reactions of biological processes, since hydrolysis of a complex, insoluble substrate depends on various parameters such as the particle size, pH, and production of enzymes, diffusion and adsorption of enzymes to particles (Denbigh and Turner, 1984). In general, kinetics model for biological processes can be addressed by microorganism growth models, characteristic of substrate used and product formation models, which are interrelated through the corresponding yield coefficients.

In the literature, several models have been introduced, employed in almost AD processes and even though their implementation can be complicated (Lindmark et al., 2011), for it requires many data to be measured. This complexity depends largely on the nature of the waste and the type of digestion process. For instance, when complex material is digested, like during co-digestion of WAS with OFMSW, it is almost impossible to obtain from them all information (Lindmark et al., 2014).

Various types of bacteria in mixed populations perform the degradation of complex organic material and their sequence steps. Thus, the determination of particular or even all microorganism concentration is unfeasible by direct methods and the limiting step great rate is often attributed to hydrolysis (Reus, 1991).

In order to facilitate the AD study and ensure estimation of all parameters the kinetic model must be simplified. So, Gelegenis et al. (2007) simplified the model based on a three-stage of methane fermentation and successfully applied to describe co-digestion of the olive-oil mill with the diluted poultry manure. The first stage, hydrolytic (hydrolyzed the organic compounds into simple soluble compounds by bacteria fermentation), second stage,

acidogenic bacteria converted simple organic compounds into volatile acids and the third stage, acetogenic bacteria together with methanogens bacteria converted VFA into CH_4 and CO_2. Table 2-7 summarizes the most studied kinetic models and used in AD. Kinetic models, such as the first-order, Gompertz model, have been frequently used to determine the kinetic constants and thus evaluate the performance of different types of digesters (anaerobic discontinuous reactor, continuous stirred tank reactor and batch reactor) (Wang et al., 2009; Lin et al., 2011; Guo et al., 2013).

Despite that numerous equations have been suggested, the adapted equation of Monod is the most accepted and most widely used in developing kinetic models. The First-order-kinetic models describe the disintegration/hydrolysis of organic matter as the enzymatic activity is not directly coupled to the bacterial (De Gioannis et al., 2009; Kumar et al., 2004) and has been reported that it is the most appropriate for complex, heterogeneous substrates and was frequently used in other more complicated models (Gavala et al., 2002). In addition, this model can simulate the biogas accumulation by an exponential rise to the maximum (Jiménez et al., 2004; Nielfa et al., 2015). Pagés Díaz et al. (2011) used the first-order kinetic model for study the evaluation and synergetic effects by co-digestion of different waste mixtures from agro-industrial activities of the discontinuous process. According to the same author, the first-order kinetic can be obtained valuable interpretation about process performance in terms of methane production.

In general, the hydrolysis kinetics concerning the hydrolysis of carbohydrates, proteins and lipids has been reported assuming first-order hydrolysis, and it can be divided into two facts: First, at the start of the digestion process, when their concentrations are still high, it can be considered that there is no significant change in the overall removal rate. However, the reaction approximates a zero-order kinetics. Second, as the substrate begins to be consumed, the reaction rate (substrate utilization) tends to decrease when the concentration of the substrate tends to reduce a minimum value (the substrate utilization rate increasing with time), the rate use becomes limited by the poor availability of substrate in the medium. Under these conditions, the kinetic occurs as a first-order process (Metcalf and Eddy, 2003; Mu et al., 2006).

Table 2-7: The most studied kinetic model of anaerobic digestion (adapted from Nielfa et al. (2015))

Yield rate model	Kinetic growth model Equation	Characteristics
Monod's model	$S = S_{0.}\, exp^{-k.t}$	Describe relationship between time and concentrations of different compounds, i.e. substrate, VFA, produced biogas.
Momoh model or First-order model	$G(t) = G_{\infty}\,(1 - e^{-kt})$	Describe relationship between substrate degradation and biogas production.
Exponential Lag phase model	$G(t) = G_{\infty}\,(1 - e^{-k(t-L)})$	Describe relationship between substrate degradation and methane grow lag phase period.
Exponential Curve factor model	$G = G_{\infty}\,(1 - exp^{-kt})^{1/C}$	Estimate the relation between volume of methane at any time and to the maximum volume of methane.
Stability/inhibition assessment model	$G = G_{\infty}\,(1 - exp^{-kt})^{n} + In$	Determine the stability /inhibition status of digesters under AD process.
Gompertz's model	$CBM = G_{\infty} \,.\, exp\left[- exp\left(\frac{Rm \,.\, e}{G_{\infty}} \,(\lambda - t) + 1\right)\right]$	Assume that the rate of gas production is proportional to the microbial activity.
Gaussian's model	$CBM = G \times exp\left(-0.5 \times \left(\frac{t-t0}{\lambda}\right)^{2}\right)$	Estimates the biogas production rates and microbial growth and decay follow the normal distribution over the digestion period.
Romero's model	$(-r_s) = \left(-\frac{dS}{dt}\right) = \mu_{MAX}\, \frac{(St-Sn)\,.(h-St)}{(S0-Sn)}$	Based on the hypotheses that microbiological process can be represented as an autocatalytic reaction as a consequence of the reproduction capacity of microorganisms.

CBM – cumulative biomethane production ; G (*t*) - the cumulative biogas production potential at time; G_{∞} - the biogas potential maximum production; Rm - the maximum daily biomethane production; L and λ – lag phase; *t* – is the time; *e* - the exp; ($-r_s$) - is the substrate consumption rate; *h* - is the maximum amount of substrate available; S_n – is the concentration of non-biodegradable substrate by microorganisms; S_t - is the total substrate concentration (biodegradable and non-biodegradable); S_0 - is the initial total substrate concentration available in the medium; μ_{MAX} - is the maximum growth rate of microorganisms; *K*- is the hydrolytic constant.

On the other hand, modified Gompertz is a structured model most commonly used by several authors to describe the main process involved in AD to convert complex organic substrates into biogas, depending on the different types of substrates and the experimental conditions (Kafle and Chen, 2016; Kim et al., 2016; Owamah and Izinyon, 2015; Yalcinkaya and Malina, 2015; Yuan et al., 2016; Zhen et al., 2015). These model describes the cell density during bacterial growth periods in terms of exponential growth rates (Gibson et al., 1987).

An assumption of biogas production rate in a batch digester is proportional to the specific growth rate of methanogenic bacteria, the Gompertz equation can be used to simulate the maximum biogas production (Nielfa et al., 2015; Kim et al., 2016; Owamah and Izinyon, 2015). However, the model presents some limitations, since it does not have the ability to evaluate in simple terms the stability /inhibition state of anaerobic digestion processes (Owamah and Izinyon, 2016) and the complexity is also the main problem of Gompertz equation as it normally necessitates specific and complex software to analyze (Igal et al., 2014; Kafle and Chen., 2016). To resolve this limitation, Igal et al., 2014 have modified the Momoh and Nwaogazie (2011) model in order to understand and estimate the maximum biogas production potential on the AcoD systems with functions similar to the Gompertz model.

3. Methodology

3.1 Introduction

During this study, several experiments were performed, and the results are presented and discussed in each experimental chapter. In Chapter 4, it was studied the biochemical acidogenic potential of different organic food wastes, including the organic fraction of the municipal solid waste (OFMSW). In Chapter 5, it was studied the co-digestion of the two main wastes in Cape Vert Islands, OFMSW and waste activated sludge (WAS) from wastewater treatment plants (WWTP), in terms of acidogenic potential (VFA production and composition) and kinetics. In Chapters 6 and 7, it was studied the acidogenic fermentation of OFMSW (first stage of a 3 stage process) and the effect of different operational conditions on the VFA production and composition of the acidified waste. In Chapter 8, it was studied the enrichment process for the selection of aerobic biomass with high polyhydroxyalkanoates (PHA) production capacities (second stage of a 3 stage process). In Chapter 9, it was studied the PHA accumulation using acidified OFMSW (third stage of a 3 stage process). In conclusion, different experiments were performed, using different biological reactors and different hydraulic behavior – anaerobic and aerobic systems, batch and semi-continuous reactors. In the following sections, it will be presented the characteristics of the different wastes under study and also the experimental conditions tested in the various studies.

3.2 Organic residues and aerobic and anaerobic inocula

In the present study, anaerobic microbial mixed culture was used as inoculum for all acidogenic assays and aerobic mixed cultures for the PHA production assays. In order to compare the potential use of different organic wastes in anaerobic digestion for valorization into bioenergy or VFA production (and, ultimately, for valorization in the form of PHA), four different types of biowastes were used as substrates, such as: organic fraction of municipal solid waste (OFMSW), waste activated sludge (WAS), coffee grounds (CG) and tomato waste (TW). After collection, all substrates were stored at 4 oC until its utilization, to preserve their characteristics. The selection of these substrates took into account their high organic load and amounts produced. In addition, they were considered, due to their inhibitory potential to the biomass present in the biological processes used in the treatment plants.

3.2.1 Inocula

3.2.1.1 Anaerobic Biomass

The inoculum (anaerobic biomass) used in the fermentation assays was obtained from a full-scale mesophilic anaerobic digester (6000 m^3) existing in a domestic wastewater treatment plant in Aveiro, Portugal. The anaerobic biomass was concentrated by gravity settling for 24 h, stored under anaerobic conditions at 4 °C until the beginning of the experiments, then it was washed, centrifuged and characterized prior to inoculation. Table 3-1 presents its characterization.

Table 3-1: Characterization of the anaerobic biomass used in the fermentation batch assays (average ± standard deviation).

SST (g L^{-1})	SSV (g L^{-1})	sCQO (mg L^{-1})
57.57 ± 0.12	31.23 ± 0.01	33.10 ± 0.11

3.2.1.2 Aerobic Biomass

The inoculum (aerobic biomass) was obtained from a full-scale activated sludge system (3000 m^3) existing in a domestic wastewater treatment plant in Aveiro, Portugal. The aerobic biomass was concentrated by gravity settling for 24 h, stored under anaerobic conditions at 4 °C until the beginning of the experiments, then it was washed, centrifuged and characterized prior to inoculation. Table 3-2 presents its characterization.

Table 3-2: Characterization of the aerobic biomass used in the biomass enrichment assays for PHA production (average ± standard deviation).

SST (g L^{-1})	SSV (g L^{-1})	sCQO (mg L^{-1})
22.38 ± 0.22	16.40 ± 0.25	42.40 ± 0.71

3.2.2 Organic residues as substrates

3.2.2.1 Waste Activated Sludge (WAS)

During biological treatment, large amounts of organics present in a wastewater are converted by microorganisms and the surplus biomass (also called waste activated sludge) is discarded, where almost 80% of it is not properly disposed (Yang et al., 2015). WAS is mainly

composed of biodegradable organic materials, heavy metals, pathogens and toxic chemicals, which contribute to the secondary environment pollution risk (Ren, W.C et al., 2015). Due to the environment impact of WAS disposal, its volume and mass reduction has been an increasing concern worldwide. To overcome the environmental problems generated by WAS disposal, the anaerobic digestion process is the common treatment for sludge stabilization (Abelleira et al., 2012; Kim et al., 2015).

In this study, WAS used as substrate was collected from the secondary sedimentation tank of a local WWTP in Aveiro, Portugal. A general scheme for the generation of WAS in wastewater treatment plants is illustrated in Figure 3-1.

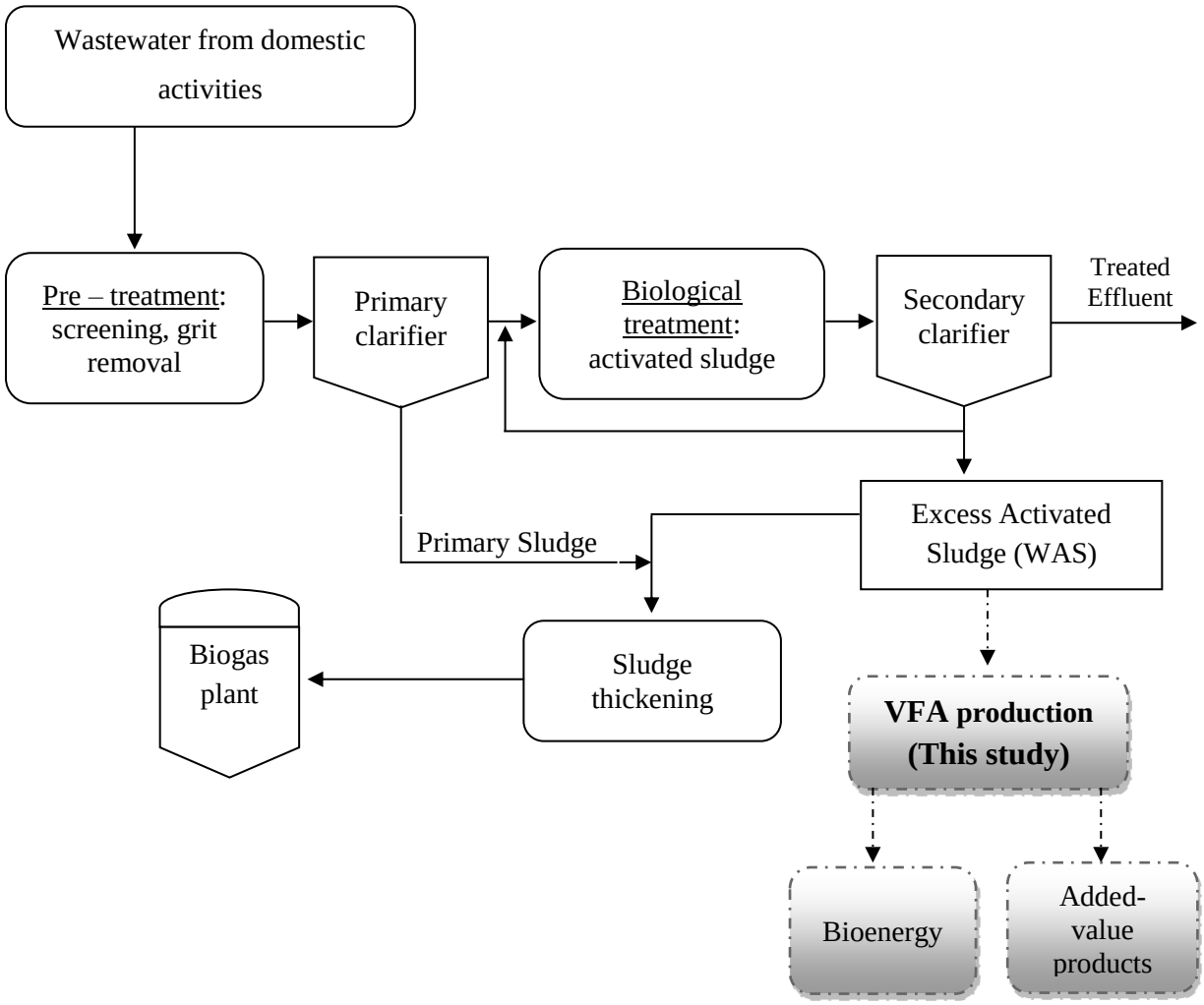

Figure 3-1: Process overview of a wastewater treatment plant, including WAS production.

Before characterization, WAS was concentrated by settling for 24 h and washed away the water layer for three times to remove solid particles. The physical-chemical characterization of WAS used in this study is shown in Table 3-3.

Table 3-3: Characterization of the WAS (average ± standard deviation).

Parameters	**Units**	**WAS**
TS	%	2.10 ± 0.23
VS	%	1.63 ± 0.20
TCOD	$gO_2 \cdot kg^{-1}$	157.01 ± 1.58
TKN	$g \cdot kg^{-1}$	8.78 ± 0.89
TP	$g \cdot kg^{-1}$	18.80 ± 0.34
pH		6.18 ± 0.01

3.2.2.2 Organic Fraction of Municipal Solid Waste (OFMSW)

In the present study, OFMSW was prepared in the laboratory, and it simulates a typical mixture based in a year collection of residues from two typical restaurants in Cape Verde Islands. The mixture was composed of rice, pasta and potatoes (45 %), kale, cabbage and lettuce (33 %), papaya, banana and apple (15 %), meat and fish (5 %) and paper (2 %). The materials such as plastic, bones and other inert materials were removed by hand. Figure 3-2 show the typical production and treatment involving industrial OFMSW and some potential applications of this waste for bioenergy or added-value materials production.

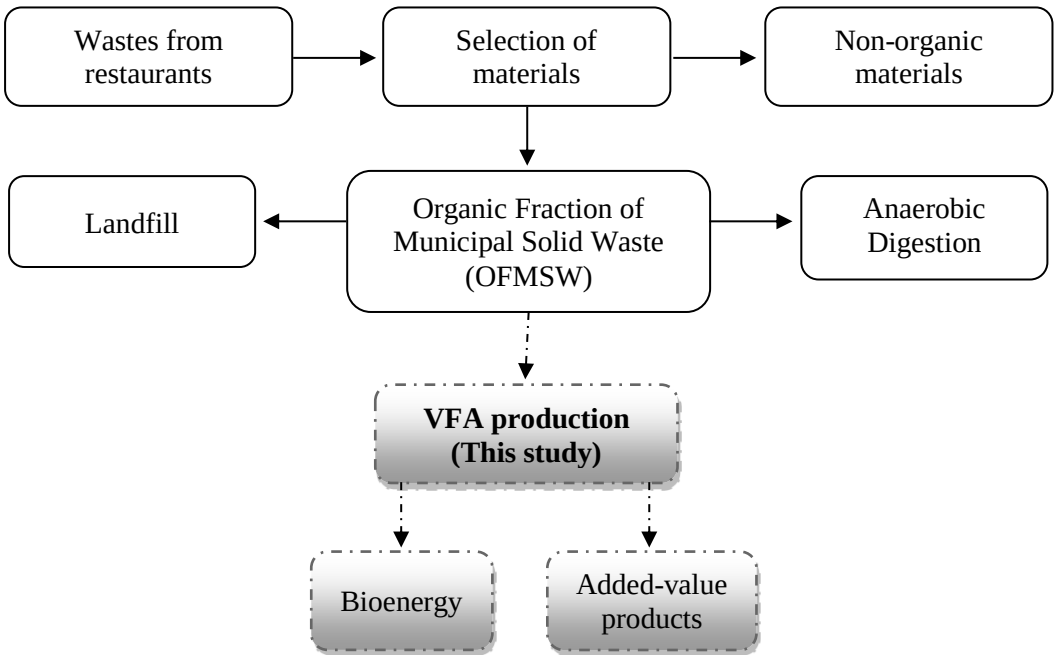

Figure 3-2: Schematic representation of the process involved in OFMSW production.

To ensure high homogeneity without changing the physical and chemical characteristics of the OFMSW, all materials were chopped into small pieces and then triturated using an automatic grinder. The biowaste obtained was sealed in a plastic bag and stored in a refrigerator at 4 °C for no longer than one week. Prior to testing, OFMSW was allowed to reach room temperature for a better characterization. The physical-chemical characteristics of this substrate are shown in Table 3-4.

Table 3-4: Characteristic of OFMSW used in this study (average ± standard deviation).

Parameters	**Units**	**OFMSW**
TS	%	23.26 ± 2.50
VS	%	21.25 ± 2.40
TCOD	$gO_2 \cdot kg^{-1}$	280.03 ± 5.4
TKN	$g \cdot kg^{-1}$	12.96 ± 0.06
TP	$g.kg^{-1}$	8.31 ± 0.01
pH		6.31 ± 0.010

3.2.2.3 Coffee Grounds (CG)

GC wastes are characterized by high carbohydrates concentration and high TS content and, consequentially, it represents an optimal alternative to be used as a source of renewable energy. Despite of this potential, very limited studies have been developed in this area using CG as substrate and only recently it has received much attention by the scientific and private organizations on the functional potential of this agricultural waste (Battista et al., 2016; Corro et al., 2013; Hernández et al., 2014; Qiao et al., 2013; Shofie et al., 2015). Figure 3-3 exemplified the typical production and treatment involving industrial coffee wastes and some potential applications of this waste for bioenergy or added-value materials production.

The CG used in the study was obtained directly from the coffee machine during the processing of coffee drinks at high temperatures (70°C), from coffee bar of Department of Environment and Planning, University of Aveiro – Portugal and transported to laboratory in cotton sacks in the same day. The main characteristics of CG used as substrate are presented in Table 3-5.

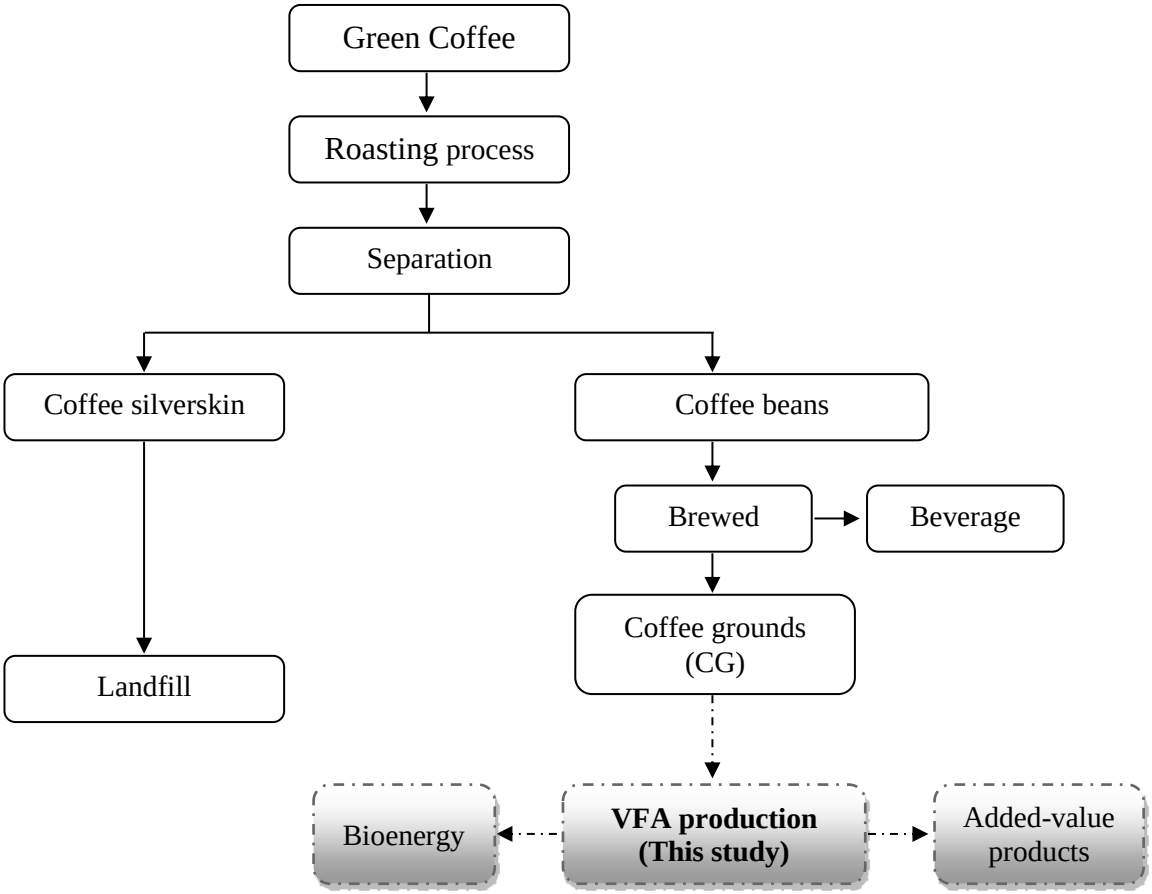

Figure 3-3: Schematic representation of the process involved in coffee industry and coffee grounds production and application (adapted from (Mussatto et al. (2011)).

Table 3-5: The coffee grounds characteristics (average ± standard deviation)

Parameters	**Units**	**CG**
TS	%	34.15 ± 4.2
VS	%	33.85 ± 4.5
TCOD	$gO_2 \cdot kg^{-1}$	551.5 ± 0.6
TKN	$g \cdot kg^{-1}$	21.58 ± 0.9
TP	$g \cdot kg^{-1}$	5.51 ± 0.01
pH		4.1 ± 0.09

3.2.2.4 Tomato Waste (TW)

Anaerobic digestion is known as a promising and suitable cost-effective technology for TW treatment (Tommonaro et al., 2007). In a study developed by Bacenetti et al. (2015), it was demonstrated that TW could be used as a potential source to produce bioenergy and fuel pellets, among other value-added products, when anaerobically digested or co-digested. Figure 3-4 represents the system of the tomato industrial production and treatment for TW.

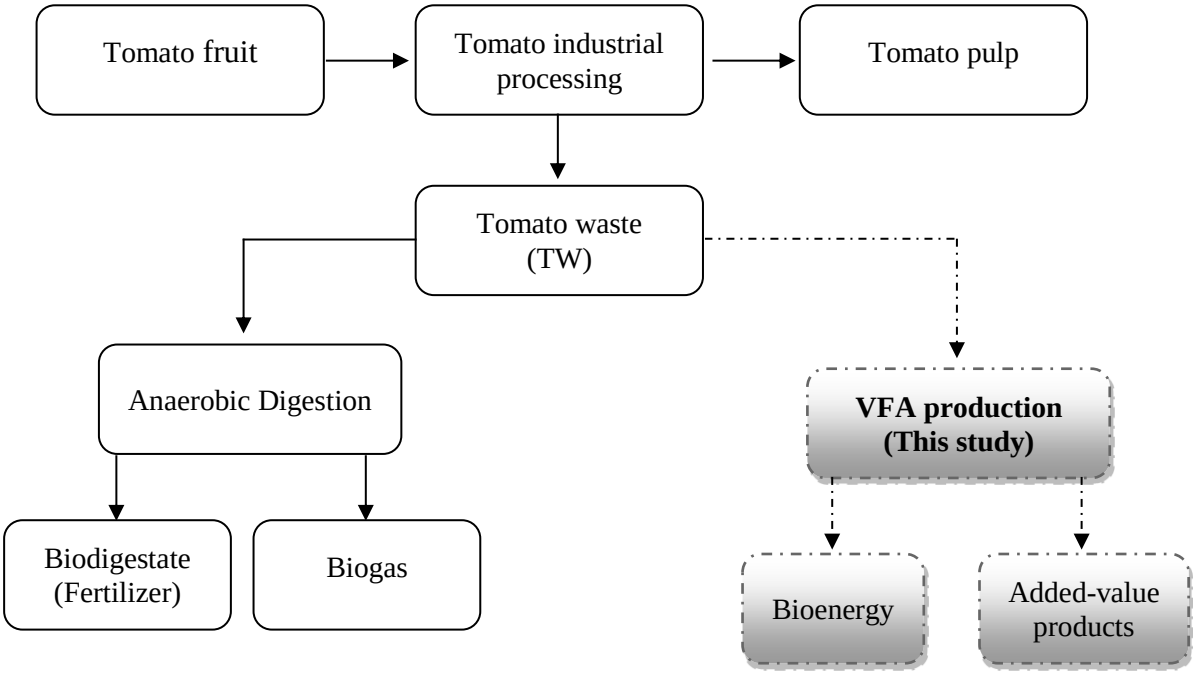

Figure 3-4: Schematic presentation of tomato industry process and possible TW biovalorization.

In this study, anaerobic co-digestion of TW was also applied. This substrate was obtained from the food market in the city of Aveiro – Portugal, and was manually mixed, and then grounded, using an electrical kitchen blender to reduce tomato waste size to less than 2 mm and produce a homogenous paste. The main characteristics of the TW are shown in Table 3-6.

Table 3-6: The main characteristics of tomato waste (average ± standard deviation).

Parameters	**Units**	**TW**
TS	%	4.14 ± 0.01
VS	%	3.73 ± 0.01
TCOD	$gO_2 \cdot kg^{-1}$	71.8 ± 10.4
TKN	$g \cdot kg^{-1}$	2.49 ± 0.06
TP	$g \cdot kg^{-1}$	1.0 ± 0.02
pH		5.1 ± 0.02

3.3 Biological reactors operation

3.3.1 Anaerobic Batch experiments

In this study, the experiments were carried out on batch laboratory scale reactor with a total volume of 5 L. The batch reactor was made of borosilicate glass and closed by a glass cover.

The reactors were equipped with a sampling tube (dipped inside the biomass) and biogas output pipe connected to a gas bubbler with a fluid level with about 2 cm (Fig.3-5).

The anaerobic batch experiments performed used four organic residues resulting from common food activities or industrial processes: organic fraction of municipal solid waste (OFMSW), coffee grounds (CG), tomato waste (TW) and waste activated sludge (WAS) from a wastewater treatment plant (WWTP) facility.

1- Glass Reactor

2- Sampling tube

3- Tube for gas flow

Figure 3-5: Reactor set-up for batch operation.

3.3.1.1 Experiment set up and operational conditions

In Fig. 3.6 it is represented a schematic set-up of the reactor used for the evaluation of acidogenic potential of different organic wastes. An anaerobic microbial mixed culture from a municipal digester was used as inoculum. Macro and micronutrients solutions were added to the reactors according to the composition described by van Lier et al. (1997). Table 3-7 shows the composition of the nutrients solutions used in the anaerobic assays to provide mineral media. A mixture of $NaHCO_3$ and $KHCO_3$ was also added to the reactors content to provide alkalinity in the pre-defined concentrations, but without pH control.

Table 3-7: Composition of inorganic nutrient solution

Component	Concentration
Macro-nutrients solution	
$MgCl_2 . 4H_2O$	1.00 g L^{-1}
$CaCl_2 . 2H_2O$	0.375 g L^{-1}
NH_4Cl	1.25 g L^{-1}
K_2HPO_4	2.18 g L^{-1}
KH_2PO_4	1.70 g L^{-1}
Micro-nutrients solution	
$FeCl_2 . 4H2O$	2.0 mg L^{-1}
$CoCl_2 . 6H2O$	0.17 mg L^{-1}
$ZnCl_2$	0.07 mg L^{-1}
$H3BO_3$	0.06 mg L^{-1}
$MnCl_2 . 2H2O$	0.05 mg L^{-1}
$NiCl_2 . 6H2O$	0.04 mg L^{-1}
$CuCl_2 . 2H2O$	0.027 mg L^{-1}
$NaMoO_4 . 2H2O$	0.025 mg L^{-1}
EDTA	5.00 mg L^{-1}

Before inoculation, the reactors were flushed with nitrogen gas for a period of 5 min to remove any residual oxygen and to maintain anaerobic conditions inside the reactors. The reactors were incubated at controlled temperature of 35 $^oC \pm 2$ in a water bath, and connected to a water displacement system, as a simple apparatus for the measurement of the produced biogas. The biogas produced in the biodegradation process was monitored daily by water displacement method. The volume of water displaced from the bottle was equivalent to the volume of biogas generated. A schematic presentation of the batch experiments assembly is given in Figure 3-6.

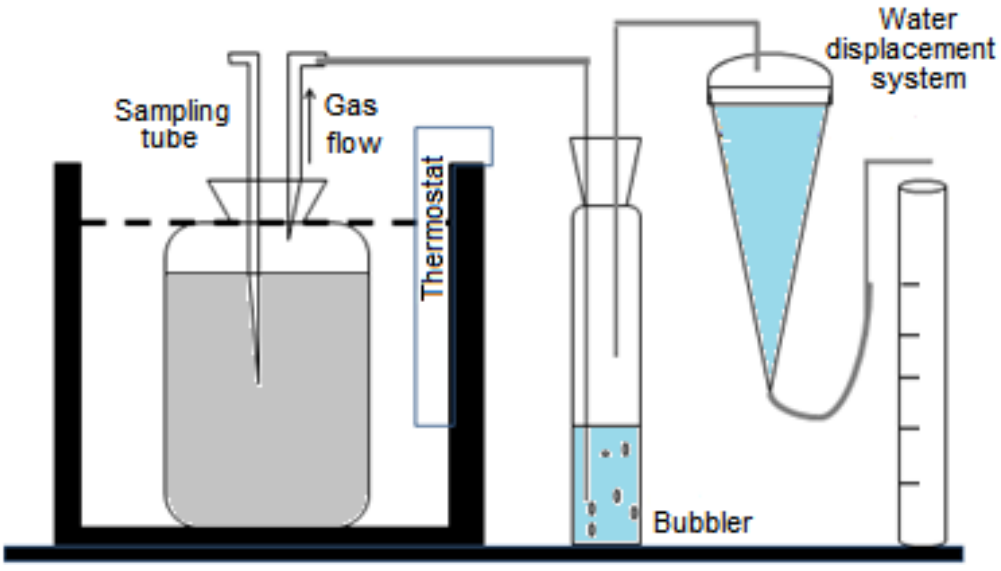

Figure 3-6: Schematic of experimental setup of the anaerobic reactors

The different assays performed under the scope of the batch reactor operation, was divided into three experiments (Experiments I, II and III), according to following Figure 3-7.

3.3.1.1.1 Experiment I

For this experiment, four different substrates were used: CG, TW, WAS and OFMSW, in order to evaluate the acidogenic potential and the performance of the anaerobic fermentation of these organic wastes. All batch assays were operated with an initial pH adjusted to 6.5 and a VS concentration inside of reactor equal 10 % (w/w). Batch experiments were conducted using a series of glass reactors with a total volume of 5 L and a working volume of 4 L. All the experiments were carried out with an F/M ratio of 2 g COD g^{-1}SSV, a value considered as an optimum, reported by Silva et al. (2013) and Spérandio and Paul (2000). In this experiment, $NaHCO_3$ was added to provide an alkalinity of 10 g $CaCO_3$ L^{-1}, considered sufficient to self-regulate the pH. The reactors were maintained at a constant temperature of 35 ± 0.5 °C controlled by a thermostat in a water bath. These batch experiments were operated over a period of 25 days and mixed manually (twice a day for 2 min).

3.3.1.1.2 Experiment II

Based on the results obtained in Experiment I, and due to low performance of batch assays with CG and WAS with respect to the acidification degree, it was decided to perform co-digestion assays using mixtures of these substrates and the other two substrates with much higher acidogenic potential, in order to evaluate the advantage of using these mixtures in co-digestion anaerobic processes to increase the acidogenic potential of the substrates and the interaction (synergisms) between selected substrates as well as to evaluate the overall stability of the different systems.

In this study, the batch co-digestion experiments were divided in four phases, as can be seen in the Fig. 3.7. In the first set (called Phase I), the reactors were fed a mixture of CG and TW; in the second set (Phase II), reactors were operated with a mixture of CG and OFMSW; the third set (Phase III) bioreactors were run with a mixture with WAS and TW; and Phase IV, studied the co-digestion performance between WAS and OFMSW.

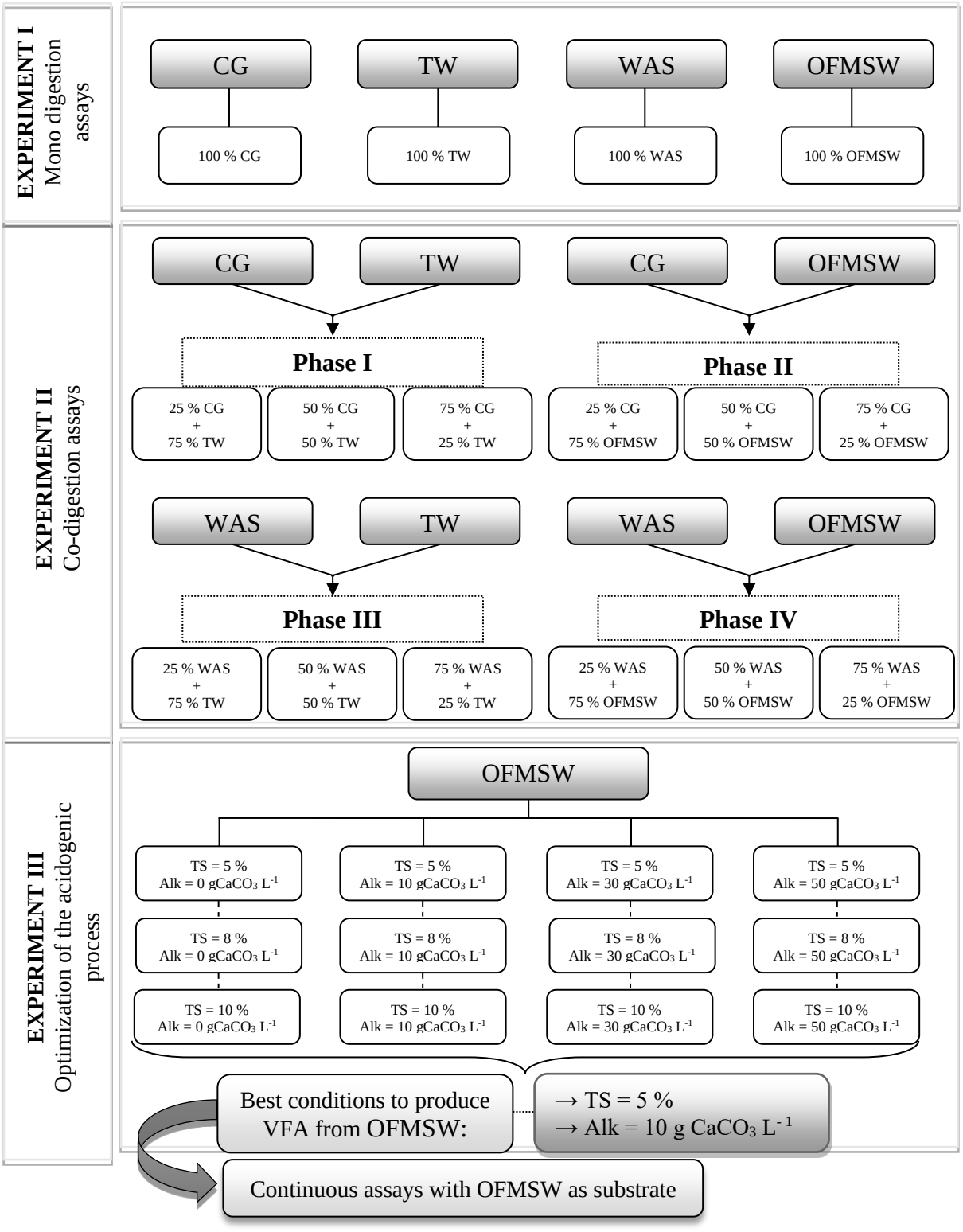

Figure 3-7: Schematic presentation of three experiments in batch reactors.

The proportions of each mixture for all batch assays were defined based on previous studies published in literature, in which the use of the referred mixtures could led to good results regarding the anaerobic digestion efficiency (Capela et al., 2008; Nielfa et al., 2015). The mixture of inoculum and substrate was performed according to the percentage referred in Figure 3.7. All experiments were carried out with the same conditions as the ones used for the mono-digestion assays and were monitored daily.

3.3.1.1.3 Experiment III

Based on the good results in the mono-digestion of OFMSW, twelve batch assays were carried out with replicates, in order to evaluate the hydrolytic and acidogenic potential of OFMSW. Anaerobic sludge and OFMSW were added to the system at different concentrations, in order to test three different total solids (TS) concentrations inside the reactors (5, 8 and 10 % of TS), combined with four external alkalinity additions (0, 10, 30 and 50 g $CaCO_3$ L^{-1}). In addition, the batch reactors were incubated at controlled temperature of 25 °C ± 2 °C in a water bath connected to a water displacement system, monitored on a daily basis. These assays were performed in a lower temperature, in order to evaluate this effect on the acidogenic potential of OFMSW. This action is considered a strategic measurement for a country like Cape Verde Islands, were this is the average temperature around all year, and consequently, this would be a cost-effective action.

3.3.2 Reactor set-up for continuous or semi/continuous experiments

The global experimental setup for the OFMSW valorization in terms of PHA production, consisted of three steps, as represented in Figure 3.8. The OFMSW acidogenic fermentation (step 1) was carried out in a CSTR operated under anaerobic conditions (the results regarding this work are developed in Chapter 7). The following step, selection and enrichment of mixed cultures with high capacity of PHA accumulation, is represented as step 2 and was performed in SBR aerobic systems, under Feast and Famine (F:F) conditions (applying the DAF regime) using the acidified effluent of the CSTR anaerobic reactor as feed. The last step, PHA accumulation in batch aerobic assays, use de biomass enriched in step 2 and the acidified effluent produced in step 1, and is represented by step 3 in Fig. 3-8.

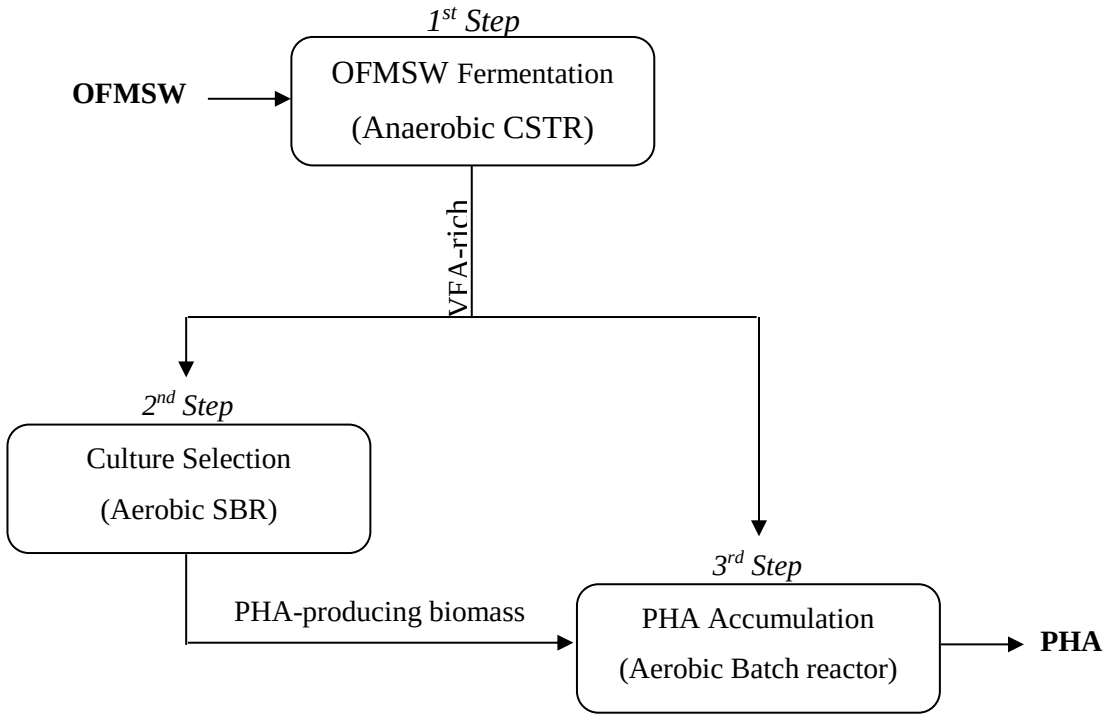

Figure 3-8: Schematic diagram of the 3 stage PHA production process using microbial mixed cultures.

For the selection of mixed cultures with high capacity of PHA accumulation (step 2), SBR systems were used with working volume of 5 L, operated in sequential mode and inoculated with aerobic sludge from the aeration tank of a local municipal wastewater treatment plant (WWTP) in Aveiro.

During the experimental period, it was tested the capacity of one operation cycle in 24 hours under the F/F regime, consisted of three discrete periods: (i) 00:00 hours, fill (20 min); (ii) 22 h 50 min, aerobiosis (feast and famine) (30 min); and (iii) draw (20 min). The liquor (the supernatant or the mixture with settled sludge) were withdrawn from the reactor stirring, maintaining the HRT at 5 days and the sludge retention time (SRT) at 10 days. The reactors were monitored by a computer system developed by the research group of the project "POLIBIO", from University of Aveiro.

The study developed in this section focused on the 3rd step of the PHA production process and comprised several batch tests to maximize the PHA content inside the cells, analyzing the accumulation capacity of and the characteristics of the PHA monomers.

3.3.2.1 Semi-continuous anaerobic experiments

The semi-continuous operation was conducted using a continuous stirred tank reactor (CSTR) as anaerobic acidogenic reactor for OFMSW fermentation during 180 days, at 25 ºC, in order to optimize both the VFA amount and composition in the acidified effluent, being this current further used in an aerobic stage as the raw material for the production of polyhydroxyalkanoates (PHA).

3.3.2.1.1 Experimental set-up

The reactor used in the semi-continuous mode was made of acrylic with a cylindrical shape, with 5.0 L of total volume and 4.0 L of active volume. The CSTR unit was equipped with: a vertical mixing system (regulated for 120 rpm) using a power-driven force mixer (Heidolph RZR-2000), a feed inlet, a liquid sampling point, a line for gas flow and sampling connected to a wet gas meter, an effluent outlet connected to a tube U- shaped, in order to inhibit air inlet and at the same time, controlling hydraulic output of the effluent. The outlet of effluent was connected to a sedimentation tank with an effective liquid volume of 2 L. The biomass in the sedimentation tank was pumped continuously back to the CSTR, promoting the re-circulation of microorganisms, and the clarified effluent was collected in a sampling tank. The reactor was operated under mesophilic conditions at 25 ± 2°C, ensured by the circulating water from a heated water bath through a jacket surrounding the reactor. More details regarding the experimental set up are shown in Figure 3-9.

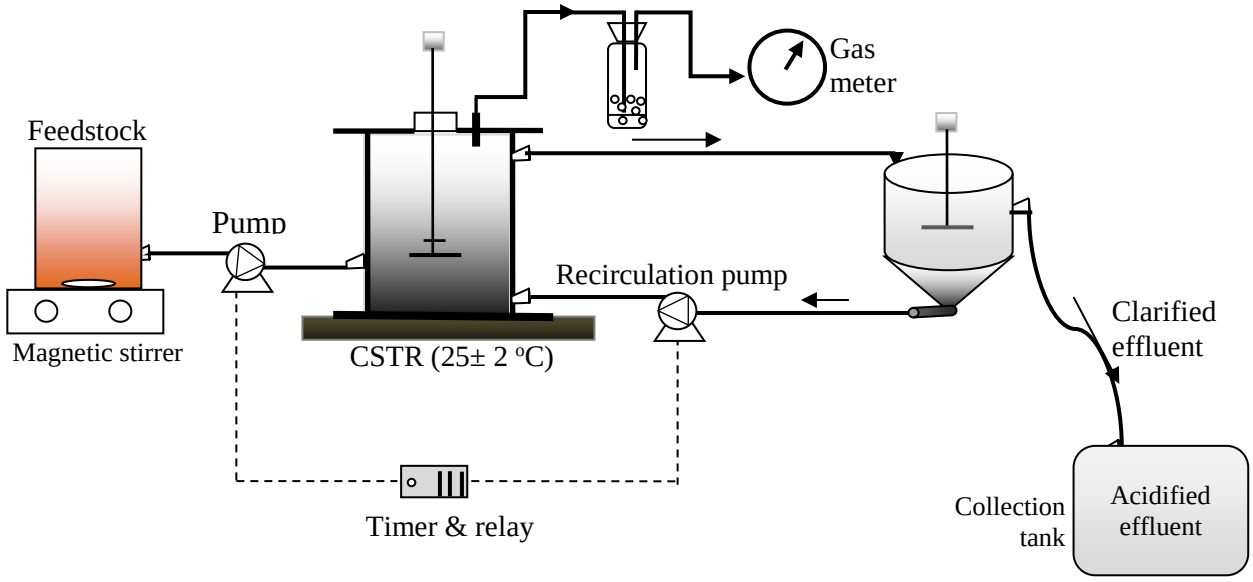

Figure 3-9: Schematic diagram of lab-scale anaerobic digester (CSTR type).

3.3.2.1.2 Operational conditions

Anaerobic microbial mixed culture with TSS and VSS concentrations of 20.84 ± 0.44 g L^{-1} and 18.33 ± 0.39 g L^{-1}, respectively, was used as inoculum. Initially, CSTR was run with 2.0 L of inoculum alone and started up in a continuous-flow mode over 1 week, using the dissolved organic matter in the anaerobic sludge as carbon source to improve the hydrolysis/acidification bacteria activity (Zahng et al., 2013). Afterwards, the reactor was only feed semi-continuously with diluted OFMSW (as a sole substrate), in order to obtain an average total solids (TS) content of 2% inside of biodigester and were mixed with a ratio of inoculum and OFMSW of 1:1 (wet weight basis). The reactor was fed daily three times a day, using a peristaltic pump controlled by a timer and a relay at 8:00 a.m, 16:00 p.m and 00:00 p.m, to maintain the established organic loading rate (OLR). The main characteristics of OFMSW used as feedstock in these assays are presented in Table 3-8.

Table 3-8: Main characteristic of the feedstock used in CSTR system.

pH	VFA (mg L^{-1})	TSS (g L^{-1})	VSS (g L^{-1})	sCOD (g L^{-1})	TCOD (g L^{-1})	C/N ratio
5.8 ± 0.1	2.1 ± 0.34	22.8 ± 0.26	19.5 ± 0.22	4.0 ± 0.12	15.8 ± 0.7	12.1 ± 0.2

The effects of increasing the OLR, maintaining the hydraulic retention time (HRT) constant and changing the alkalinity on the performance of the continuous process were investigated. After the start-up, OLR was gradually increased from 3 g COD L^{-1} d^{-1} until reaching the planned OLR of 6.5 g COD L^{-1} d^{-1}, as schematized in Figure 3-10.

The HRT and effluent flow rate were maintained constant at 2.5 days and 1.6 L d^{-1}, respectively, during the CSTR operation. Total chemical oxygen demand (TCOD) was used as a parameter for OLR calculation. Regularly, the HRT and the flow rate were controlled by adjusting the pump speed. The macro and micronutrients solutions (described previously in Table 3-5) were also added in this experiment, in the ratio of 1 mL of solution per kg of waste in the feed solution and the pH value was adjusted to 6.5 with a 1M NaOH solution. The system was monitored in the influent and effluent in terms of pH, TCOD, sCOD, VFA, alkalinity, TSS, VSS, and biogas composition. The system was considered to be in a steady state when the concentrations of sCOD and VFA in the draw-off were stable (sCOD and VFA were found to fluctuate within 2 – 5 % for several days). Biogas volume was measured

with a wet gas flow meter and the composition of the biogas was determined daily. The experimental conditions with respect to the feed COD and the imposed alkalinity concentration and the OLR applied are summarized in Table 3-9.

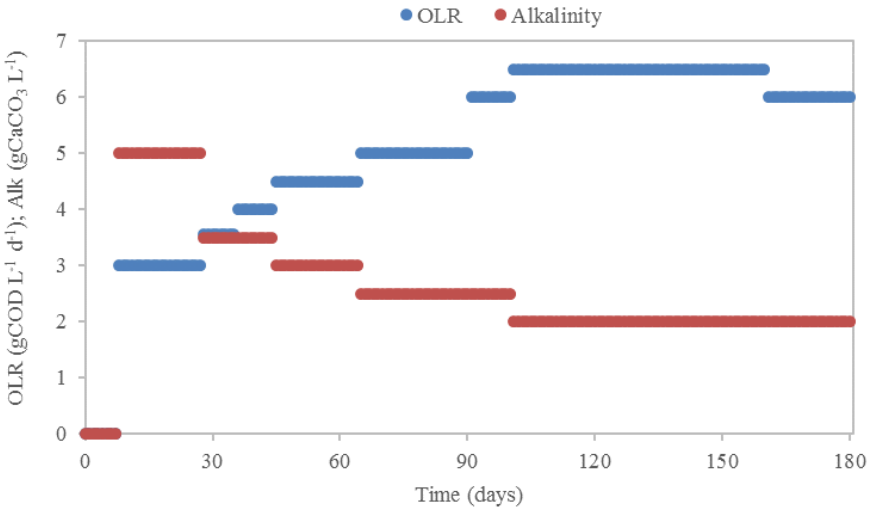

Figure 3-10: Variation of OLR and Alk concentration applied in the CSTR reactor during time.

Table 3-9: Experimental phases tested in the acidogenic CSTR reactor

Stage	Operation (days)	S_0 feed (g COD L^{-1})	OLR (g COD L^{-1} d^{-1})	Alkalinity (mg $CaCO_3L^{-1}$)
Stage 0	0 - 7	--	0.0	0.0
Stage I	8 - 35	7.5 ± 1.0	3.0 ± 0.1	5.0 ± 0.1
Stage II	36 - 64	9.9 ± 2.0	4.0 ± 0.2	3.5 ± 0.1
Stage III	65 - 90	12.5 ± 2.0	5.0 ± 0.1	2.5 ± 0.1
Stage IV	91-130	15.0 ± 1.0	6.0 ± 0.2	2.5 ± 0.1
Stage V	131-160	16.2 ± 2.0	6.5 ± 0.3	2.0 ± 0.1
Stage VI	161-180	15.0 ± 2.0	6.0 ± 0.3	2.0 ± 0.1

3.3.3 Aerobic assays for PHA accumulating biomass enrichment

The volatile fatty acids (VFA) produced in the acidogenic fermentation assays were used as carbon sources for the PHA accumulating microbial enrichment in aerobic conditions. Thus, in order to valorize the VFA mixture produced in the anaerobic assays to produce PHA, it was performed several sequencing batch tests in parallel to the operation of the CSTR system, studying the effect of the substrate feeding composition on the selection of biomass with high capacity to accumulate PHA. The synthesis of PHA by aerobic mixed biomass obtained in full-scale activated sludge systems could be enhanced by applying the dynamic aerobic feeding (DAF) procedure (feast-famine mode), when sequencing batch reactors

(SBR) are operated for culture enrichment, as referred by Albuquerque et al. (2007) and Serafim et al. (2004). The DAF strategy was conducted in the operation of a SBR by using the acidified stream obtained in the OFMSW fermentation as carbon source (substrate).

To do the enrichment of the mixed aerobic culture, it was operated 3 SBRs, at different times for a total of four different operational conditions analyzed (see Table 3-10). The SBRs were fed with clarified fermented effluent produced in the operation of the CSTR. Thiourea was added to the feedstock in order to inhibit nitrification and pH was adjusted to 8 ± 0.1.

Table 3-10: SBRs operational conditions for biomass selection and enrichment

Assay	Inoculum	Operation time (d)	OLR (g COD $L^{-1}d^{-1}$)	VFA_{in} (%)	C:N ratio (Cmol:Nmol)	pH	HRT (d^{-1})	SRT (d^{-1})
SBR_1	WAS	70	1.7	75 %	14	8	5	10
SBR_2	WAS	41	3.0	75%	14	8	5	10
SBR_3	WAS	35	1.4	69 %	5	8	5	10

The SBR were aired by an air pump through ceramic diffusers, which also allowed the agitation of the reactor content. The SBR stood in a temperature controlled (25 ± 2 °C) for a period of 35 at 70 days. Figure 3-11 schematize the installation of the experimental system.

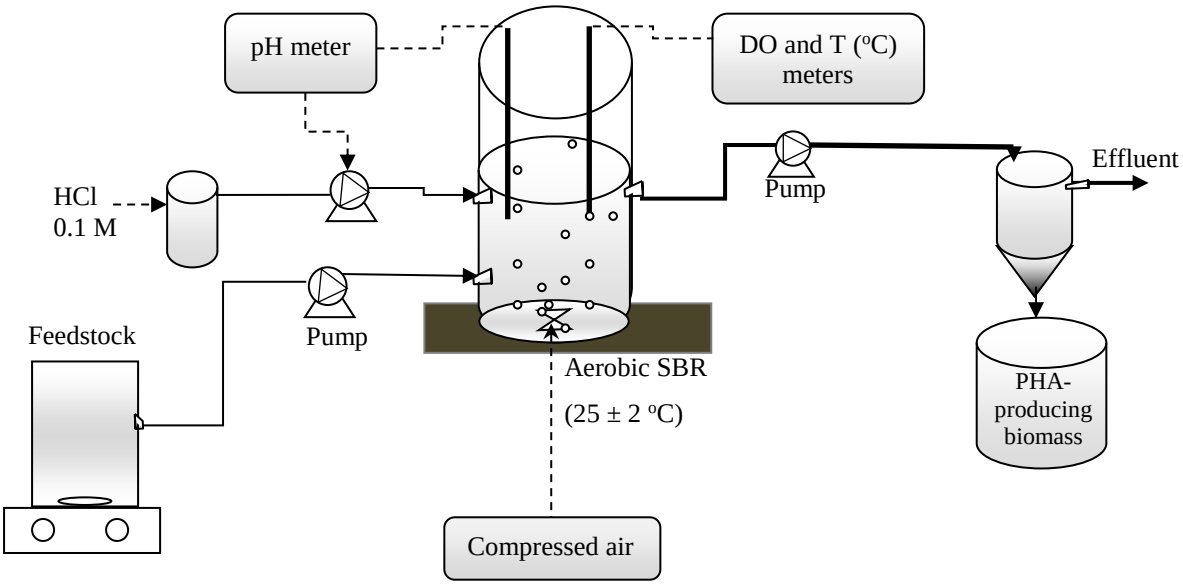

Figure 3-11: Schematic diagram of lab-scale for selection and enrichment of PHA accumulation culture in aerobic reactor (SBR type).

Air supply to the reactor during aeration phase, as well as the cut-off air (settling period), was programmed according to experimental design through an electro valve in the air supply line connected to a timer. The feeding pump and the pump for effluent output were also

connected to a timer. pH was controlled with a higher limit (set-point) of 8.0. The system has also a system for data acquisition and recorder for pH, OD and temperature.

For the selection of mixed cultures with high capacity of PHA accumulation (step 2), SBR systems were used with a working volume of 5 L, operated in sequential mode and inoculated with mixed aerobic activated sludge from the aeration tank of a local municipal wastewater treatment plant (WWTP) in Aveiro.

During the experimental period, it was tested a total operation cycle of 24 hours under the F/F regime, consisting of four discrete periods: (i) 00:00 hours, fill (20 min); (ii) 00:20 hours, aeration (feast and famine) (22h 50 min); (iii) 23:10 hours, settling (30 min); and (iv) 23:40 hours, draw (20 min). The liquor (supernatant of the reactor content after sludge settling) was withdrawn from the reactor, maintaining the hydraulic retention time (HRT) at 5 days and the solids retention time (SRT) at 10 days. The reactors were monitored (pH, OD and temperature) by a computer system software developed by the research group of the project "POLIBIO", from University of Aveiro.

3.3.3.1 Aerobic batch assays for PHA accumulating

PHA accumulation (step 3) assays were carried out in batch reactors with a total volume of 500 mL, inoculated with enriched biomass collected from the SBR aerobic system (step 2), at the end of an operating cycle, i.e., at the end of the famine phase, as reported in Serafim et al. (2004). The substrate used was the acidified effluent collected in the anaerobic acidogenic system for OFMSW fermentation (step 1). The biomass collected (100 mL) was concentrated gravimetrically during 45 min of settling, after removing the supernatant, in order to reduce the nutrient level. For all assays, 100 mL of substrate from step 1 were also added, as well as the micronutrients solution, according to Table 3-11. Ammonia was not added in this stage and the residual concentration of ammonia in the biomass sample collected in the SBR was always inferior to 0.1 mg $NH_3 - N\ L^{-1}$. The aeration of the reactors was maintained with a ceramic diffuser to provide high oxygen diffusion to the microorganisms and the stirring was maintained with a magnetic stirrer. The continuous agitation and aeration provided the maintenance of dissolved oxygen (DO) level always between 2-5 mg L^{-1}. PHA accumulation assays were conducted in ambient temperature. Figure 3-12 schematize the installation of PHA accumulation assays.

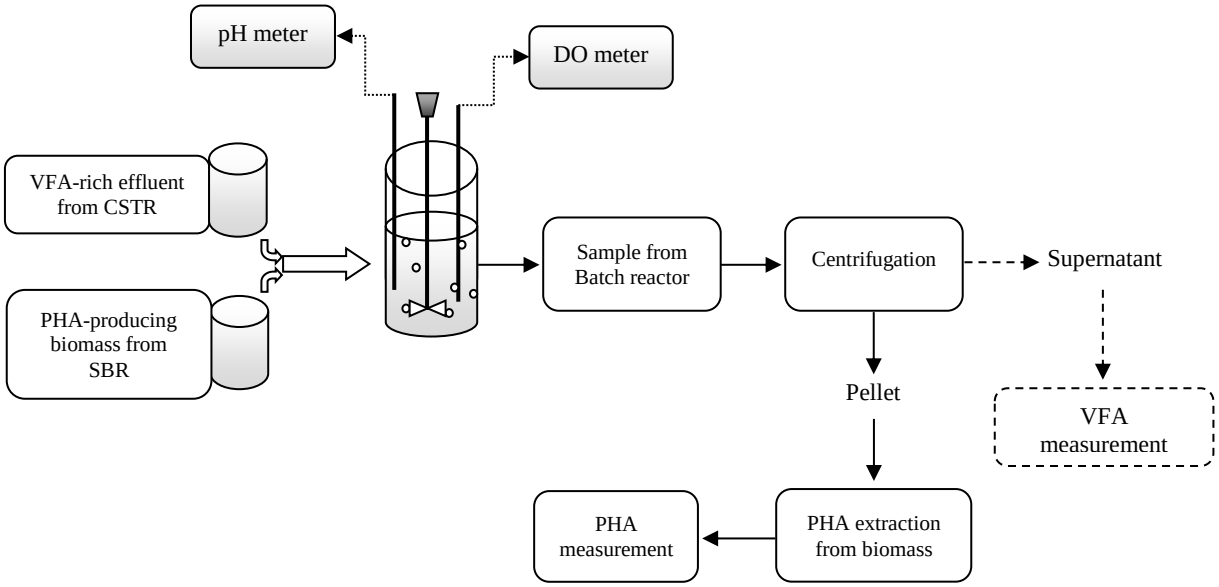

Figure 3-12: Schematic diagram from PHA accumulation assays.

Table 3-11: Micronutrients concentration added SBR and batch reactors

Component	Concentration
Mineral- solution	
$MgSO_2.7H_2O$	100 mg L^{-1}
EDTA	3.0 mg L^{-1}
$CaCl_2.2H_2O$	5.0 mg L^{-1}
FeCl3.6H2O	2.0 mg L^{-1}
Micro-nutrients solution	
H_3BO_3	0.3 mg L^{-1}
$MnCl_2$. 4H2O	0.03 mg L^{-1}
$CoCl_2$. 6H2O	0.2 mg L^{-1}
$ZnCl_2$	0.1 mg L^{-1}
$(NH_4)_6Mo_7O_2$. 4 H2O	0.05 mg L^{-1}
$CuCl_2$. 2H2O	0.01 mg L^{-1}
$NiCl_2$. 6H2O	0.02 mg L^{-1}

Three different accumulation assays (A1, A2 and A3) were carried out in a 400 mL working volume reactor in order to examine the PHA accumulation capacity of the biomass and investigate the polymer composition produced. For this purpose, test A1 was performed with pH control (pH between 7.0 to 7.3), test A2 was carried out without pH control, whereas A3 was made with controlled pH (pH=8) with 1M HCl. In order to evaluate the effect of substrate composition (type of VFA) on the final biopolymer, different acidified effluents with a high concentration of organic matter obtained in the CSTR operation, with pH values ranging from 5 to 6.5 were used in these tests. For all assays, the increase on the DO level

was used as an indicator of substrate exhaustion. During these batch tests, the progress of the experiments was monitored via online data acquisition (namely for DO, pH, acid and base dosage, off-gas CO_2 and O_2) and measurement of VFA uptake over time.

3.4 Analytical Procedures

In this work, several parameters were determined for the characterization of the used biowastes and to evaluate the performance of all reactors. All physical-chemical analyses were carried out according to the recommendations of the standard methods described in the Standard Methods for the Examination of Water and Wastewater (APHA, 2005) with the exception of the analysis of TCOD for samples with high solids content.

3.4.1 Solids

Total solids (TS) and volatile solids (VS) were determined following the guidelines given by the standard methods (method 2540-G). The solids analyses were performed with glass microfibre filters (Reeve AngelTM grade 403), analytical balance PrecisaTM XB120, drying oven WTCTM Binder E28 and muffle furnace TermolabTM Fuji PXR-9 (methods 2540-B, 2540-D and 2540-E). Total solids (TS) contents were determined in triplicates with a certain volume or weight of the sample residue (about 1 g) placed in a ceramic crucible and dried in a drying oven at 105oC for 24 hours. After cooling in the desiccators about 3 hours, the samples were weighed for TS determination in percentage (%), according the following Equation:

$$TS\ (\%) = \frac{A-B}{C-B} \times 100 \qquad (1)$$

Where: A - weight of dried (residue + crucible) after drying at 105 °C

B - weight of pre-dried crucible

C - weight of pre-dried crucible + sample before drying

The dried (residue + crucible) were put in a muffle furnace and ignited at 550 °C for 2 hours for VS determination. Analysis of VS content in a sample has an important application once it gives a rough estimation of the amount of organic matter present in the samples under

analysis. The VS content in percentage was determined in triplicate and presented as average values. These values were obtained according to Eq. (2):

$$VS\ (\%) = \frac{A - D}{C - B} \times\ 100 \quad (2)$$

Where, A - weight of dried (residue + crucible) after drying at 105 °C

B - weight of pre-dried crucible

C - weight of pre-dried crucible + sample before drying

D - weight of dried (residue + crucible) after drying at 550 °C

Similarly, total suspended solids (TSS) and volatile suspended solids (VSS) were determined using procedure described in Standard Methods (2540-D, 2540-E). To obtain the TSS, the sample solution (5 mL) was filtered and dried for 24 hours at 105 oC, and after then, the sample was burned for 2 hours at 550 oC to determine the amount of VSS, as represented in following Equations:

$$SST\ (mg\ L^{-1}) = \frac{A - B}{V_{LS}} \times\ 100 \quad (3)$$

$$SSV\ (mg\ L^{-1}) = \frac{A - D}{V_{LS}} \times\ 100 \quad (4)$$

Where: VLs - volume of filtered sample (mL)

3.4.2. Chemical Oxygen Demand (COD)

3.4.2.1 Closed reflux method

For liquid samples, the soluble COD (sCOD) was measured by the colorimetric method SM 5220-D, using the supernatant of samples after filtration (APHA, 2005). In this method, organic matter is oxidized with potassium dichromate ($K_2Cr_2O_7$) with a mixture of sulfuric acid and mercuric sulfate (H_2SO_4 + $HgSO_4$) and silver sulfate (Ag_2SO_4). The preparation of the solutions for COD determination is detailed in Annex I. After sample digestion in a thermos block Selecta® DQ06 at 150 °C for 2 hours, the built green Cr^{3+} ions concentration was measured by a colorimetric method using a spectrophotometer equipment Aqualytic™ at 620 nm, COD Vario PC compact (method 5220-D). This measurement was then converted to COD concentration taking into account the calibration performed previously (Annex II),

the dilution factor and the value of absorbance (in $mgO_2\ L^{-1}$) obtained for each sample, according to Eq. (5):

$$COD\ (gCOD\ L^{-1}) = \frac{1}{d_f} \times\ abs \quad (5)$$

Where: d_f – sample dilution factor

abs – absorbance value at 620 nm

3.4.2.2 Open reflux method

For samples with high solids content, TCOD was determined by an open reflux method described by Raposo et al.(2008). The proposed method consists of a wet oxidation with potassium dichromate (as the oxidant) and silver sulfate (as the catalyst) in a strong sulfuric acid solution. The preparation of these reagents is presented in Annex II. This method is considered more suitable and precise, namely for samples as OFMSW and WAS. The digestion apparatus was composed by a condenser connected to the digestion vessels which contain the digestion solution and the samples. The digestion vessels were placed in the block heater at 150 ºC for 2 hours, and cooled down to room temperature. After this, the digested solution was diluted to double the final volume with distilled water and titrate with excess potassium dichromate and ferrous ammonium sulfate (FAS) at 0.5 N using ferro in as indicator.

The TCOD of samples with TS content > 10 % (solid sample), was calculated using the following Equation:

$$TCOD\ (mg\ O_2\ g^{-1}\ VS) = \frac{(FAS_{BI} - FAS_{Ss}) \times N_{FAS} \times 8}{W_{Ss}} \quad (6)$$

For samples with high solids content, but with TS content < 5% (liquid sample), TCOD can be expressed using the following Equation:

$$TCOD\ (mg\ O_2\ L^{-1}) = \frac{(FAS_{BI} - FAS_{Ls}) \times N_{FAS} \times 8000}{V_{sample}} \quad (7)$$

Where FAS_{BI} – volume of FAS used in titration of the blank sample (mL)

FAS_{Ss} – volume of FAS used in the titration of solid sample (mL)

N_{FAS} – concentration of reducing reagent (N)

W_{Ss} – weight of dry sample (mg)

FAS_{LS} – volume of FAS used in the titration of the liquid sample (mL)

V_{sample} – volume of liquid sample (mL)

3.4.3. pH and Alkalinity

The analyses of pH and alkalinity were performed according to methods 2320B and 4500-H^+B, with an automatic titrator Mitsubishi™ GT, calibrated properly before each sampling. For anaerobic systems, alkalinity is essential to avoid sudden drops on pH, due to accumulation of volatile fatty acids when a high organic load is applied. To determine the alkalinity, 50 mL of sample from the reactor was titrated with hydrochloric acid (HCl 1.0 M) until pH value reach 4.5. Alkalinity of each sample was determined by the following Equation:

$$Alkalinity\ (g\ CaCO_3\ L^{-1}) = \frac{A\ \times N\ \times 50000}{V} \quad (8)$$

Where: A – volume of hydrochloric acid used in titration (mL)

N –concentration of hydrochloric acid solution (normality)

V – volume of sample (50 mL)

3.4.4. Total Organic Carbon (TOC)

TOC was determined in dilute filtered samples, previously stored at -4 ºC, by using a TOC/TN_b Analyzer, Analytic Jena™ multi N/C 2100, according to the differential method, which can be described with the following Equation:

$$TOC\ (mg\ L^{-1}) = TC\ (mg\ L^{-1}) - TIC\ (mg\ L^{-1}) \quad (9)$$

Where: TOC – total organic carbon

TC – total carbon

TIC – total inorganic carbon

Two sequential measurements are performed in the same sample to determine TIC and TC, respectively, and the difference between those values is considered as TOC. The differential

method detects not only volatile but also non-volatile organic carbon compounds. The TOC analysis should be used when the samples contain easily purgeable organic substances as benzene, cyclohexane, chloroform and other. On the other hand, the TOC analysis should not be used when the TIC content of the sample is significantly higher than the TOC content (Analytik Jena AG, 2011).

3.4.5. Total Kjeldahl Nitrogen (TKN)

To perform the Total Kjeldahl Nitrogen (TKN) analysis, a defined amount of the solid sample was directly digested using selenium as catalyst to accelerate the oxidation of some persistent organic substances. This method is used to determine the sum of both organic and ammonia nitrogen, involving a preliminary digestion to convert the organic nitrogen to ammonia at temperatures above the boiling point of sulfuric acid (340 ºC). The TKN can be deduced from the Eq. (10) as follows:

$$TKN\ (mg\ L^{-1}) = \frac{Vtotal_{sample}}{Vtotal_{blank}} \times M_{N_2} \times 1000 \times 2 \times C_{H_2SO_4} \quad (10)$$

Where: $Vtotal_{sample}$ – total sample volume used in titration (mL)

$Vtotal_{blank}$ – total blank volume used in titration (mL)

M_{N2} – Nitrogen concentration (g mol^{-1})

C_{H2SO4} – sulfuric acid concentration (mol L^{-1})

3.4.6. Volatile Fatty Acids (VFA)

The VFA mixtures obtained in anaerobic assays and used in aerobic production of PHA, were determined by gas chromatography. The mixtures were composed of mainly acetic acid [H-Ac], propionic acid [H-Pr] and *n*-butyric acid [H-Bu], but iso-butyric acid [H-*i*Bu], iso-valeric acid [HiVal], *n*-valeric acid [HVal] and *n*-caproic acid [HCap] were also quantified, although in lower amounts. The determination of VFA was performed in a gas chromatograph, injecting 0.5 µL of filtered sample containing 10 % (v/v) of formic acid (Panreac™) in a gas chromatograph PerkinElmer™ Clarus 480 with an injector set to 300 °C, a flame ionization detector set to 240 °C, a 25 m × 0.53 mm SGE™ ID-BP1 5.0 µm column and helium as carrier gas. The temperature program used was as follows: 1 min at

70 °C, rise of 20 °C min^{-1} to 100 °C and then kept for 2 min; rise of 10 °C min^{-1} to 140 °C and kept for 1 min; rise of 35 °C min^{-1} to 235 °C, and kept for 6 min (18.21 min of total running time). Calibration curves were obtained by injecting nine standard solutions of acetic, propionic, iso-butyric, *n*-butyric, iso-valeric, *n*-valeric, and *n*-caproic acids (Riedel-de Haën™). The calibration curves for VFA determination are presented in Annex III. Additionally, acid concentrations were converted into COD according to the following oxidation stoichiometry: 1.067 mg COD mg^{-1} acetic acid, 1.514 mg COD mg^{-1} propionic acid, 1.818 mg COD mg^{-1} *n*-butyric or iso-butyric acid, 2.039 mg COD mg^{-1} *n*-valeric or isovaleric acid, and 2.207 mg COD mg^{-1} caproic acid.

3.4.7. Biogas

The volume of biogas production in the anaerobic reactors was measured daily by water displacement method. The composition (CH_4 and CO_2) of biogas was analyzed by a gas chromatograph (SRI™ 8610C) equipped with a thermal conductivity detector (TCD) set to 75 ºC using 80/10 x 2.5 m CRS Hayesep™ column set to 61 °C and helium as the carrier gas at a flow of 10 mL min^{-1}. After injecting 2 mL of gas samples using pressure syringe into GC, the composition of the biogas (regarding CH_4 and CO_2 gases) produced was determined with reference to the peak area standard of each sample obtained from the chromatograms. The calibration curve was obtained with pure gases of CH_4 and CO_2 and also a molar mixture of the same gases to perform a validation process. In this work, it was just calculated the relative percentages of CH_4 and CO_2 and discharged other components not quantifiable by this column (such as N_2, H_2, H_2S). The relative percentages of CH_4 and CO_2 in biogas were determined using Eq. (11) and (12) respectively:

$$CH_4\ (\%) = 0.9896 \times \frac{Area_{CH_4}}{Area_{Total}} \times 100 \quad (11)$$

$$CO_2\ (\%) = 0.9924 \times \frac{Area_{CO_2}}{Area_{Total}} \times 100 \quad (12)$$

The volume of the produced methane was obtained from the triplicate average for each bottle and were expressed as the net volume of methane per g of VS added (mL_{CH4}/g_{VS} added).

3.4.8. Polyhydroxyalkanoates (PHA)

PHA concentrations were determined by gas chromatography in an equipment PerkinElmer^{TM} Clarus 480 equipped with thermal column SGE BP20 (WAX) 60 m x 0.32 mm x 0.5 mm. PHA quantification was done according with the methodology described by Serafim et al. (2004) and Lemos et al. (2006). Lyophilized biomass was incubated for methanolysis in chloroform and a solution of 20 % sulfuric acid in methanol. After the digestion step, the organic phase (methylated monomers dissolved in chloroform) of each sample was extracted and injected into a gas chromatograph coupled to a Flame Ionization Detector (GC-FID). The injection was performed at 280 ºC with a split ratio of 10. The oven temperature program starts at 40 ºC; then rise of 20 ºC min^{-1} until 100 ºC; then rise of 3 ºC min^{-1} until 175 ºC; and finally 20 ºC min^{-1} until 220 ºC. The detector temperature was set at 250 ºC. Hydroxybutyrate (HB) and hydroxyvalerate (HV) monomers concentrations were determined using two calibration curves, one for hydroxybutyrate and another for hydroxyvalerate, using standards (0.1–2 mg/mL) of a commercial P(HB-HV) polymer (88% of HB composition) and corrected using a heptadecane internal standard (concentration of approximately 1 mg/mL). The calibration curves for monomers determination are presented in Annex IV.

The PHA storage capacity of the biomass was determined based on the PHA content, represented in Eq. (13):

$$PHA\ content(\%) = \frac{PHA}{VSS} \times 100 \quad (13)$$

VSS is considered to be constituted by both active biomass (X) and PHA

The active biomass, X, (in mol L^{-1}) was estimated in according to Bengtsson et al. (2008), assumed to be represented by the typical molecular formula $C_5H_7NO_2$ and was calculated as:

$$VSS(mol\ L^{-1}) = \frac{VSS\ (g.L^{-1})}{113\ (\ g.mol^{-1})} \quad (14)$$

PHA yield ($Y_{\frac{PHA}{VFA}}$) was calculated by dividing the amount of PHA produced and the total amount of VFA consumed,

$$Y_{\frac{PHA}{VFA}} = \frac{PHA\ produced}{VFA\ consumed} \quad (15)$$

Specific substrate (VFA) uptake rate ($-q_{VFA}$) was calculated by the VFA consumed divided by the product of the initial active biomass concentration and the duration of batch PHA production (h):

$$-q_{VFA} = \frac{\Delta\ VFA}{X_{initial}.\ \Delta\ h} \quad (16)$$

Maximum specific PHA production rate (q_{VFA}) was calculated by the maximum PHA produced divided by the product of the initial active biomass concentration and the duration of batch PHA production (h).

$$q_{PHA} = \frac{\Delta\ PHA}{X_{initial}\ .\ \Delta\ h} \quad (17)$$

3.5 Microscopic Analysis

3.5.1 Direct observation of samples and Fluorescence

In order to visualize the PHA granules accumulated inside the bacterial cells and to estimate the capacity of accumulation of the culture, Nile-blue staining was applied to fresh samples recovered from SBR systems, at the end of the feast phase. According to Oshiki et al. (2011), fluorescence emitted from PHA granules with red color was easily observed using Nile-blue staining. The staining procedure was employed as described previously by Ostle and Holt (1982).

3.6 Indirect Calculations

3.6.1 Degree of Solubilization (DS)

The solubilization of the organic matter present in the substrate expresses the hydrolysis rate of particulate organic compounds (proteins, carbohydrates or fats) and this first step of anaerobic digestion enhances the further uptake by the microbial population of some

compounds, such as amino acids, sugars or fatty acids, being the sCOD the main product for the evaluation of the bioavailability of the organic material (Zhen et al., 2014). The degree of solubilization (DS) was determined through the quotient between the amount of solubilized COD, measured as sCOD at the end of the assays and the initial tCOD, measured without inoculum, deducting to both parameters the sCOD existing prior to fermentation, according to the following Equation:

$$DS\ (\%) = \frac{COD_{solubilized}}{TCOD_{in} - SCOD_{in}} = \frac{SCOD_{out} - SCOD_{in}}{TCOD_{in} - SCOD_{in}} \quad (18)$$

3.6.2 Degree of Acidification (DA)

The degree of acidification (DA) was the main parameter used to evaluate the acidogenic potential of the organic wastes under study and also the behavior of the acidogenic reactors. According to Gameiro et al. (2015), the DA was determined through the quotient between the sum of each individual VFA produced during the fermentation, expressed as COD equivalents, and the initial total COD (measured without the inoculum) for each assay, deducting to both parameters the amount of VFA existing prior to fermentation and was calculated as:

$$DA\ (\%) = \frac{TVFA}{tCOD_{in}} \quad (19)$$

The effluent quality in terms of VFA (express in gCOD-VFA g^{-1} COD) was determined as the amount of VFA (as COD equivalents) produced during the biological acidogenic fermentation, divided by the amount of soluble COD present in the liquid phase, as represented in Equation (20):

$$Y_{VFA/COD}\ (gCOD - VFA\ g^{-1}COD) = \frac{TVFA_{out} - TVFA_{in}}{SCOD_{out}} \quad (20)$$

3.6.3 Odd-to-Even ratio of VFA (Odd-to-even)

In order to evaluate the quality of the acidified fraction of the waste in terms of individual VFA, an additional parameter was considered besides the DA and the yield of VFA. The odd-to-even ratio of VFA was defined as the sum of odd-equivalent carboxylic acids formed

(propionic and *n*-valeric acids) divided by the sum of even-equivalent carboxylic acids formed (acetic, *iso*-butyric, *n*-butyric, *iso*-valeric, and *n*-caproic acids), according to Eq. (21):

$$Odd - to - even = \frac{[HPr] + [HVal]}{[HAc] + [HiBu] + [HBut] + [HiVal] + [HCap]} \quad (21)$$

The designation of odd or even is not related with the number of carbon atoms present in each carboxylic chain, but with the metabolic products of each VFA. In this sense, nonlinear acids (HiBu and HiVal) were considered in this study as even-equivalent acids according to their metabolic products, both resulting on acetic acid after β-oxidation pathway (i-but) or degradation (i-val) (Matthies and Schink, 1992), and not odd-equivalent according to the number of carbon atoms.

3.6.4 Total solids (TS) or volatile solids (VS) removal

The TS or VS removal rate during the experiences was calculated based on total mass removal from the testing reactors using Eq. (22).

$$TS\ or\ VS\ removal\ (\%) = \frac{I - F}{I} \times 100 \quad (22)$$

Where: *I* - initial TS or VS added to reactor (g)

F – final TS or VS in the reactor (g)

3.6.5 Hydraulic Retention Time (HRT)

HRT is the average residence time of the waste suspension inside the reactor. HRT has been calculated based on liquid volume of reactor and effluent flowrate.

$$TRH\ (d) = \frac{V_r}{Q_e} \quad (23)$$

Where: V_r – liquid volume of the reactor (mL)

Qe – effluent flow rate (mL)

3.6.6 Organic Loading Rate (OLR)

OLR has been calculated based on the mass of TCOD of the substrate fed to the reactor, according to the following Equation:

$$OLR\ (gCOD\ L^{-1}) = \frac{S_0 \times Q_e}{V_r} \quad (24)$$

Where: S_0 – concentration of feed substrate

3.7 Kinetic Model

In this study three different kinetic models were used to study and understand the performance and the evaluation of the methane production from WAS and OFMSW anaerobic co-digestion.

First-order kinetic models of the anaerobic digestion process used in this work (Eq. 25) were previously described by Sajeena et al. (2015) and provide a simple basis for comparing of hydrolysis/acidification process performance under practical conditions. The relationship between VS or COD and methane production can be described by Eq.27. Exponential Lag phase period model (Eq. 28) and Exponential Curve factor model (Eq. 29) were used to describe the kinetics of methane production from the co-digestion of WAS and OFMSW and also used to compared the evaluation of predicting methane production. In this study, the maximum biogas production potential and stability assessment (MBPPSA) model developed by Owamah and Izinyon (2015) was used to describe the stability/inhibition evaluation of mixture digestion process (Eq. 30).

The Curve Expert Professional 2.2 software and Software SigmaPlot™ 11.0 was used to obtain the graphical representation of the main operational parameters.

3.7.1 Hydrolysis kinetic coefficient of the first order

The hydrolysis rate constant was determined by the variation of the complex biodegradable substrate concentration with time and was expressed by the Eq. (25):

$$S_{(t)} = S_0 \cdot exp^{-k_H \cdot t} \quad (25)$$

Where $S_{(t)}$ represents the cumulative hydrolyzed substrate (namely the sum of the concentration of soluble substrate produced and CH_4 production during digestion at each time *t*), S_0 represents the initial biodegradable substrate concentration at time zero (0) and k_H is the first-order hydrolysis rate constant (d^{-1}).

3.7.2 Methanogic kinetic coefficient

The general equation for the first-order kinetic model shown in Eq. (25) can be correlated with methane production (represented as *G*), as shown in Eq. (25), where $G\infty$ represents the ultimate methane production:

$$\frac{G_\infty - G}{G_\infty} = \frac{S}{S_0} \tag{26}$$

From equations (25) and (26), the integrated equation for the first order model gives the relationship between the amount of methane produced and the digestion time (Eq. 27).

$$G_{(t)} = G_\infty(1 - exp^{-k_M \cdot t}) \tag{27}$$

Where the k_M represents the methanogenic rate constant (d^{-1}) and *t* represents the digestion time (day).

Representative models such as Exponential Lag phase period model (Sahito et al., 2013), which was set on an exponential relationship between specific bacterial growth curves, (Eq. 28) and also represented as first order exponential model with Lag phase period.

$$G_{(t)} = G_\infty\left(1 - exp^{-k_M (t-L)}\right) \tag{28}$$

The Eq. (27) was modified and elevated to variable 1/C, to give Eq. (29) described previously by Sahito et al. (2015) was used in this study. The equation has been recognized as a reliable tool in the co-digestion of residues with two different substrates for determination the ratio of volume of methane at any time to the maximum volume of methane.

$$G = G_\infty\,(1 - exp^{-kt})^{1/C} \tag{29}$$

Where *C* is the dimensionless curve factor.

The o study the inhibition / stability of the co-digestion is added the viability determination factor (*In*) to Eq. (29) and is represented by the following Eq.30, where to make the equation conform with general form of linear equations (y = mx + c).

$$G = G_{\infty}\,(1 - exp^{-kt})^{n} + In \quad (30)$$

In can obtained from the intercept of the Eq. $(1 - exp^{-kt})^{n}$.

Where: *n* represents the ratio of substrates mixed in co-digestion scenarios and *n* = 1 for equal proportion of substrates mixture. If (*In*) is negative, indicates feasible or non-inhibited process and positive (*In*) represents inhibited or non-feasible for methanogen process (Owamah and Izinyon, 2015).

3.8 Statistical Study

Statistical methods are the most frequently used in biotechnology to help the evaluation of the effective factors and building models to study the interaction between different experimental conditions and outputs obtained and to select the most suitable conditions to operate those biological processes with higher yield and efficiency. In this study, the optimization of the parameters of the process was carried out using response surface methodology.

3.8.1 Response surface methodology (RSM)

Response surface methodology (RSM) consists of a group of most common mathematical and statistical techniques used to investigate a combined effect of several combined variables and to find optimum conditions for a multivariable system (Baş et al., 2007). The RSM defines the relationships between the response and the independent variables alone or in combination on the processes. In this study, RSM was also applied to understand the effects of the two predictor variables (namely the total solids content in reactors and the alkalinity addition) and to evaluate the importance of each response parameter (as VFA production and VFA composition), in order to predict trends or optimize conditions for a potential scale-up of the use of the waste under study in an acidogenic anaerobic full-scale process. Design Expert Software StatSoft Statistica™ was used to perform the regressions and to obtain graphical representations of the response curves for the variables under study, so attempting to maximize the response. Results for maximum total VFA production, degree of acidification, effluent quality in terms of VFA and methane production were modelled according to Eq. 29 (as described by Myers et al., 2009):

$$E\ (z) = \ \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{1,2} x_1 x_2 + \beta_{1,1} x_1^2 + \beta_{2,2} x_2^2 \qquad (31)$$

where *E(z)* is the response variable, x_1 is the total solids content in reactors (in %), x_2 is the alkalinity added (in g L^{-1} as $CaCO_3$), β_0 is the model constant, β_1 and β_2 are linear coefficients (main effects), $\beta_{1,2}$ is a cross-product coefficient (interaction) and $\beta_{1,1}$ and $\beta_{2,2}$ are quadratic coefficients (Myers et al., 2009). The simulation is performed by solving the equation and find the values of the parameters β.

3.8.2 Quadratic model validation

For the validation of the significance of the experimental results obtained, it was applied the test analysis of variance (ANOVA) where the correlations were considered statistically significant at a 95% confidence interval ($p < 0.05$). The coefficient of correlation (R^2) was adopted to validate the quadratic models, which was calculated from prediction error sum of squares using software StatSoft Statistica™ and adjustment test for several degrees' numbers was obtained in according the following regression Equation (Ferreira et al.,2007).

$$R_a^2 = 1 - (1 - R^2)\frac{n-1}{n-p} \qquad (32)$$

Where: *n* is the number of the assays and *p* is the number of coefficients in the model.

4. Acidogenic biochemical potential of various organic wastes in mono and co-fermentation processes

4.1 Introduction

Due to the fast population growth, the agricultural activities and the industry related with these activities had become one the most important environmental problem worldwide. Every year, this sector generates million tons of wastes, resulting from both production and processing activities. The wastes generated by restaurants, markets, greenhouses or derived from other food and agricultural activities, had become the main constituents of municipal solid wastes (Salhofer et al., 2008). Recently, the use of food wastes for biovalorization processes had increased (Grisel et al., 2013; Yu et al., 2014) and anaerobic digestion (AD) has been the preferential technology applied for the treatment of several organic waste streams. The valorization processes for these wastes maximize recycling practices, converting a waste into added-value products and at the same time helping in the reduction of the environmental impacts due to their disposal (Bouallagui et al., 2004).

The aim of the work presented in this chapter was the maximization of the VFA production in mono and co-fermentation processes of various organic wastes, namely organic fraction of municipal solid waste (OFMSW), tomato waste (TW), coffee grounds (CG) and waste activated sludge (WAS). Hence, it was evaluated the acidogenic potential of these selected substrates either in mono or co-digestion processes and also the interaction (synergisms) between substrates, as well as the overall stability of each system under study. For these purposes, several co-digestion mixtures were selected (see Table 3.6, in sub chapter 3.2), in order to cover a wide range of possibilities which may occur in waste treatment plants, and to identify optimum mixtures in terms of high VFA productivity. A kinetic analysis was also conducted in order to evaluate the synergies of the co-substrates for VFA production and the performance of each reactor.

These four different co-substrates were selected due to the environmental problems caused by their management and disposal, either in generation or in treatment processes (Arroja et al., 2003; Wu et al., 2016)., The biodegradation process of these wastes will contribute to natural environmental contamination if not handled efficiently. Hence, the AD process applied to these wastes would minimize the negative impact on their disposal, helping the reduction of the pollution and, at the same time, to obtain added-value materials.

Due to their high organic content, the fermentation process of wastes such as OFMSW, TW, CG or WAS can offer many benefits such as the increase of the biodegradability of

substrates, with a cost-effective mass reduction for final disposal and a decrease on the carbon foot-print (Jian et al., 2011; Borowski et al., 2013). Characterization of these wastes and inoculum in terms of TS, VS, alkalinity, tCOD and sCOD were performed at the beginning of the experiments and were presented in chapter 3 (see subchapter 3.1).

To evaluate the acidogenic biochemical potential, in terms of total VFA production and individual VFA content, of each one of the four different substrates under study, anaerobic discontinuous reactors were used, because they are easy to handle and economic when it is needed a battery of tests (with different mixtures of substrates or operational conditions).

4.2 Results and Discussion

4.2.1 Mono substrates digestion and performance

Figure 4.1 (a and b) illustrates the variations on pH values and VFA concentrations measured during the whole experimentation stage for mono digestion assays for the four different substrates under study. In this experiment, pH values of almost all reactors dropped during the first 7 days, and later remaining nearly constant, except for the bioreactor with WAS where pH remained nearly constant at a value of 7 since the beginning until the end of the experience, showing low hydrolytic/acidogenic steps. Comparing pH evolution curves for the other three reactors (OFMSW, TW and CG), it can be seen that they present pH values approximately stable after day 7 until the end of the fermentation process. For these reactors, the minimum pH achieved ranged from 5.0 to 5.5 (final pH value), indicating high hydrolytic-acidogenic performance in all these batch reactors.

Generally, the decrease on the pH values is due to the easily digestible fraction of organic matters present in the waste, where hydrolyses and acidification processes can occur in the digester, due to the convention of complex organic matter, as it is observed by the accumulation of VFA (Wang et al., 2014). The low pH values achieved, without any external pH control, showed that the three substrates (OFMSW, TW and CG) used in the experiences are suitable for acidogenic fermentation, i.e., they have potential for VFA generation, with the CG waste presenting a much lower biodegradability than OFMSW and TW. In the opposite, the digester with WAS did not show favorable conditions for the growth of the hydrolytic-acidogenic bacteria, where the pH did not decrease neither there was a considerable amount of VFA produced. It is know that pH affects significantly the growth

rate of the microorganisms, either acidogenic or methanogenic. For the acidogenic phase, the optimum pH is considered generally between 4.5 ± 2 and 6.5 ± 2 (Fang and Liu. 2002; Wang et al., 2014), which were the pH values found in this study in the fermentation processes for three of the substrates (OFMSW, TW and CG). Hence, these three substrates were considered to be appropriate for VFA production.

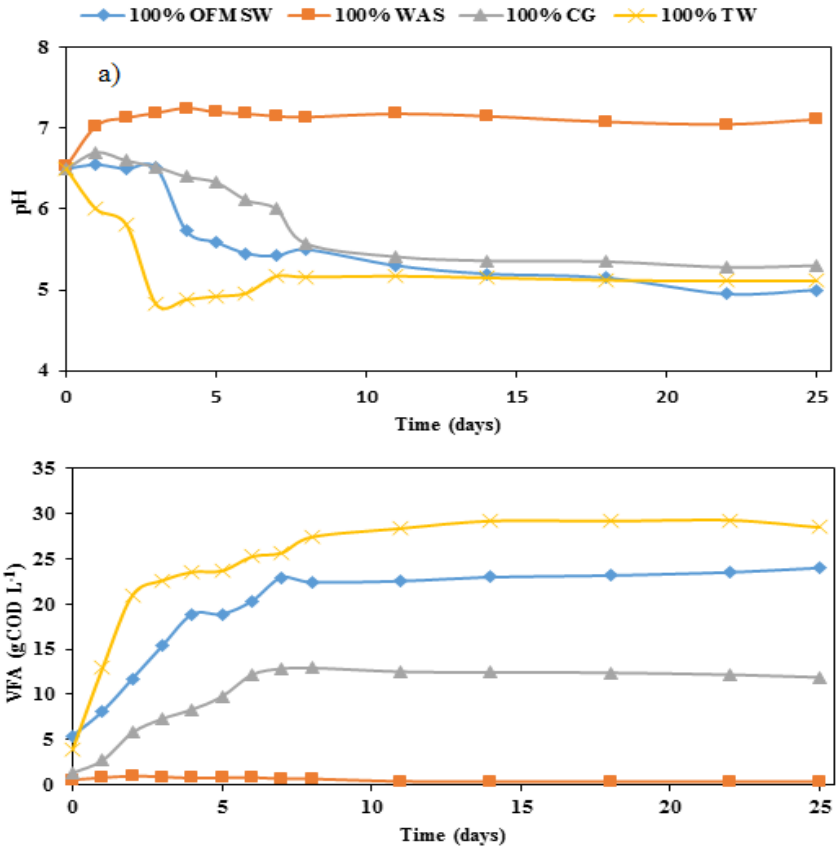

Figure 4-1: Plot showing the evolution of: a) pH values and b) VFA concentrations during all mono assays

Besides pH, total VFA production was also evaluated for the experiments with the four substrates and their evolution during the experiments are presented in Figure 4-1b. It is clear that a considerable amount of organic matter was converted into VFA production in the three reactors with OFMSW, TW and CG. The highest total VFA concentrations of 29.20 and 22.96 g VFA-COD.L^{-1} were observed in mono-digestions with TW and OFMSW respectively, followed by CG digester with 12.18 g VFA-COD.L^{-1}. The highest value obtained in the assay with TW may be explained by the high humidity and the low content of metal elements in its composition (Rossini et al., 2013), as well as the presence of high

amounts of easily biodegradable compounds (Jang et al., 2016). Although less biodegradable than TW and OFMSW, the presence of biodegradable carbohydrates in CG waste, which are its main components, favor the acidogenesis phase of the anaerobic process (Li et al., 2015). However, in the case of the WAS reactor, VFA production was very low (0.98 g VFA-COD L^{-1}) during the test, probably due to the fact that methanization phase was not inhibited together with a much lower hydrolytic-acidogenic activity. These results for WAS are consistent with the very small variation of the pH value found in this assay (Fig. 4-1a).

Fig. 4-2 shows the evolution of the degree of acidification over time (DA in percentage) and the maximum DA obtained for each experiment. DA is one of the most important parameters when evaluating acidogenic anaerobic systems.

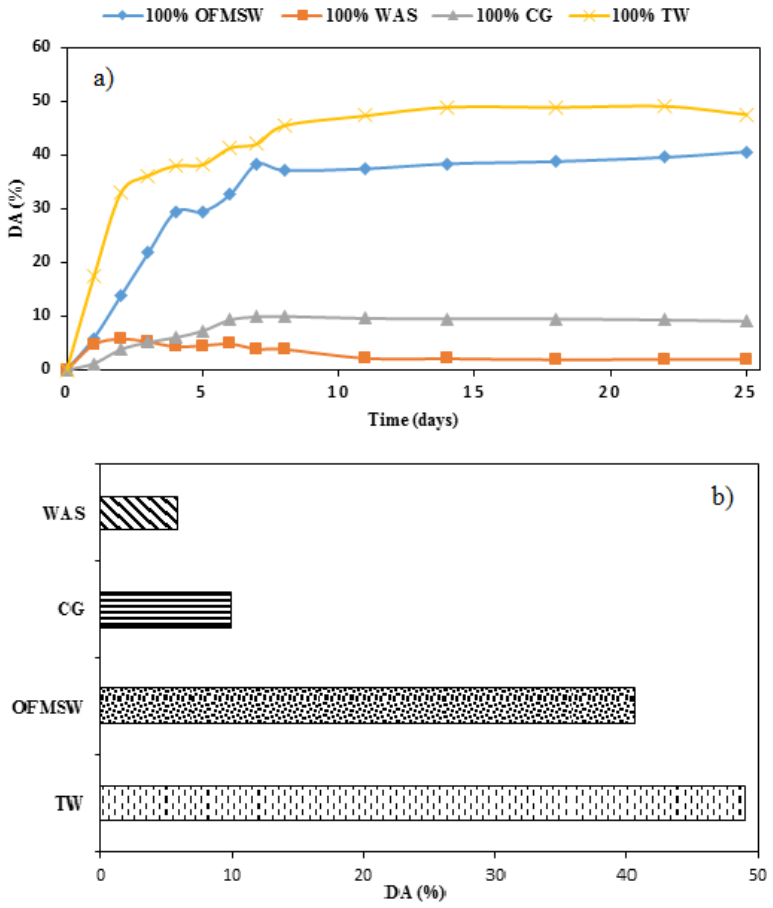

Figure 4-2: DA measured for the four substrates: a) DA in percentage with respect to time, b) maximum DA.

A clear distinction can be observed in Fig. 4-2 a), between the four substrates under study, with a significantly higher DA for two of the them. It is observed that TW and OFMSW

bioreactors yielded a much higher DA (49.0 % and 40.6 % respectively) when compared to the CG reactor (9.9 %) and WAS reactor, which presented the lowest DA of all digestion tests (5.7 %) (Fig.4.2 b), indicating a very low fermentable organic fraction in this waste.

Based on these results, low acidogenic performance in the mono-digestion of CG and WAS, and on the characteristics of the four substrates (see sub-chapter 3.1), it was decided to perform co-digestion experiments with CG and WAS and adding as co-substrate the other two wastes with higher acidogenic performance, in order to optimize VFA production, due to a potential increase of synergies. In addition, as shown in Table 3-5 (in chapter 3), pH value of the CG waste is 4.1, value which is lower than pH of TW (5.1), OFMSW (6.3) or WAS (6.8), indicating another potential synergy, which could be a favorable complementation for mixing substrates (Zhang et al., 2015). Thus, four mixtures were prepared for further VFA production study, as shown in the following schematic diagram (Fig. 4-3) and, for each mixture, three different proportions of each co-substrate were tested.

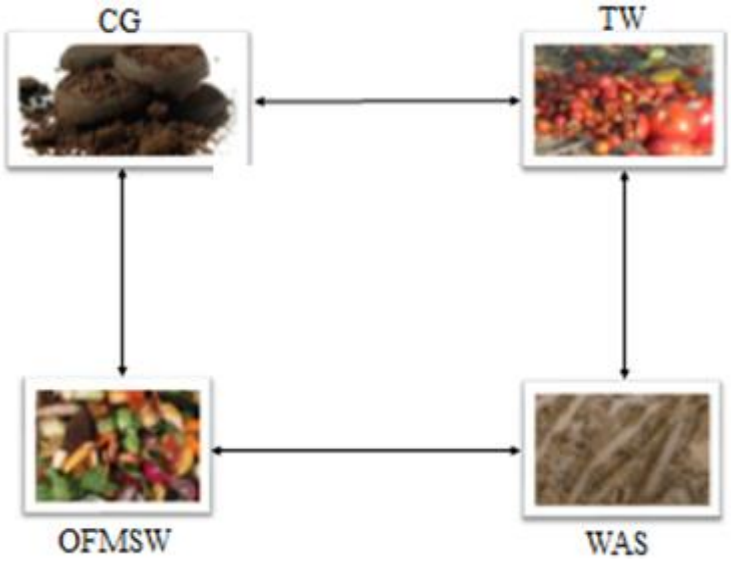

Figure 4-3: Schematic of organic waste mixture in this study for co-digestion

4.2.2 From mono-digestion to co-digestion of organic solid wastes

The anaerobic co-digestion (AcoD) study was divided into four phases: **phase I** - studies of the AcoD of CG and TW; **phase II** - assays of AcoD of CG and OFMSW; **phase III** – assays of AcoD of WAS and TW; and **phase IV** – assays of AcoD of WAS and OFMSW. Two assays with a mixture of higher biodegradable substrates (OFMSW and TW) and lower biodegradable substrates (GC and WAS) were also performed, but the results were

discharged due to practical problems occurred during experiments. In particular, due to the rapid hydrolysis and consequently the fast acidification observed in the bioreactor containing a mixture of OFMSW and TW, it was impossible to control in practice the process due to excessive foaming, with a consequent blockage of all tubing, including the one for biogas collection. After a few trials, it was decided not to consider this mixture in the study, due to the impossibility to obtain accurate results. The same decision was taken with the mixtures with CG and WAS, but for opposite reasons (very low hydrolysis and consequently very low acidification in the bioreactor).

Hence, based on the four mixtures selected for the anaerobic co-digestion studies, only the results using three of that mixtures will be discussed in this chapter: CG + TW, CG + OFMSW and WAS + TW. The discussion of the results obtained in the co-digestion assays with the mixtures composed of WAS and OFMSW will be done separately in chapter 5, because of their importance to Cape Verde Islands waste management strategies, reasons previously reported in sub chapter 2.1.2. Hence, the following discussions will be focused only in the first three phases of this experimental plan (phase I, phase II and phase III).

All batch co-fermentation assays were operated under the same conditions as mono digestion assays (see sub chapter 3.2.1). In addition the co-substrates were not pretreated, in order to evaluate the basic performance of the co-digestion process under study.

The main experimental results in terms of maximum TVFA concentration achieved and the DA obtained during the AcoD of the mixtures correspondent to Phases I, II and III, are reported in Table 4.1.

For the three mixtures discussed in this chapter (phases I, II and III), both co-digestion assays with CG (phases I and II) obtained higher DA (19.7 to 57.6 %) and higher TVFA (18.9 to 32.5 g VFA-COD L^{-1}) than the assays with WAS and TW (phase III), where it was obtained DA (10.3 to 28.7 %) and TVFA (4.2 to 10.9 g VFA-COD L^{-1}). Although it was expected to have higher performance for assays with CG when compared with assays with WAS, the synergies obtained were much higher than were predictable.

For phases I and II (assays with CG waste), it can be observed that, DA for all assays, varying between 19.7 to 57.6 % were considerably higher (at least doubled) than mono-digestion assay with just CG (9.9%). In addition, in the case of the assay with 25 % CG and 75% TW, it even showed a higher DA (57.6 %) than the mono-digestion assay with just TW (49%),

which indicates a high synergy effect on the mixture of the two co-substrates (CG and TW). With respect to the assay with 25 % CG and 75% OFMSW, although it did not show a higher DA (36.6 %) than the mono-digestion assay with just OFMSW (41%), it showed a higher TVFA (27.9 g VFA-COD L^{-1}) than the mono-digestion assay with just OFMSW (22.9 g VFA-COD L^{-1}), which also indicates a good synergy effect on the mixture of the two co-substrates (CG and OFMSW).

Table 4-1: Soluble and total COD in the beginning of the experiment, maximum total VFA concentration, DA in percentage, and VFA/ALK ratio, in the co-digestions assays (mean ± standard deviation)

Experiment/composition Co-substrates	Parameters				
	sCODin (g COD L^{-1})	TCODin (g COD L^{-1})	TVFAmax (g VFA-COD L^{-1})	DA (%)	VFA/ALK
Phase I					
75 % CG + 25% TW	30.5 ± 0.7	56.5 ± 0.9	18.9 ± 1.4	32.5 ± 0.4	1.6 ± 0.1
50 % CG + 50 % TW	33.4 ± 0.6	51.2 ± 0.6	25.9 ± 1.9	49.6 ± 0.2	2.4 ± 0.1
25 % CG + 75% TW	45.7 ± 0.7	55.5 ± 0.7	32.5 ± 1.3	57.6 ± 0.2	2.9 ± 0.2
Phase II					
75% CG + 25 % OFMSW	28.1 ± 0.6	48.0 ± 0.9	20.5 ± 1.4	19.7 ± 0.4	2.1 ± 0.4
50 % CG + 50 % OFMSW	30.8 ± 0.4	47.1 ± 1.5	22.4 ± 1.8	36.5 ± 0.4	2.3 ± 0.2
25 % CG + 75 % OFMSW	32.9 ± 0.4	45.2 ± 1.4	27.9 ± 1.8	36.6 ± 0.3	2.6 ± 0.2
Phase III					
75 % WAS + 25 % TW	11.73 ± 1.0	37.6 ± 1.8	4.2 ± 0.7	10.3 ± 0.2	0.3 ± 0.1
50 % WAS + 50 % TW	11.13 ± 0.9	34.1 ± 1.9	7.2 ± 0.9	20.2 ± 0.4	0.4 ± 0.1
25 % WAS + 75 % TW	16.27 ± 0.9	37.2 ± 1.8	10.9 ± 0.9	28.7 ± 0.5	0.6 ± 0.1

The results of phase III (assay with WAS), show that DA for all assays, varying between 10.3 and 28.74 % were considerably higher (at least double) than mono-digestion assay with just WAS (5.7%). In addition, TVFA for all assays, varying between 4.2 and 10.9 g VFA-COD L^{-1}, were also considerably higher (at least four times) than mono-digestion assay with just WAS (0.98 g VFA-COD L^{-1}), which indicates a high synergy effect on the use of TW as a co-substrate for WAS.

The variation of pH and VFA concentrations profile throughout the co-fermentation tests for all assays are shown in Figure 4-4. As it can be seen in Fig. 4-4 a) and b), for assays with CG, all reactors achieved a pH lower than 6.0, at the end of experiments. For that assays, the higher the amount of TW or OFMSW as co-substrate (50% or higher), the quicker they achieve low pH (from day 5 onwards). For the assays with just 25% of the higher biodegradable co-substrate (TW or OFMSW), the pH only achieves that low values later on, after day 10. Hence, the use of these two co-substrates (TW and OFMSW) accelerated the

acidification process of CG, as can be seen in Fig. 4-4 b) and d), reaching much higher TVFA and DA (Table 4-1).

For the assays with WAS, Fig. 4-4 e), all co-digestion experiments did not achieve a pH lower than 6.0, at the end of experiments, except the assay with just TW. For the assay with the highest amount of TW as co-substrate (75%), the pH was the lowest in the co-digestion assays, but remained around 6.5 during all experiment. However, the use of this co-substrate (TW) accelerated the acidification process of WAS, as can be seen in Fig. 4-4 f) for all assays, reaching the highet TVFA (10.9 g VFA-COD L-1) and DA (28.7%) in the assay with 25% WAS and 75% of TW, as can also be seen in Table 4-1.

It can also be observed in the Fig. 4-4 e) and f) phase III assays, that the higher the proportion of WAS in the mixture the higher the pH increase in the begging of the assay. In addition, the minimum pH value reached in these assays (phase III) were the highest for all assays (higher than 6), varying between 6.7 for the assay with 75 % of WAS and 6.5 for assay with 50 % of WAS, indicating a lower acidification extension.

In this study, all reactors showed an initial stage with hydrolytic and acidogenic activity during the first 15 days, with the increase on the VFA amount produced (Fig. 4-4). After the initial hydrolytic-acidogenic phase, the VFA concentrations remained constant until the end of the experiments and this profile was observed in all reactors. The increase on the VFA concentration of the mixtures under study reflected the extension of the acidification process where it was observed a gradual increase on VFA concentrations during the first ten days of the experiments. These results are in accordance with the ones obtained by several authors, where slightly acid conditions were considered as optimal for hydrolysis/acidogenisis steps (Rajagopal et al., 2014; Shofie et al., 2015; Li et al., 2015).

Comparing the VFA concentrations obtained in all experiments (Table 4-1), it can be seen that in the co-digestion assays with CG were obtained much higher VFA concentrations (18.9 – 32.5 g VFA-COD L^{-1}) than in the co-digestion assays with WAS (4.2 – 10.9 g VFA-COD L^{-1}). In addition, the assays with CG and TW, showed a better performance and synergy than the mixtures of CG and OFMSW.

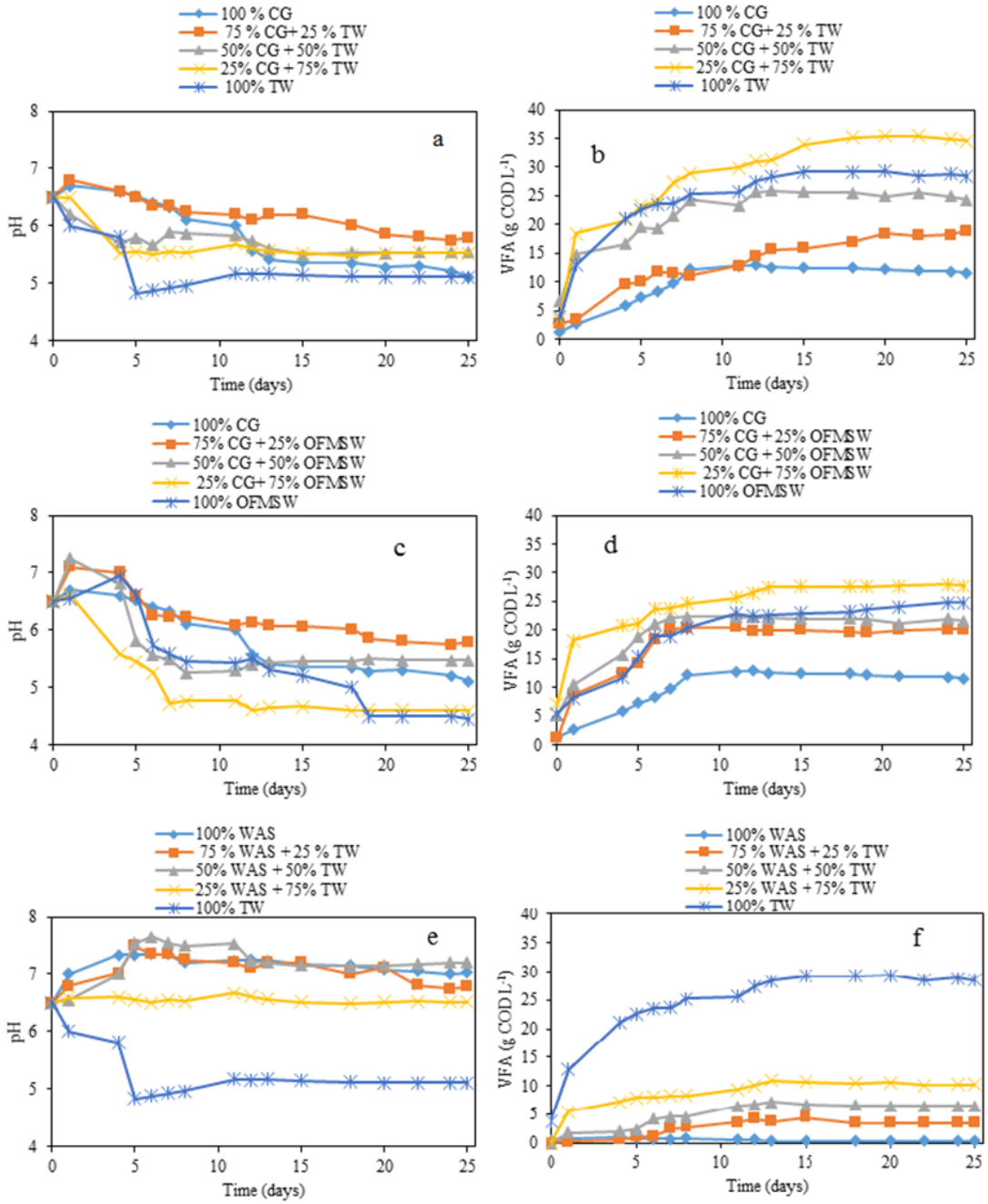

Figure 4-4: Plot for the evolution of pH and VFA production during the mono and co-digestion assays: a) e b) CG and TW; c) e d) CG and OFMSW and e)e f) WAS and TW.

Similar conclusions were observed in the co-digestion assays of CG with TW and OFMSW, as represented in Fig. 4-4 a) and c), showing better performances in the mixtures than in the mono-digestion assays. For example, in the co-digestion assays with OFMSW, the one with 25 % CG + 75 % OFMSW, showed the best performance with respect to VFA production, reaching also a very low pH of 4.5, as can be seen in Fig. 4-4 c) and d). This behaviour is

even better than the one presented by the reactor with just OFMSW, which emphasizes the synergy of mixing OFMSW with a small amount of a lower biodegradable material such as CG.

The increase on VFA concentration during the batch assays (phases I and II) suggests that the three substrates under study (CG, TW and OFMSW) have high acidogenic potential which consequently can cause the inhibition of the methanogenic activity. Contrarily, in phase III, co-digestion assays with WAS and TW showed a low performance for the acidogenic potential. In particular, when it was mixed just 25% of WAS to TW, the acidogenic potential dropped abruptly to less than half the value, when compared to the assay with just TW, which suggests that, combining a small amount of WAS to TW, decreases the inhibitory effect to the methanogenic microorganisms with a probable increase in the methane generation.

4.2.3 VFA composition during the acidogenic co-fermentation assays

The composition of the VFA mixture obtained in an anaerobic co-fermentation process is important as it can provide not only useful information regarding the degree of hydrolysis and fermentation but also it determines the further application of the mixture (Wang et al., 2014). Hence, with the aim of further application, besides the amount of VFA produced it is very important to know the individual VFA composition obtained in the acidogenic fermentation processes under this study, so it is crucial to assess the synergies that may occur from mixing two different substrates. Fig. 4-5 a) to i) illustrate the VFA composition observed during all co-digestion assays (phases I, II and III), specially the main in dividual volatile fatty acids produced, namely acetic (H-Ac), propionic (H-Pr) and butyric (H-Bu) acids, which accounted to 75 to 90% of total VFA (TVFA) produced. Occasionally, small amounts of valeric (H-Val) or caproic (H-Cap) acids were also observed in same reactors, but accounting less than 10% of TVFA produced. In these co-digestion assays, acetic and butyric acids were always the major products, being the butyric acid found in higher amounts when the pH was in range of 5.0 – 6.0. H-Ac and H-Bu acids concentrations measured were higher in the co-digestions assays with CG and TW than in the other co-digestion tests,

reaching almost double concentration in the former case. This result is in accordance with the previous studies by Rent et al. (2007) and Wag et al. (2014).

Similar trends with respect to the evolution of H-Pr concentration were observed on the co-digestion assays of CG with TW and OFMSW, with the concentration of H-Pr being rapidly increased, especially in the assay with higher proportion of CG in the mixture (75 % to 100%). In the assay with 100 % CG, in contrast to the other reactors, H-Pr was even the main component of the VFA mixture, where its concentration increased at beginning of the experience and remained almost constant until the end of the digestion assay. This result is in accordance with the finds achieved by Luo et al. (2013) and Li et al. (2015), who reported that at the low pH, the conversion of higher molecular weight compounds to lower molecular weight volatile fatty acids ($C_2 – C_6$) was efficient, leading to the accumulation of H- Pr acid in a high ratio. In the addition, according to Yu and Fang (2002), pH 4.0 – 4.5 favoured the production of H-Pr and in this study the highest propionic concentration was archived at a slightly higher pH of 4.5 – 5.0.

Relatively to the production of H-Bu, and comparing the three phases (see Fig. 4-5), it can be observed that H-Bu was detected in much higher concentrations in the assays of phase I (GC and TW), especially for the assays with lower CG (25% and 50%). In the opposite, assays with higher amounts of CG (75% to 100%) showed very small amount of H-Bu. Similar amounts of H-Bu, but much lower than the assays in phase I, were observed in all assays in the phase II (CG + OFMSW) with the exception of the assay just CG were the values were even lower. For the phase III, all co-digestion assays with WAS and TW exhibited very low production of H-Bu (less than 2 g VFA-COD L^{-1}), showing a low performance for the acidogenic potential as already stated before. It is interesting to note that lower H-Bu production contributed to lower acidification values (see Table 4-1). This is in agreement with Dahiya et al. (2015), who stated that the degree of acidification is largely influenced by the type of the VFA produced in the system.

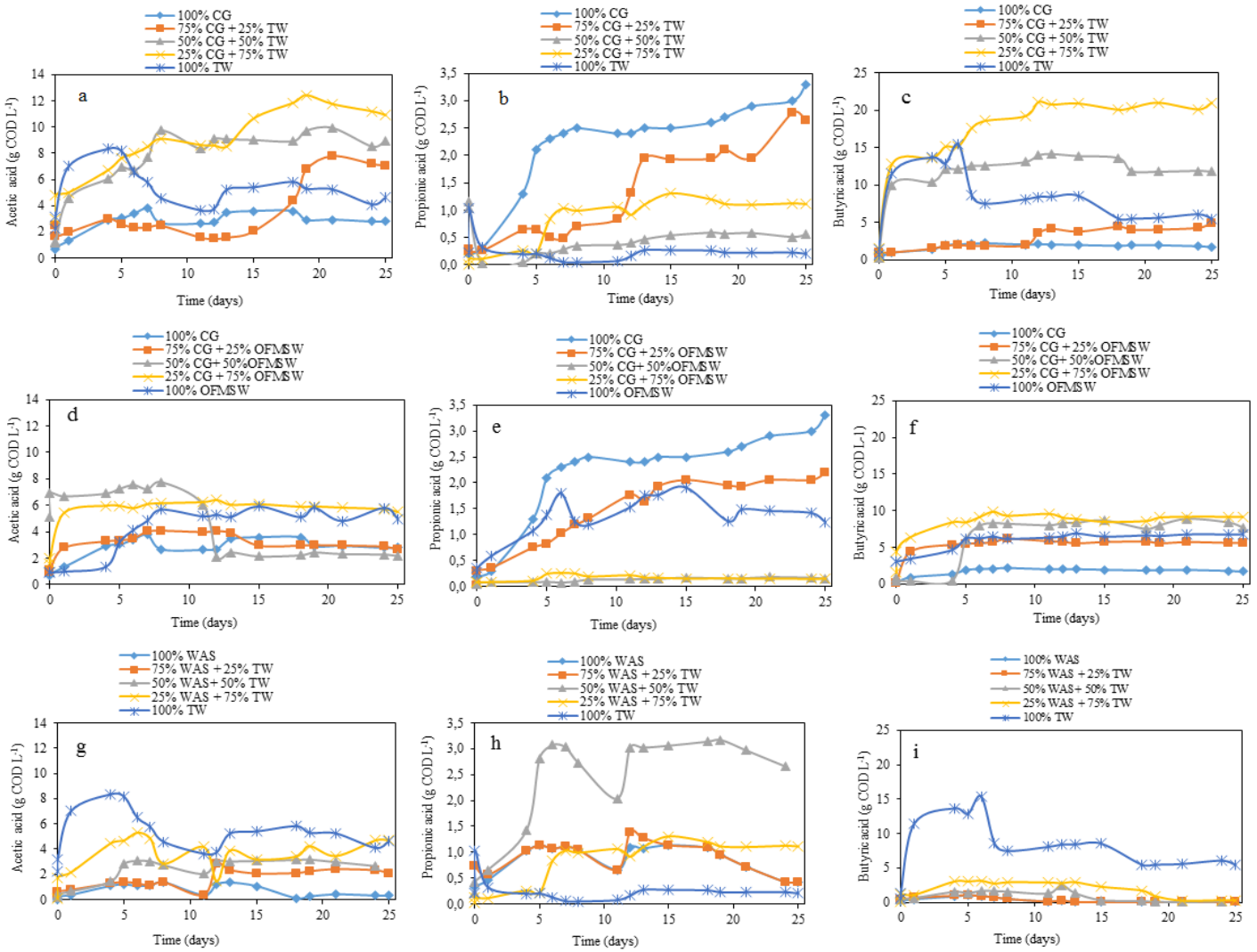

Figure 4-5: Individual VFA composition; acetic acid, propionic acid and butyric acid from mono and co-digestion assays: a), b) and c) CG and OFMSW; g), h) and i) WAS and TW.

4.2.4 Digester stability on VFA production

Some environmental parameters, as VFA concentrations and pH or alkalinity values have been identified as the most important indicators to evaluate the stability of the anaerobic co-digestion system (Liu et al., 2012) in terms of methane production. To evaluate the stability of an anaerobic system during the biodegradation process, the average value of VFA/Alkalinity (VFA/ALK) ratio is typically used. In this work, the stability of the systems was evaluated based on the study describe by Rincon et al. (2008) and Fonoll et al. (2015). According to some authors, when the VFA/ALK ratio is less than 0.4 the methanogenic step of the process is stable, without the risk of acidification, but when VFA/ALK ratio is high, the process is potential acidogenic (Rincon et al., 2008; Fonoll et al., 2015; Shofie et al., 2015). Hence, several authors have proposed that the optimum VFA/ALK ratio should be determined for each specific situation, as it is dependent on the constitution of the waste and their physical-chemical characteristics. For the VFA/ALK ratio determined in the present study, it can be concluded that the values for phases I and II are in accordance with an acidogenic process (Table 4-1), corroborated by the pH values observed (see Fig. 4-1a) and b)) at the end of assays for all digesters, confirming the existence of favourable environmental conditions for the occurrence of acidogenesis. VFA/Alk ratios in all tests assays of phases I and II (1.0 – 2.9) are much higher than the recommended values by some authors for methanogenic performance stability (Rincon et al., 2008; Bernard et al., 2001; Liu et al., 2012; Shofie et al., 2015) , which makes then favourable for acidogenic process predominance. In this study, negligible methanogenic activity was detected in the assays of these two phases, confirming the previous observation that higher VFA production at low pH or alkalinity could be toxic to methanogenic microorganisms, causing the inhibition of methanogenic microbial population (Borga et al., 2004; Zhang et al., 2015). In addition, the increase in stability and performance in the assays where it was added a substrate (TW or OFMSW) to the GC can also be sustained by the fact CG waste contains caffeine, hemicellulose, free phenols and tannins, which might be toxic to microorganisms, not only to the highest sensitive methanogenic microorganisms but also to the acidogenic bacteria (Fan et al., 2003). Hence, these finding further suggest that the co-digestion of CG with other substrates, such as TW, had synergistic effects on the performance of the digestion process. Contrarily, phase III assays (WAS and TW) showed low VFA/ALK ratio (values between 0.3 and 0.6) which, together with the higher pH values found in these co-digestion assays,

indicate favourable conditions for methanogenic activity, which might have prevented the dominance of acidogenic bacteria in most assays (Riau et al., 2010).

4.2.5 Biogas production and cumulative methane efficiency

The different mixtures of biodegradable food wastes used in this study influenced the evolution of biogas components (mainly CH_4 and CO_2). Fig. 4-6 presents the cumulative CH_4 and cumulative biogas production under the time, obtained in all batch phases assays. The amount of methane generated was calculated in terms of COD equivalent per unit of reactor volume.

The Figure 4.6 (b, d and f) show the evolution of cumulative biogas production. Biogas production started immediately from the first day for almost all digestion tests. The highest maximum cumulative biogas was obtained for the assay with 100 % TW which amounted to 24.5 L at day 15 of experience, most probably due to the higher sCOD content (Table 4-1), which could be rapidly biodegraded since the first day. This value is still higher than those obtained for the co-digestion assays with WAS and TW (phase III), where the peak values were 17.5 L and 14.7 L for the assays with 25 % WAS + 75 % TW and 50 % WAS + 50 % TW respectively, obtained at the end of the experiment after the methanogenic activity predominance prevailed.

It can also be seen that the majority of the methane produced, although in a very small amounts, was generated in the first five days of the experiment, with the exception of the assays in phase III (Fig.4-6 i), where higher CH_4 production was observed after day 15 and until the end of experiment. The decrease in pH values (see figure 4-4 a) and 4-1 b)) and the accumulation of propionic acid, were probably the most responsible for the low CH_4 content in the biogas produced (Li et al., 2015; Zhang et al., 2015). The results for methane production confirmed the achievements already observed previously that, in phases I and II acidogenic bacteria activity was dominant as opposed to the verified in phase III. In this phase (WAS+TW), it was reached a maximum of 15.3 and 16.2 g COD L^{-1} reactor in the batch assay with 50 % WAS + 50 % TW and 16.2 g COD L^{-1} reactor for the batch assay of 25 % WAS + 75 % TW.

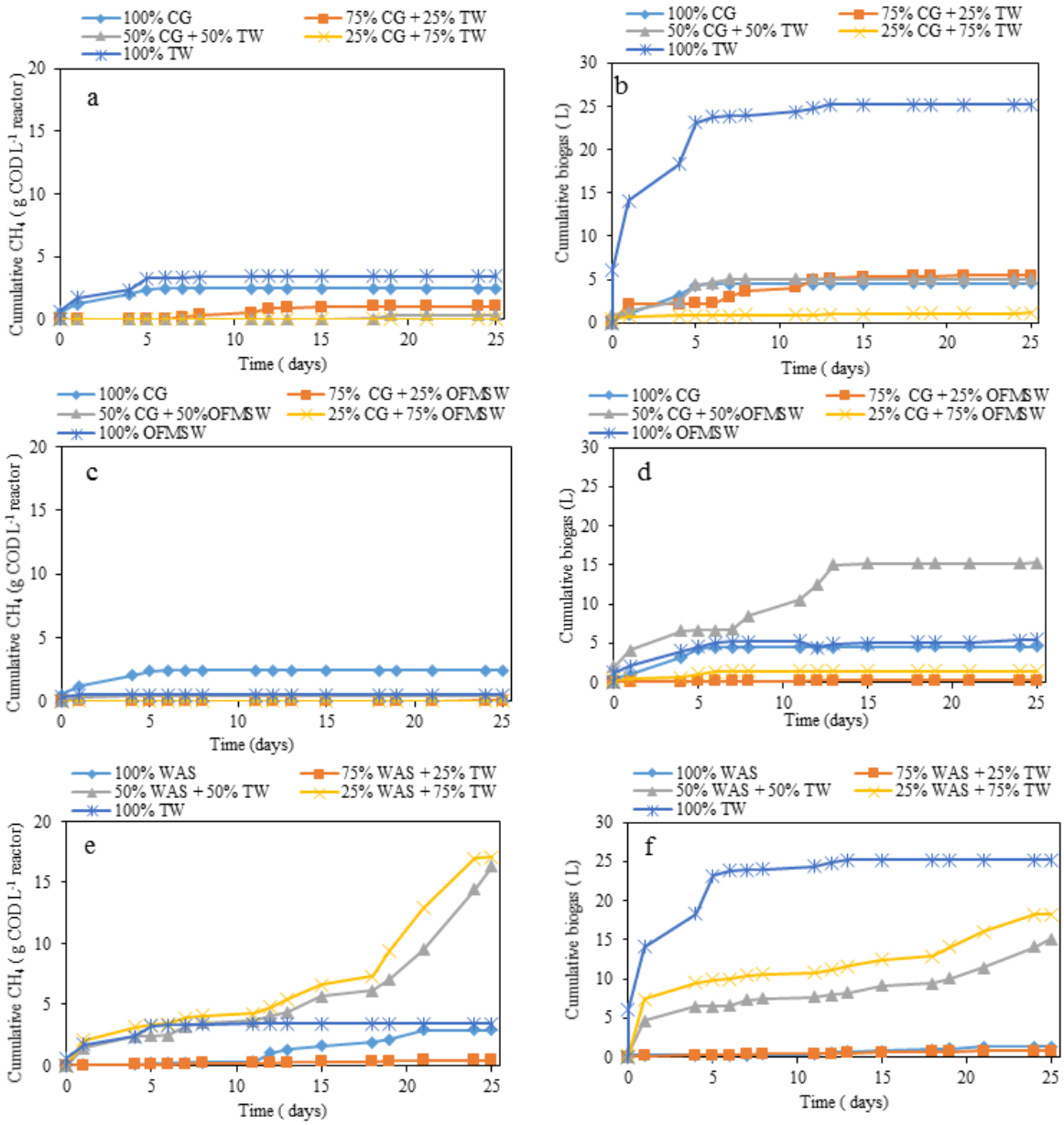

Figure 4-6: Evolutions of cumulative biogas and methane production from mono and co-digestion under experience.

Hence, for all the experimental conditions in phases I and II, the reactors presented an average acidification degree between 19.7 % and 57.6 % over the 25 days of operation and a stabilization phase after day 10, indicating that acetogenesis was the limiting step, because the majority of the accumulated VFA species were not converted into acetate, affecting consequently the further methane production (Li et al., 2015).

4.2.6 Kinetic study

Understanding the kinetics of the anaerobic digestion of substrates is crucial to optimize the design of AD systems. The accumulation of some VFA species in anaerobic treatment is an important indicator of the extension of the acidogenic fermentation. The kinetics of overall acidogenic fermentation process should be described considering two fundamental steps: first, the hydrolytic bacteria convert the complex organic compounds into simple soluble compounds; second, the acidogenic bacteria convert the simple soluble compounds into VFA (Lin et al., 2015).

To evaluate the performance of the fermentation process, considering the mono and co-digestion of CG, TW, OFMSW and WAS (phases I and II), it was determined kinetic parameter for VFA production from different mixtures of substrates, using the first-order kinetic model (Eq. 25). The model was used to estimate disintegration-hydrolysis kinetics from batch assays. Tables 4-2 summarize the results of the kinetic study performed using all data obtained either in mono or co-digestion assays. Analyzing the data in Table 4-2, it can be observed the advantages caused by mixing two substrates instead of using just CG, TW, OFMSW or WAS. Regarding CG (VFA yield of 0.1 $gCOD.L^{-1}.d^{-1}$), it is evident the higher VFA yields obtained in the mixtures either with TW (0.2-0.6 $gCOD.L^{-1}.d^{-1}$) or OFMSW (0.2-0.4 $gCOD.L^{-1}.d^{-1}$). Regarding TW or OFMSW, although the VFA yields are not higher in the mixtures, the amount of VFA produced in the two assays with the smallest amount of CG (25%), are higher than in the mono assays: 31.2 $gVFA\text{-}COD.L^{-1}$ (with TW) and 20.6 $gVFA\text{-}COD.L^{-1}$ (with OFMSW) when compared with 24.6 $gVFA\text{-}COD.L^{-1}$ (TW) and 19.4 $gVFA\text{-}COD.L^{-1}$ (OFMSW).

In the addition, according to the VFA yields calculated and presented in Table 4-2 co-digestion assays between CG + TW resulted in values slightly higher than the results obtained in co-digestion assays using CG + OFMSW.

The AcoD between WAS and TW (phase III) showed much lower kinetic VFA yields (0.08-0.1 $gVFA\text{-}COD.L^{-1}$) than the other two phases, confirming that fermentation activity in these assays was not predominant.

The highest hydrolytic/acidogenic activities were observed in assays where pH achieved lower values (phases I and II), as also reported by Jiang et al. (2013), which obtained higher VFA yields at pH 6.0.

Table 4-2: Maximum predicted and experimental VFAs produced in batch experiments of mono and co-digester acidogenic fermentation and kinetic parameters obtained in this study.

Assays experimental	Predicted VFA ($gCOD.L^{-1}$)	Experimental VFA ($gCOD.L^{-1}$)	R^2	VFA yield ($gCOD\ L^{-1}\ d^{-1}$)
100 % CG	11.1	11.6	0.98	0.10
100 % TW	24.7	24.6	0.99	0.35
100% OFMSW	19.3	19.4	0.99	0.32
100% WAS	1.9	1.7	0.96	0.01
75 % CG + 25 % TW	16.3	16.2	0.97	0.20
50 % CG + 50 % TW	18.6	17.8	0.99	0.24
25 % CG + 75 % TW	31.3	31.2	0.99	0.26
75 % CG + 25 % OFMSW	19.1	19.4	0.97	0.22
50 % CG + 50 % OFMSW	16.8	17.2	0.98	0.21
25 % CG + 75 % OFMSW	20.3	20.6	0.98	0.24
75 % WAS + 25 % TW	3.6	4.2	0.96	0.08
50 % WAS + 50 % TW	6.7	7.2	0.97	0.10
25 % WAS + 75 % TW	10.3	10.9	0.97	0.10

The determination coefficients (R^2) values for all curves were ranging of 0.96 - 0.99 for all mono and co-digestion assays, suggesting that the first-order kinetic model was able to adequately describe the fermentation potential of single or mixed substrates, showing a good fit between the experimental data and the predicted values with a relative error below 5 %.

4.3 Conclusions

The acidogenic fermentation of four different substrates (CG, TW OFMSW and WAS) single or combined in different mixtures was investigated in order to evaluate the VFA production potential. The main conclusions from this study are:

Carbohydrates and proteins present in CG, TW and OFMSW were converted into VFA, which are added-value intermediates that are considered as suitable carbon sources for various bioprocesses.

Anaerobic co-digestion of CG with TW or OFMSW was beneficial to increase the VFA production and yield, showing the importance of synergistic effects in mixing two substrates, when one of them (CG) has potential inhibitory compounds.

VFA production was significantly improved using TW as co-substrate in CG co-digestion in comparison to similar process with CG and OFMSW.

The highest VFA production was obtained during co-digestion of CG and TW at pH 5.7, which can be considered an optimum pH for higher fermentative microbial activity.

Acetic, propionic and butyric acids were the dominant species in the VFA mixtures.

Anaerobic co-digestion between WAS and TW showed unfavorable conditions for the acidogenic process.

The kinetic study of mono and co-digestion of CG with TW or OFMSW indicated that the highest VFA yield was observed when 25% CG and 75 % of TW were digested, confirming the synergy occurring at this mixing substrate ratio.

5. Performance and kinetic assessment of Co-fermentation of WAS and OFMSW

5.1 Introduction

This chapter focus on the anaerobic co-fermentation process of simulated OFMSW produced in Cape Verde with waste activated sludge (WAS) from domestic wastewater treatment plant, at different percentages of each substrate (see sub chapter 3.2.2, for the detailed composition), determining the influence of the substrate mixture to improve either the methane production efficiency, or the hydrolysis/acidogenesis step, measured as hydrolyzed COD and VFA production.

The aim of the work in this section was to apply kinetic studies to substrate mixtures, in order to provide a simple basis to evaluate the stability of the digestion process, without the inhibition of the OFMSW digestion process, due to the presence of high amounts of readily biodegradable organic matter, and with the improvement of the WAS digestion process, due to the addition of a highly biodegradable organic substrate. Kinetic studies of AD process are not only useful to predict the performance of digesters and design appropriate digesters, but also helpful in understanding inhibitory mechanisms of biodegradation.

In chapter 3.2, it has been presented the main characteristics of both substrates used in this experiment. Five batch reactors and replicates with a working volume of 4 L (R1, R2, R3, R4 and R5) and different mixtures of the substrates under study were used being incubated at 35 ± 2 ºC.

Due to internal legislation of Cape Verde and with the difficulty to transfer the wastes to outside, the OFMSW used was simulated in the laboratory, as described in chapter 3. Since it was not also possible to transport the waste sludge (WAS) from a wastewater treatment plant for domestic wastewater in the country, it was decided to use the WAS from a treatment plant located near the laboratory.

5.2 Results and Discussion

5.2.1 Performance of batch anaerobic reactors

During all experiments pH was monitored, although not controlled, as its evolution along the assay is considered to be an important indicator to evaluate the performance of the anaerobic co-digestion process under study. Figure 5-1a) shows the pH profiles obtained during all experiments for mono and co-digestion assays of OFMSW and WAS. It can be

observed that the performance of most reactors was similar, with the exception of reactor R1, which was operated with just OFMSW without adding any WAS. For this reactor, pH increased during the first days of the experiment, achieving a maximum of 7.7 on the third day and decreased afterwards to values below 6 at day 5, and further decreasing to below 4.5 at day 10, maintaining these low values until the end of the experiment. All the other reactors had similar pH profiles throughout the experiments, maintaining always values higher than 7, and achieving at the end values between 7.1 and 8.1. Hence, it can be observed that the conditions in these reactors are favorable to the activity of methanogenic microorganisms inside the reactors (Liao et al., 2014), whereas the conditions verified in R1 were more favorable for the activity of acidogenic microorganisms (low pH and methane and high VFA during most of the experiment). These pH profiles observed can be related to the nature of the waste used as substrate. Hence, the presence of high amounts of readily fermentable compounds in OFMSW (reactor R1), led to a easily conversion of the organic matter into VFA, decreasing rapidly the pH to values lower than 6, which inhibited the activity of the methanogenic microorganisms (Silva et al., 2013).

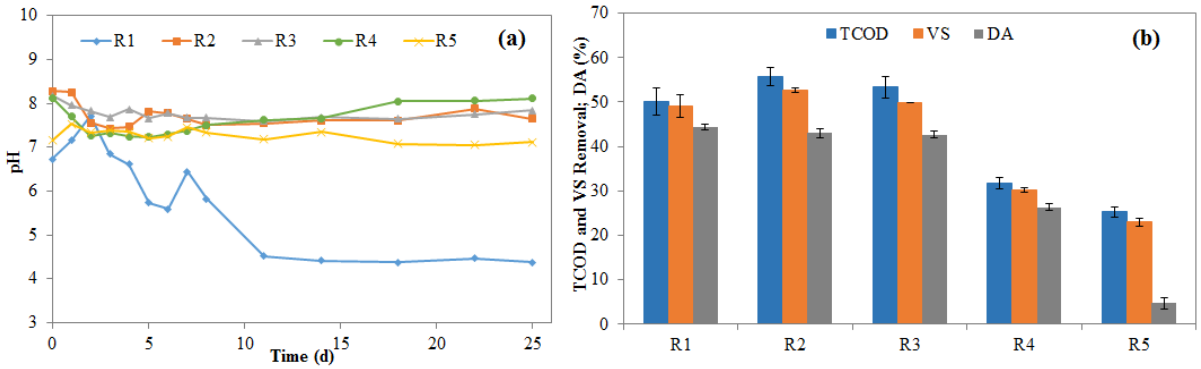

Figure 5-1: (a) pH variation for co-digestion assays; (b) TCOD and VS removals and DA for all assays (in percentage). R1: 100 % OFMSW; R2: 75 % OFMSW + 25 % WAS; R3: 50 % OFMSW + 50 % WAS; R4: 25 % OFMSW + 75 % WAS; R5: 100 % WAS.

Fig. 5-1b shows the removals of both TCOD and VS and also the degree of acidification (DA) determined for all assays with different proportions of OFMSW and WAS in the mixture. The DA is also presented in order to determine the acidogenic potential of each mixture.

In general, the increase of OFMSW in the mixture had a significant beneficial effect on the VS and TCOD removals, presenting an increase in both parameters. However, for the assay with just OFMSW (R1), it was not obtained higher values for these parameters when

compared with the assay R2 with a lower amount of OFMSW (75%), most probably due to the inhibition of the methanogenic microorganisms in the assay with just OFMSW, due to high acidification activity, leading high VFA and very low pH values. Considering the assays where co-digestion was studied (R2, R3 and R4), the highest values for TCOD and VS removals were obtained for the highest amount of OFMSW in the mixture (75 % for R2), being higher than 50 % for both parameters (55.7 % ± 2.0 for TCOD removal and 52.7 % ± 0.22 for VS removal), being even higher than the ones obtained in the assay with just OFMSW (R1), which confirms the beneficial effect of adding a small amount of WAS. On the other hand, for the co-digestion assay where the amount of OFMSW was the lowest (R4, with 25 % of OFMSW), removal efficiencies lower than 35 % were obtained, namely 31.5 % ± 1.2 for TCOD removal and 30.3 % ± 0.54 for VS removal.

Comparing the two sets of experiments for single substrate digestion, namely R1 (100 % OFMSW) and R5 (100 % WAS), the assay with OFMSW obtained much higher TCOD and VS removals (50.12 % ± 3.09 and 49.2 % ± 2.58) than the assay with WAS (22.93 % ± 1.0 and 25.3 % ± 0.94), which reflected the higher biodegradability of OFMSW. However, for the reactor with just OFMSW, it was not possible to maintain a stable reactor for methane production, as also reported by Flor et al. (2003). In addition, comparing R1 (100 % OFMSW) and R2 (75 % of OFMSW) it was observed a better performance with higher values for organic matter removal in the assay with lower amount of OFMSW (R2), which showed the benefit of the addition of 25 % of WAS, for the reactor performance in terms of controlling methane inhibition.

Hence, as it can be observed (Fig. 5-1b), the reactor with just OFMSW acidified rapidly (very low pH and high VFA) which contributed to the inhibition of the methanogenic microorganisms (low methane), as can also be seen by the pH values achieved during most of the experiment (lower than 4.5). The values for DA reached by Silva et al. (2013) (32.4 %) or reached by Wang et al. (2015) (42.7 %) using OFMSW were lower at mesophilic conditions (35 ^{o}C), than those obtained in this study (44.4 %), which confirms the importance of the substrate composition in the performance of biological process. It is also observed that the increase in the amount of OFMSW in the mixture with WAS resulted in higher acidification degrees. Reactor R5 Reactor R5 achieved the lowest value of DA (4.8 % ± 1.24), possibly because WAS is a waste with a much lower biodegradability. The average value for DA in R3 (50% OFMSW+50% WAS) was 42.0 % ± 0.76, very similar to the value

obtained for R1 (100% OFMSW) which also showed the beneficial synergies of mixing both substrates. Hence, the co-digestion of this two substrates is not only beneficial to the digestion of OFMSW (avoid methanogenic inhibition) but also to WAS (biodegradability increase).

5.2.2 Fermentation products from anaerobic co-digestion assays

Figure 5-2 shows the hydrolyzed SCOD, the TVFA and CH_4 production determined as COD equivalents with time for all conditions. The trend of the hydrolyzed SCOD shows that the particulate organic compounds present in the substrates were first hydrolyzed and converted into soluble monomers, being further converted into VFA. These transformations are performed by hydrolytic and acidogenic bacteria respectively and the results of these transformations are perceptible by the increase of TVFA concentration in all reactors. As it can be seen in Fig. 5-2, the trend for TVFA concentration is to increase until day seven of incubation maintaining this level afterwards until the end of the experiment, with the exceptions for R4 and R5. For these reactors, TVFA concentration decreased after seven days, most probably due to its utilization for methane production, as it can be observed in Fig. 5-2 by a sudden increase on the cumulative methane curves.

The maximum TVFA concentration achieved in all experimental conditions decreased greatly with the increase of WAS addition, ranging from 14.89 g L^{-1} in R1 to 0.41 g L^{-1} in R5. The highest VFA concentrations occurred in reactors R1 and R2, since they contained higher OFMSW percentages in the mixture than the other assays. The environmental conditions needed to obtain high VFA concentration, as in the case of the mono digestion of OFMSW, could be further optimized in order to use these produced intermediates (VFA) to stimulate biogas production or to obtain other added-value products (Capela et al., 2008; Reis et al., 2003; Zhang et al., 2015). The lowest VFA concentration obtained in R5 can be attributed not only to the presence of compounds with lower biodegradability, as it is the case of WAS (the only substrate in reactor R5), but also to their further degradation to methane.

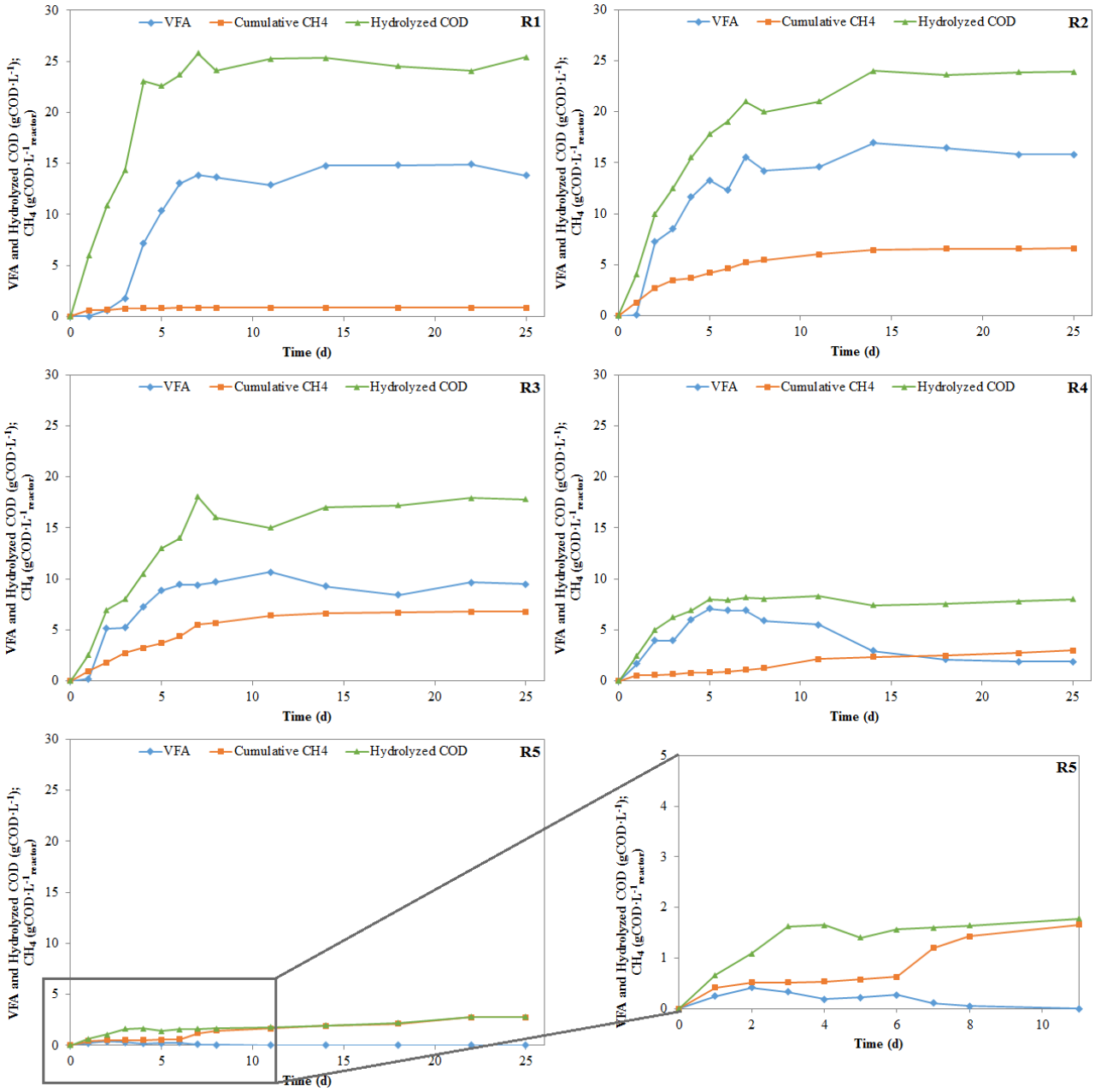

Figure 5-2: Cumulative production of VFA, CH4 and hydrolyzed COD for all assays: R1: 100 % OFMSW; R2: 75 % OFMSW + 25 % WAS; R3: 50 % OFMSW + 50 % WAS; R4: 25 % OFMSW + 75 % WAS; R5: 100 % WAS

In all assays, the hydrolyzed SCOD and the methane concentration tended to increase with time and to stabilize after day 11, except for R1, the batch assay with OFMSW as substrate, where methane production stopped much earlier, most probably due to inhibition, caused by high VFA concentration (Fig. 5-2) and low pH (Fig. 5-1a).

The ultimate methane productions obtained during the experiments with single substrates digestion were the lowest in both assays (0.87 and 2.81 gCOD as CH_4, per liter of reactor in R1 and R5 respectively) caused by the inhibition of the methanogenic phase induced by high VFA concentration (Figure 5-2) and low pH values (Fig. 5-1a), for just OFMSW (R1) or caused by the low biodegradability of the substrate as in the case of just WAS (R5). On the other hand, higher ultimate methane productions were obtained in the co-digestion assays, when compared with the mono-digestion assays, where R2 and R3 achieved high productions (6.59 gCOD as CH_4 and 6.80 gCOD as CH_4, respectively, per liter of reactor).

Observing these results, it is clear that the optimum proportions of OFMSW and WAS to obtain a good and stable performance in anaerobic co-digestion, in terms of methane production at ambient temperature, were those with 75 % (R2) and 50 % (R3) of OFMSW. These proportions of OFMSW and WAS could provide the appropriate conditions, in particular with regard to the buffer effect achieved, in order to obtain high methane productivity from OFMSW, promoting both the hydrolysis and the acidogenesis of the organic matter present in the substrates, without methanogenesis inhibition.

5.2.3 Methane yields for the single and mixed substrates

The experimental data of methane production obtained in each batch experiment carried out in this study was used for the determination of the methane yields for each assay. The methane production results, presented in Fig. 5-2, are an important indicator to assess the performance of the reactors, taking into account the methanogenic phase. Observing the curves obtained for each assay, it is possible to verify the positive effect on the methane production by the increase of OFMSW in the mixture. However, it can also be seen that the assay with just OFMSW became inhibited, being the one with the lowest methane production. The increase on OFMSW amount in the mixture resulted in an increase on the maximum methane production, reaching the highest values of 6.59 and 6.80 gCOD as CH_4 per liter of reactor for R2 (75 % of OFMSW) and R3 (50 % OFMSW) respectively.

Table 5-1 presents the main experimental results of methane production and yields through 25 days of digestion operating period for the single and mixed substrates under study. Regarding the methane productivity (determined as the methane production in terms of COD equivalent with respect to the amount of substrate in terms of $VS_{initial}$ concentration), the

assay with OFMSW as single substrate obtained 0.029 gCOD-CH4 $gVS_{initial}$, which is the lowest value obtained in all assays, even lower than the ones obtained for the assays with WAS as single or co-substrate.

Table 5-1: Summary of digester performance of co-digestion WAS with OFMSW under different conditions.

Description	R1	R2	R3	R4	R5
Cumulative volume of methane (L)	0.46	3.18	3.12	1.37	1.25
VS removal fraction (%)	49.2 ± 2.58	52.7 ± 0.22	49.8 ± 0.07	30.3 ± 0.54	25.3 ± 0.94
Total methane production rate ($L.g^{-1}$ $VS_{initial}$)	0.10	0.57	0.55	0.41	0.29
Total methane production rate ($gCOD\text{-}CH_4.g^{-1}$ $VS_{initial}$)	0.029	0.190	0.212	0.124	0.119
Total methane production rate ($L.g^{-1}$ $VS_{removed}$)	0.10	1.14	0.97	0.19	0.13
Total methane production rate (g $COD\text{-}CH_4.g^{-1}$ $VS_{removed}$)	0.04	0.29	0.27	0.22	0.20

For all assays, the methane productivity tended to generally decrease with the increase of WAS content in the reactor. Thus, it was obtained a methane productivity of 0.190 gCOD-CH4 $g^{-1}VS_{initial}$ in R2 (25 % WAS), 0.212 gCOD-CH4 $g^{-1}VS_{initial}$ in R3 (50 % WAS), 0.124 gCOD-CH4 $g^{-1}VS_{initial}$ in R4 (75 % WAS) and 0.119 mg COD-CH4 $g^{-1}VS_{initial}$ for R5 (100 % WAS). Similar tends of total methane production rate per $L\text{-}CH_4.g^{-1}VS_{removed}$ in same reactors were observed. The higher CH_4 production can be attributed to the anaerobic co-digestion R2 and R3 with 1.14 and 0.92 $L.g^{-1}$ $VS_{removed}$ respectively, which enhances the positive synergistic effect and promotes microorganism activities. Another potential reason for these results, is the higher TCOD and VS removal (see Fig. 5-1b) in the digester R2 and R3 resulting in a higher methane production. Based on the above-mentioned analysis and discussion, it clear that for all experiments are in agreement on the superiority of co-digestion to mono-digestion. It also shows, however, that OFMSW is an excellent substrate when co-digested with WAS. Furthermore, all of the co-digestion experiments demonstrated a positive effect and strengthened the process stability of AD.

In the addition, the values obtained for the methane productivity are closely related with the nature of the substrate and with the efficiency of the hydrolysis step. In the assay with only OFMSW (R1), the high concentration of TVFA and low pH achieved during the process (Fig. 5-1 and 5-2) affected the methanogenic activity, causing inhibition to the process. For all the other assays, the results demonstrate that methanogens and acetogens could handle

the VFA accumulation inside the reactor, ensuring stable conditions for methane production, which enhanced the efficiency of the anaerobic process (Montañés et al., 2013).

5.2.4 Kinetic assessment

The purpose of this study is to assess the anaerobic kinetics of digestion of OFMSW with WAS residue. Due to the complexity of the biological process under study, the model to determine the kinetic constants was simplified, considering a uniform system in terms of biomass. The assessment of the kinetics was carried out by using first order kinetic model for hydrolysis step and first order exponential model, exponential lag phase model and exponential curve factor model for methanogenic step.

In this study, the substrates (OFMSW and WAS), the intermediate (VFA) and the final product (biogas) were defined by their carbon contents. The kinetic constants were determined for all reactors, with different substrate mixtures. The hydrolysis rate constant was determined considering the incubation period from the beginning until reaching the peak concentration of hydrolyzed COD, while the methanogenic rate constant was determined considering the total time of incubation. The biomass yield was difficult to determine due to the mixture complexity, so the biomass concentration was taken as constant during the process (Gavala et al., 2003).

5.2.4.1 Determination of first-order hydrolysis rate constants

The hydrolysis rate constant (k_H), also called the biodegradability constant (Owamah and Izinyon, 2015), was determined by the integration of the first-order kinetic Eq. 25 and estimated using the linear regression Curve Expert Professional 2.2 software. It has been reported that the first-order kinetic model can be appropriate for complex substrates and it is used for other more complicated models (Gavala et al., 2003). Figure 5-3 shows the plot used for the determination of first-order hydrolysis rate constants, for all assays. As it can be seen by the slopes obtained by linear regression, the value for the hydrolysis rate constants decreased with the decrease of OFMSW content, from 0.37 d^{-1} in R1 to 0.03 d^{-1} in R5. The high value for the constant obtained in R1 is related with the origin of the substrate, since OFMSW has high organic matter content that can be easily hydrolyzed. On the other hand, R5 obtained the lowest value for the hydrolysis rate constant, indicating a slower biodegradability of this substrate under the tested conditions. These results of the hydrolysis

rate constant emphasizes that, besides environmental conditions, the composition of the waste used as substrate for anaerobic digestion is of great importance.

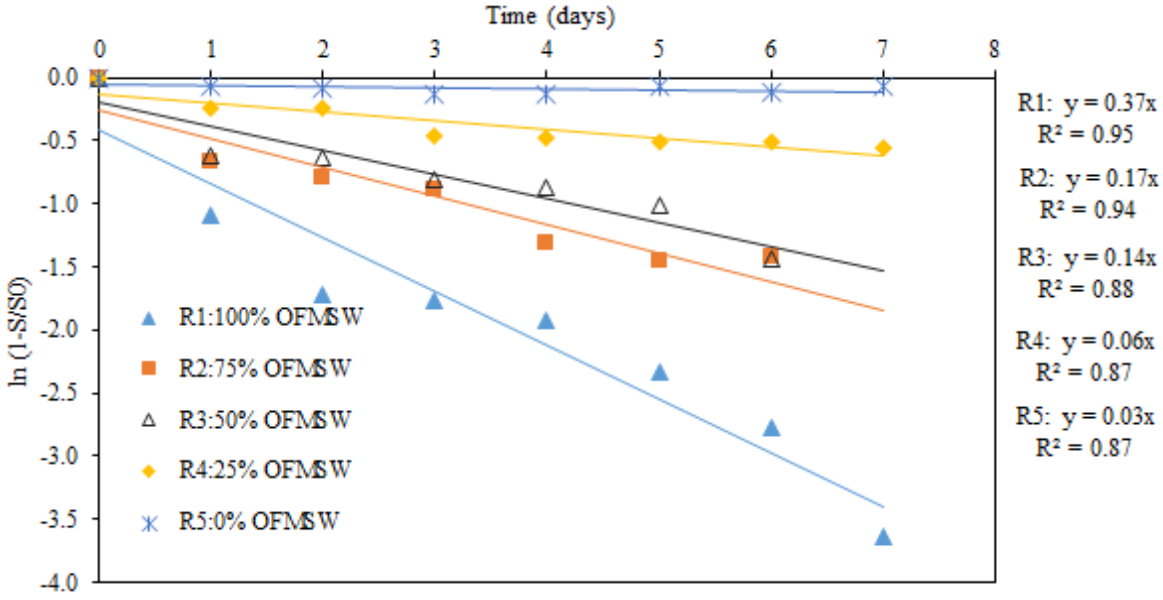

Figure 5-3: Plot for determination of hydrolysis rate constant (k_H): R1: 100 % OFMSW; R2: 75 % OFMSW + 25 % WAS; R3: 50 % OFMSW + 50 % WAS; R4: 25 % OFMSW + 75 % WAS; R5: 100 % WAS.

Some authors also studied the hydrolysis step of the anaerobic digestion process, determining the biodegradation rate or the hydrolysis rate constant for similar substrates as the ones used in this study, mostly at mesophilic temperature, and the most relevant results are summarized in Table 5-2.

Table 5-2: Values of hydrolysis rate constants (k_H) obtained with different subtracts in different studies

Proportion of substrates	**Temperature** (°C)	**k_H** (d^{-1})	**Reference**
100 % OFMSW	MT	0.37	This study
100 % OFMSW	MT	0.30	Sosnowski et al. (2008)
75 % OFMSW + 25 % WAS	MT	0.17	This study
75% FW + 25 % MH	MT	0.11	Owamah et al. (2015)
50 % OFMSW + 50% WAS	MT	0.14	This study
25 % OFMSW + 75 % WAS	MT	0.06	This study
25 % OFMSW + 75% WAS	MT	0.17	Sosnowski et al. (2008)
100 % WAS	MT	0.03	This study
100 % DWAS	MT	0.04	Zhang et al. (2014)

MT: mesophilic temperature; TT: thermophilic temperature; FW: food waste; MH: maize husk; DWAS: dewatered waste activated sludge

The values found in this study for the hydrolysis constant (0.06-0.37 d^{-1}) are in the range reported by the literature for comparable organic wastes. The differences in the hydrolysis rate constant for OFMSW found for mesophilic conditions (Table 5-1), although in the same range, k_H = 0.30 d^{-1} (Sosnowski et al., 2008) and k_H = 0.37 d^{-1} (this study) must be mostly related with the composition of the substrate, which in most cases attempts to simulate a particular composition of wastes produced in a specific region. . In this study, the region under study was Cape Verde Islands. However, at thermophilic temperature, OFMSW hydrolysis rate constant decreased drastically to 0.025 d^{-1}, as determined by Beevi et al. (2015), hence, it can be concluded, that temperature is a more important factor affecting the hydrolysis rate, being favored when working at a mesophilic range. Vavilin et al. (2008), and Li et al. (2013), have also determined a number of kinetic coefficients in mesophilic conditions, among which kitchen waste (0.34 and 0.18 d^{-1}), food waste (0.55 d^{-1}) and slaughterhouse waste (0.35 d^{-1}) which can be compared with the substrate mix present in OFMSW. Furthermore, Elbeshbishy & Nakhla (2012) have reported hydrolysis constants for single particulate substrates, as protein (k_{prot} 0.65 d^{-1}) and carbohydrate (k_{hyd} 0.78 d^{-1}), which presented higher values.

On the other hand, for the digestion of WAS, which is the substrate studied with the lowest biodegradability, the value for the hydrolysis rate constant obtained (0.03 d^{-1}) is much smaller than the one obtained by Zhang et al. (2015), at equivalent temperature (0.04 d^{-1}), using dewatered waste activated sludge (DWAS). This is also reflected in the assays with 75 % of WAS and 25% of OFMSW, where it was obtained a hydrolysis constant of with 0.06 d^{-1} in this study and 0,17 d^{-1} (Sosnowski et al., 2008), both at mesophilic temperature.

Owamah and Izinyon (2015), determined the hydrolysis rate constant of a different substrate, a mixture of 75 % of food waste (FW) and 25 % of maize husk (MH) at mesophilic temperature and obtained a value of 0.11 d^{-1}. This value could be compared with the value obtained in this study for the assay with 75 % OFMSW and 25% WAS (0.17 d^{-1}). This comparison also shows that the composition of waste is a parameter of great importance in the hydrolysis step of the anaerobic digestion process, besides temperature, and consequently the extent of hydrolysis will affect the later methanogenic stage.

5.2.4.2 Assessment of methanogenic kinetics and comparison of models

The methane production for each mixture was determined from the methane production obtained for each experiment (represented in Fig. 5-2), after subtraction of the average background methane generated by the inoculum. The obtained values were than used for the determination of the ultimate methane productivity evaluation using three models: the first order exponential model, the first order exponential lag phase model and exponential curve factor model.

Based on the obtained methane production for each mixture with time, the ultimate methane production for the first order exponential model (represented as G∞) was determined using the Curve Expert Professional 2.2 software. From the equations, 25 and 26 it was plotted the results of Eq. 27, and the graph is shown in Figure 5-4. This plot also shows the linear regression to estimate the values of methanogenic rate constants (k_M) and the determination coefficients (R^2) obtained. The methanogenic rate constant for R1 was not determined due to reactor acidification (pH values lower than 5 during most of the experiment) and consequently the very low amount of methane obtained in the experiment.

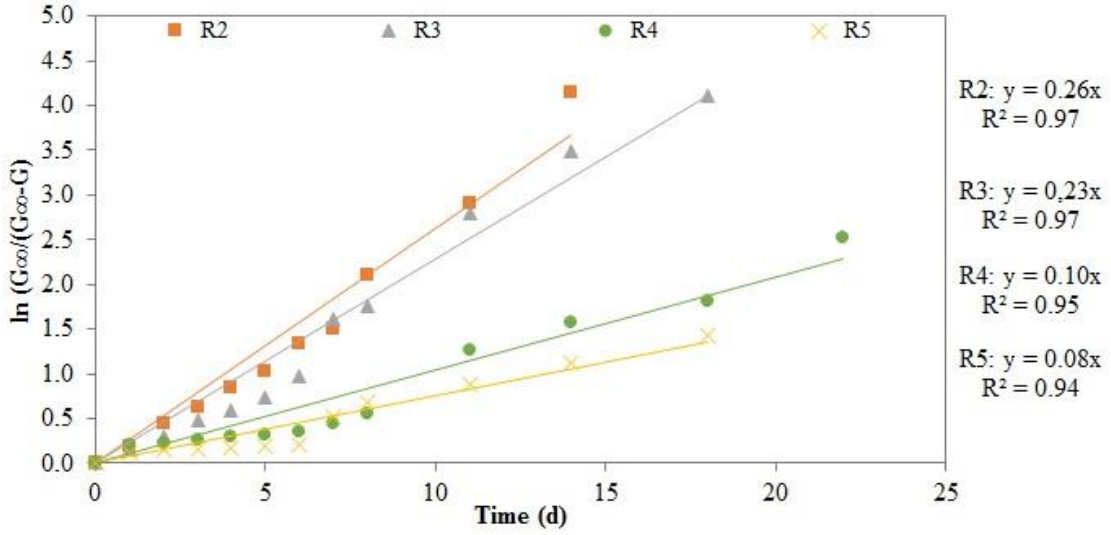

Figure 5-4: Plot for determination of methanogenic rate constants (K_M) for all assays: R2: 75% OFMSW + 25% WAS; R3: 50% OFMSW + 50% WAS; R4: 25% OFMSW + 75% WAS; R5: 100% WAS

As observed for the hydrolysis rate constants (Fig. 5-4), the values for the methanogenic rate constant decreased with the decrease of OFMSW content, from 0.26 d^{-1} in R2 to 0.08 d^{-1} in

R5. These results show that the increase on WAS content decreased the values of methanogenic rate constant which can be related with the type of substrate and consequently with the presence of high amounts of not readily biodegradable compounds, derived from WAS. The lowest value for the methanogenic rate constant in R5 (100 % WAS) can also be related with the lower hydrolysis rate obtained for this substrate, confirming that in this case the hydrolysis step of the anaerobic digestion of WAS is the limiting step for methane production.

Based on the obtained methane production for each mixture with time, the kinetic parameters and statistical analysis were determined for the three types of models and the results are presented in Table 5-3. Figure 5-5 shows the maximum methane production (G_∞) assay and simulated by three different models. To evaluate the models statistical indicators such as the coefficient of determination (R^2), the methanogenic production rate (k), the exponential Lag phase (L) and exponential Curve factor (C) relative for each model were studied.

Table 5-3: Parameters estimated from non-linear regression for of kinetic parameters and statistical analysis predicted by three models.

		Assay			
Kinetics Model	**Parameters**	**R2**	**R3**	**R4**	**R5**
First-order Exponential model	Measured exp. G_∞ ($L.g^{-1}VS$)	0.318	0.312	0.132	0.125
	Predicted Model. G_∞ ($L.g^{-1}VS$	0.317	0.311	0.125	0.108
	k (day^{-1})	0.26	0.23	0.10	0.08
	R^2	0.97	0.97	0.95	0.94
Exponential Lag model	Measured exp. G_∞ ($L.g^{-1}VS$)	0.318	0.312	0.132	0.125
	Predicted Model. G_∞ ($L.g^{-1}VS$	0.318	0.310	0.118	0.106
	k (day^{-1})	0.27	0.24	0.12	0.11
	L (day)	0.74	0.78	1.35	1.62
	R^2	0.98	0.97	0.97	0.96
Exponential Curve factor model	Measured exp. G_∞ ($L.g^{-1}VS$)	0.318	0.312	0.132	0.125
	Predicted Model. G_∞ ($L.g^{-1}VS$	0.318	0.311	0.126	0.110
	k (day^{-1})	0.27	0.26	0.14	0.12
	C	0.98	0.95	0.89	0.85
	R^2	0.99	0.99	0.98	0.98

As can be seen in Table 5-3 and Fig.5-5, for all studied models, the G∞ predicted production decreased with decreased portions of OFMSW in the mixture. The addition of the WAS at high percentage obviously inhibit the methanogenic activity and reduced the methane production.

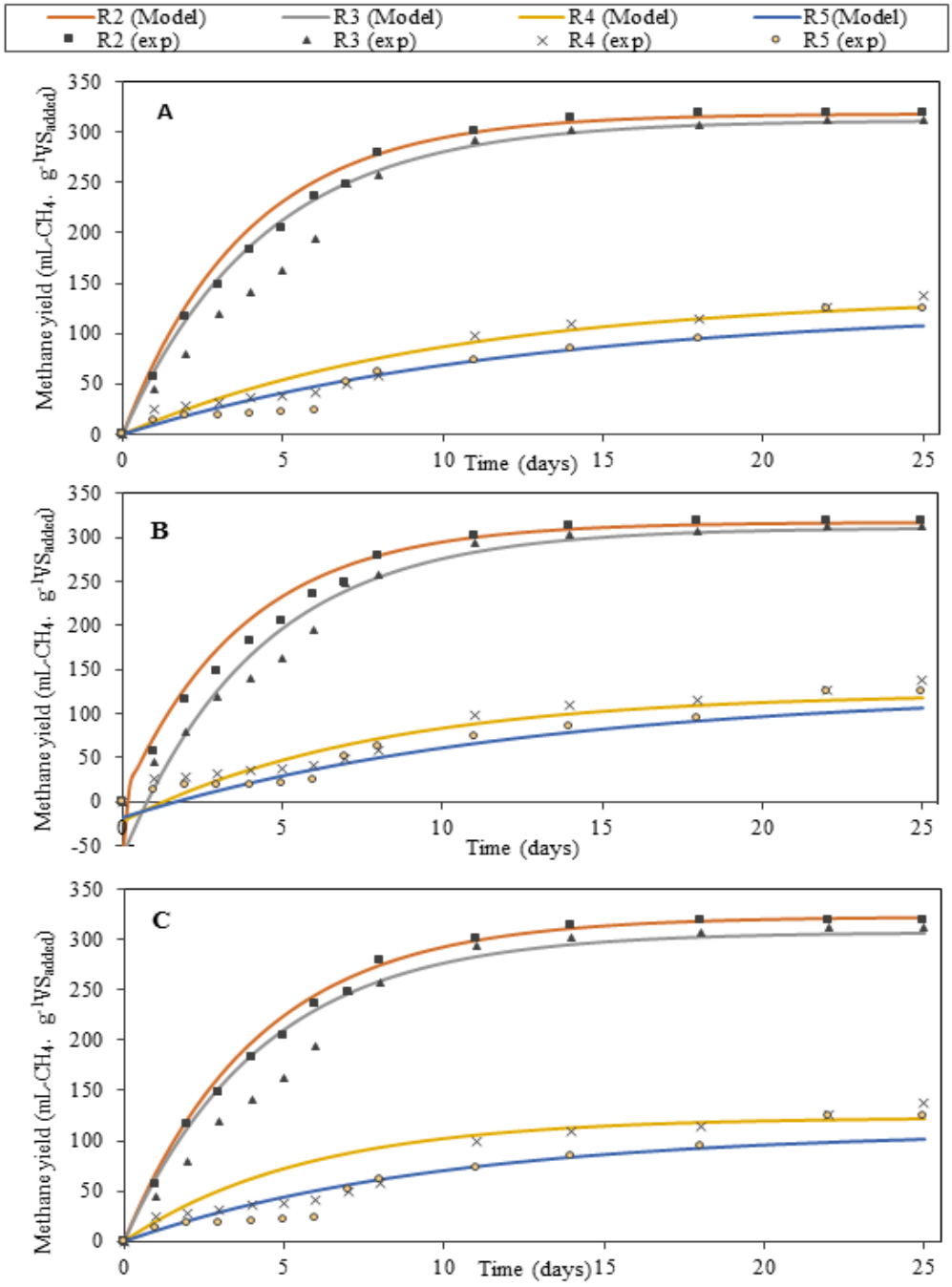

Figure 5-5: Simulated of experimental data assays by three models: A: First-order exponential model; B: Exponential Lag model; C: Exponential curve factor model.

Observing the Fig. 5-5, it can also be seen that, the exponential Curve factor model exhibited the better correlation between the experimental and predicted model methane yield (0.0 – 4.5%) in all co-digestion reactors R2, R3 and R4, followed by first order Exponential model with (0.3 – 5.0%) of difference, and the exponential Lag phase model between (0.0 – 10.0%).

Mono-digestion (R5) showed the highest difference between the measured and predicted methane production yield (13.0 –15.2%) of the three kinetic models used as can be seen in Table 5-3. Also, it is evident that Lag (L) given by exponential Lag phase model, increased with decrease of OFMSW fraction in the reactors. For example, R4 and R5, corresponding to 25% and 0% of the OFMSW addition respectively, resulted in some noticeable increase in the L (1.35 and 1.62 respectively), values higher than the R2 (L= 0.74) and R3 (L= 0.78), with higher amount of OFMSW. On the other hand, the estimated Curve factor (C) values obtained from the exponential Curve factor model of the four reactors were decreased when the OFMSW mixing decreasing (0.85 – 0.98) with an opposite trend for Lag model.

Out of the three models, the simple exponential model was the poorest model to estimate the methane potential rate, which is in agreement with 0.26 – 0.08 day^{-1}. Due to the involvement of the Curve factor, it estimates even higher values of the methane production rate constant, which ranged from 0.12 – 0.27 d^{-1}. The exponential Curve model gives better accuracy compared to the first-order model and exponential Lag phase model as shown in Table 5-3. In the addition, the higher R^2 value (0.98–0.99) were calculated for the Curve model for all digestion experiments, which suggested that Curve model was well fit to the biogas production curve in this study. The advantage of this model is the inclusion of wiggling effect in the estimated curve, or in other words, it increases the inflection point, which cause reduction of sum of squares, thus fitting better than the other models.

Table 5-4 summarizes the k_M values that were determined from Momoh and Nwaogazie (2011) kinetic model (Fig. 5-4) compared with values for methanogenic rate constants obtained in other studies, related with single and co-digestion experiments using similar type of substrates to those used in this study.

In the co-digestion assays it was obtained a decreasing value of the methanogenic rate constant with the increase on WAS content. Nielfa et al. (2015) studied the co-digestion of simulated OFMSW with wasted biological sludge at mesophilic temperature and obtained for a mixture of 20 % OFMSW and 80 % WAS a value of 0.31 d^{-1} for methanogenic rate constant. This value could be compared with the assay R4 (25 % OFMSW and 75 % WAS) at the same temperature range (0.14 d^{-1}). Hence, it can be concluded that the performance of the digestion process may be mostly affected by substrates composition, where WAS has an inhibitive effect on the methanogens and this inhibitive effect increases with increasing the

WAS % in the co-digestion mixture. This result was confirmed again with the digestion of WAS (R5) originated the lowest values for the methanogenic rate constant (k_M = 0.12 d^{-1}) as it was also observed by Flor et al. (2003), which achieved a k_M in the same range (k_M = 0.09 d^{-1}). The anaerobic digestion of WAS as single substrate was affected by its low hydrolysis rate and hence the methanogenic rate constant was also the lowest when compared with other substrates. According to the results obtained in this study, helped much in concluding the k_M could be favored by addition of OFMSW % on the solubilizing the co-digestion mixture (higher k_H) and consequently higher constant k_M, proved to significantly improve biogas production.

Table 5-4: Values of methanogenic rate constants (k_M) obtained with different subtracts in different studies

Proportion of substrates	**Temperature (**ºC)	**k_M** (d^{-1})	**Reference**
100% OFMSW	MT	0.34*	This study
100 % OFMSW	MT	0.30	Sosnowski et al.(2008)
100 % OFMSW	MT	0.29	Flor et al. (2003)
100 % OFMSW	TT	0.024	Beevi et al. (2015)
75 % OFMSW + 25 % WAS	MT	0.27	This study
50 % OFMSW + 50 % WAS	MT	0.26	This study
25 % OFMSW + 75 % WAS	MT	0.14	This study
20 % OFMSW + 80 % WAS	MT	0.31	Beevi et al. (2015)
100 % WAS	MT	0.12	This study
100 % WAS	MT	0.09	Flor et al. (2003)

*Value directly determined by the equation obtained in Fig. 5-8 b.

To validate the kinetic model for the methanogenic phase of the anaerobic digestion process using OFMSW and WAS as co-substrates, a representation of experimental data and simulated values using Eq. 27 was performed and is showed in Fig. 5-6 (a) for all assays were methanogenesis was not inhibited (R2 to R5). Fig. 5-6 b) show the results obtained for OFMSW digestion (R1), simulated and real, where it can be confirmed the methanogenic phase inhibition, achieving a methane production less than 20% of the ultimate methane potential.

Based on the results obtained from the Fig. 5-6 (a), and in order to compare the performance of the mono and co-digestion reactors, the developed maximum biogas production potential and stability assessment (MBPPSA) model proposed by Owamah and Izinyon (2015), was

applied to experimental data to explore the stability/inhibition evaluation between the digesters (R1 – R5).

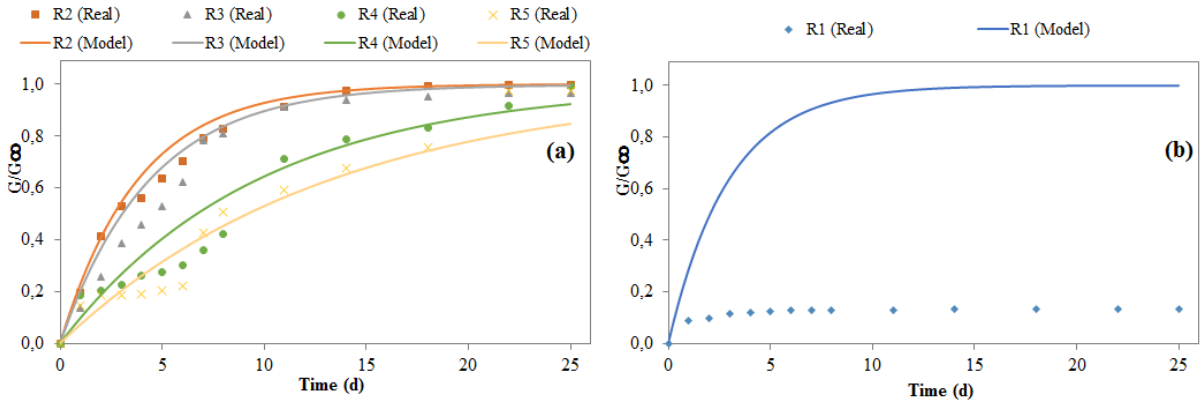

Figure 5-6: (a) Adjustment of experimental data to the model determined, for methane production in R2: 75% OFMSW + 25% WAS; R3: 50% OFMSW + 50% WAS; R4: 25% OFMSW + 75% WAS; R5: 100% WAS; (b) Experimental data and model determined for methane production in R1: 100.

5.2.4.3 Assessment of digester stability/inhibition

The MBPPSA model can be used for simple prediction behavior of the methane production of the anaerobic co-digestion process, as well as to assess of process feasibility in terms of stability /inhibition (Owamah and Izinyon, 2015). In their study, Owwamah and Izinyon compared two different models; modified Gompertz (MG) equation, and the MBPPSA model and were found an overall agreement between the two models and the experimental data from both methane assays with a relatively small difference between the experimental methane yields and model methane yields by MG.

The stability/inhibition (*In*) system were determined intercepting the $(1 - e^{-kt})^n$ equation (Eq. 29) to give Eq. 30 (see section 3.7.2). A negative value of (*In*) indicate that the digestion process is stable. Contrarily, positive (*In*) values show inhibition or instability (Owamah and Izinyon 2015). Yusuf et al. (2011), was used similar stability assessment classification in their study of biogas production from co-digestion of cow dung and house dung.

Figure 5-7 illustrates the results of digester stability/inhibition evaluation for five digesters (R1– R5). It is evident that, R1 with lowest performance in terms of methane production, as expected was found to be inhibited/unstable or inhibitory effects, due to the rapidly acidification process (see Table 5-1). In addition, the positive *In* value during 25 days of

experiment for anaerobic digestion from OFMSW as a sole substrate was about 0.19, describing as more inhibited/unstable than other digesters. Furthermore, the digesters (R2 – R5) showed an negative *In* values (Table 5-5), suggesting that, the all mixing ratios of OFMSW and WAS tested in this the study have a stable effects on reaction kinetics, provided that no inhibitory effects occur during digestion process, exactly the same tend evolution observed in Fig. 5-6a.

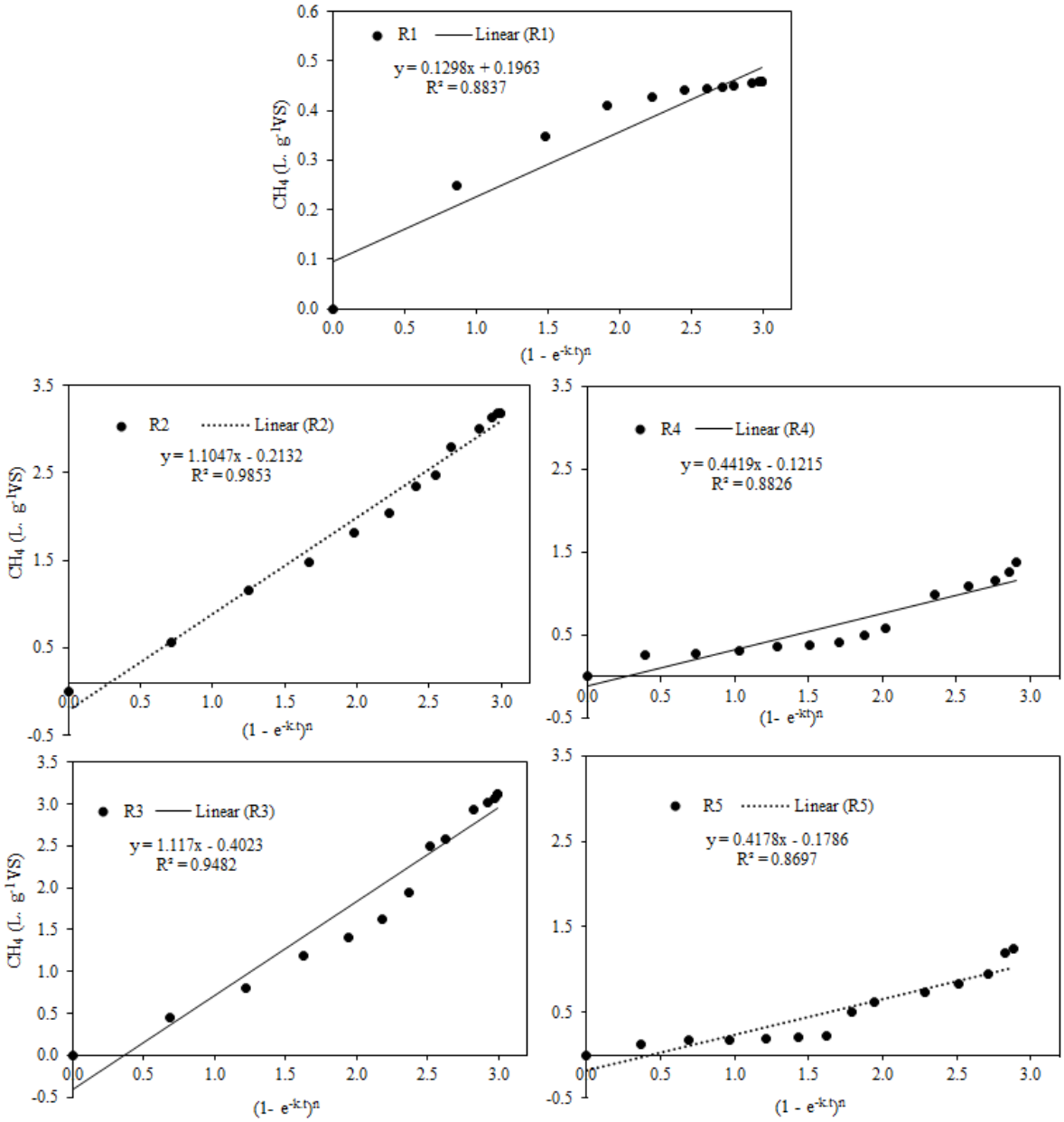

Figure 5-7: Results of MBPPSA model parameter determined for digesters: R1: 100% OFMSW; R2: 75% OFMSW + 25% WAS; R3: 50% OFMSW + 50% WAS; R4: 25% OFMSW + 75% and R5: 100% WAS.

However, it was observed that for all co-digester reactors R2, R3 and R4, were found high the absolute value of the negative *In* (- 0.20, -0.40 and -0.12 respectively). The reactor R3, with low *n* value (*n* = 1) observed the high *In* absolute value (-0.4) showing to be more stable and efficient than the co-digesters reactors R2 and R4. Similarly, Owamah and Izinyon (2015) also reported in there study about the co-digestion of food waste and maize husk that, when I/S ratio is low (n equal a 1) the reactor found high biogas production and stability. Comparing the digesters R1 and R5 with as solo substrates (Fig. 5-7), in terms of stability and efficiency for methane production, it can see that the reactor R1 with 100% of OFMSW, obtained the higher *In* value (0.19) showing the unstable and high inhibition reactor than the mono-digestion reactor R5. This results can be explained due to the high VFA concentration in the reactor, while is it the main parameter for stability effect. In addition, reactor R1, had low pH value than R5 (see Fig. 5-1a), that may be toxic for methanogens and consequently, as expected, the instability process. In turn, R5 had better performed than R4. This result can be explained due to the low *n* value in R5 (see Table 5-5), and can be described as being more stable than R4. This observation is in accordance with the result obtained by Boulanger et al. (2012) in their study on the effect of the inoculum on biogas production from municipal solid. Based on the results obtained in this study, it is noticeable that adding OFMSW less than 50%, the inhibition of the methanogens is only observed as a lower k_H (Fig.5-6) and a low methane production (Fig. 5-7a).

The linear regression of the MBPPSA model for maximum biogas production are shown in the Table 5-5. The correlation coefficients R^2 values fell within the range of 0.87 – 0.99, showing to have a very good fit to the experimental data with the MBPPSA model. It can also be concluded that, and based on the results obtained in this study, that the MBPPSA model can therefore be used to complement the first-order model, for anaerobic digestion experiments and feasibility studies.

Table 5-5: The co-digestion of OFMSW and WAS mixing ratios (*n* value) and inhibition/stability variation (*In* values).

Reactor: proportion of substrates	***n* values**	***In* values**	**Total.biogas production[a)]**	**R^2 (from MBPPSA)**	**Stability status**
R1. 100% OFMSW	1	0.19	0.12	0.88	Unstable/inhibited
R2: 75% OFMSW + 25% WAS	3	-0.21	1.10	0.99	Stable
R3: 50% OFMSW + 50% WAS	1	-0.40	1.12	0.95	Stable
R4: 25% OFMSW + 75% WAS	3	-0.12	0.44	0.88	Stable
R5: 100% WAS	1	-0.17	0.41	0.87	Stable

a): Maximum biogas production potential of the MBPPSA model in gCOD L^{-1} reactor

5.2.5 Mixture ratio assessment

The values obtained in this study for hydrolysis (Fig. 5-8) and methanogenic (Fig. 5-4) rate constants can be linearly correlated with the increase of OFMSW amount in the mixture (or, consequently, with the decrease on the amount of WAS in the mixture), as it is represented in Fig. 5-4a (hydrolysis rate constants) and Fig. 5-4b (methanogenic rate constants). In the figure it is also presented the equations relating both parameters (kinetic constants and the amount of OFMSW in the mixture and the respective regression coefficients (R^2) obtained by linear regression. The values of R^2 obtained in both cases, indicated a well-correlated relationship between the kinetic parameters obtained in this study and the amount of OFMSW in the mixture, which is an important output for reactor design and methane predictions under practical conditions. Furthermore, for all regressions performed in this study, besides the observation of regression coefficients, it was considered the test of significance of the regressions and the standard error below 5 %. At that significant level, the adequacy of the regressions was tested with a confidence level of 95 %. Thus, the results for kinetic models confirm a good correlation between simulated values and experimental data, ensuring its validity.

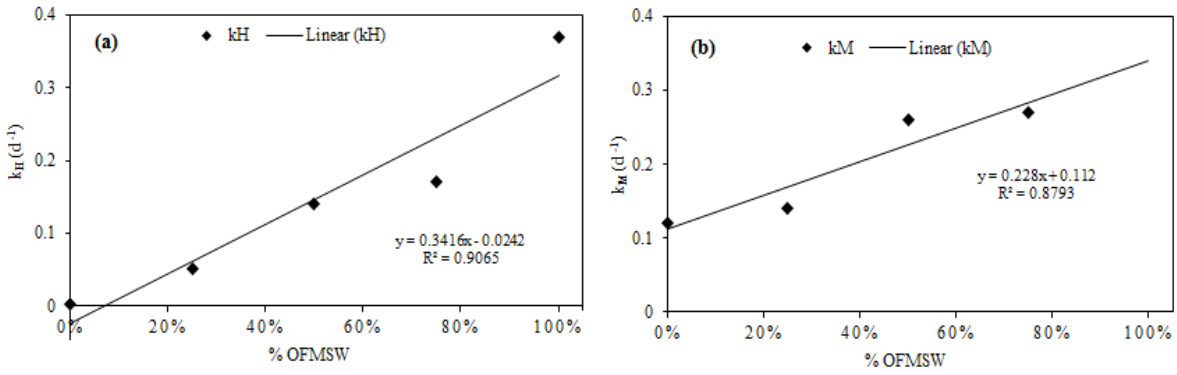

Figure 5-8: Relation between k_H (a) and k_M (b) and OFMSW percentage in substrate mixture and their respective linear regressions.

In conclusion, with the equations presented in Figure 5-8 it is possible to predict the kinetic constant values when a different mixture of OFMSW and WAS is used in the co-digestion assays of these two types of substrates. These predictions are crucial for reactor design and also vary important for the optimization of both single or co-digestion process, in order to achieve higher methane productivities and more sustainable processes, under practical

conditions. As a direct application of this achievement, by using the linear equation obtained in Fig. 5-7b it is possible to determine the value of k_M for the assay with just OFMSW (0.34 d^{-1}), value not determined experimentally in this study due to reactor acidification. In order to validate this value, a comparison is done with the values presented in Table 5-2, at mesophilic temperature, for Sosnowski et al. (2008) and Flor et al.(2003) that obtained a value of k_M of 0.30 d^{-1} and 0.29 d^{-1}, respectively. For thermophilic temperature, Beevi et al. (2015), obtained the lowest value for the methanogenic rate constant for the digestion of OFMSW (0.024 d^{-1}), which supports the previous hypothesis that the values of the methanogenic rate constant for OFMSW as single substrate is very depends to the composition of the substrates used and tended to decrease with the increase in temperature, similarly to what was observed with the hydrolysis rate constant.

5.3 Conclusions

Analyzing the experimental results of the anaerobic co-digestion process using OFMSW and WAS it can be concluded that the proportion of OFMSW in the mixtures has a great influence in terms of reactor stability and performance, as well as in the methane ultimate potential. Thus, the main conclusion can be as follows:

The low pH values and high TVFA concentrations, together with a degree of acidification of 44 % showed that the assay with OFMSW as single substrate had an acidogenic behavior, leading to the inhibition of the methanogenic phase. All the other assays with WAS as a single or co-substrate had a methanogenic behavior.

First-order kinetic models are appropriate for complex substrates as it is the case of both OFMSW and WAS. The high correlation between the first order kinetic model and the experimental data validate this model with a confidence interval of 95%, being a useful tool for reactor design and for the prediction of the process performance and behavior under practical conditions.

The values for the hydrolysis rate constant increased with the increase of OFMSW in the mixture, due to the high biodegradability of this substrate, and hence the maximum value of k_H (0.37 d^{-1}) was obtained when OFMSW was used as single substrate.

The values for the methanogenic rate constant increased also with the increase of OFMSW in the mixture, where the maximum experimental value of k_M (0.26 d^{-1}) was obtained when 75 % OFMSW was used as co-substrate. A value of k_M of 0.34 d^{-1} is predicted by the proposed model for OFMSW as single substrate. These results will allow for the maximizing of methane yields when undertaking OFMSW/WAS co-digestion.

Among the three kinetic models used, the exponential Curve factor model was found to be the best model for predicting methane production. The relationship between the kinetic parameters and the amount of OFMSW obtained in this study are crucial for reactor design because with that equation it is possible to predict kinetic constants for different mixtures other than the ones used in this study.

The MBPPSA model was used to evaluate the stability of anaerobic digesters through its inhibition factor (*In*) and also to estimate the maximum biogas production potential of co-substrates. This model could therefore assist in the design of anaerobic digestion plants. This in turn may increase the GHG mitigation potential and commercial viability of AD systems co-digesting OFMSW and WAS.

6. Study of acidogenic fermentation of OFMSW for volatile fatty acids production in batch reactors

A modified version of this chapter was published as: Gameiro T., Lopes M., Marinho R., Vergine P., Nadais H., Capela I. (2016). "Hydrolytic-Acidogenic fermentation of organic solid waste for volatile fatty acids production at different solids concentrations and alkalinity addition". *Water, Air & Soil Pollution*, 227:391.

6.1 Introduction

Intermediate products from anaerobic fermentation, such as volatile fatty acids (VFA), are the preferred carbon sources for the production of added-value products, namely polyhydroxyalkanoates (PHA) or bioenergy. Organic fraction of municipal solid waste (OFMSW) can be valorized through the application of a hydrolytic-acidogenic stage, thus reducing its pollutant content and at the same time that it is obtained high-value products (VFA).

In this context, the aim of the work presented in this section was the production of VFA mixtures from OFMSW by hydrolytic-acidogenic fermentation, taking into account the influence of two main operational parameters: the initial addition of external alkalinity and the total solids (TS) content inside the reactor. The composition of the VFA mixtures to be obtained should be optimized according to ensuing application, i.e. the type of the final added-value material to be chosen determines the optimal conditions to be applied in the anaerobic acidification of the organic residue under study. On one hand, a high quality PHA production could be enhanced when an acidified residue with high propionic acid content is produced whereas, in the other hand, the biomethane and biohydrogen production are favored when a high acetic and/or butyric acids content is obtained. In conclusion, the manipulation of the operational parameters will then lead to mixtures with different VFA compositions and hence the hydrolytic-acidogenic process can be optimized taking into account the final purpose of VFA application prior to industrial exploitation. Response surface methodology was used to explore the relationships between the two predictors under study (TS reactor content and initial alkalinity addition) and the response variables (total VFA concentration, degree of acidification and effluent quality in terms of VFA).

6.2 Influence of TS concentrations and alkalinity addition

Alkalinity addition and total solids (TS) concentration inside the reactor are two of the most important parameters to be considered for high performance of anaerobic digestion process. Alkalinity is essential to avoid sudden pH drop due to VFA accumulation. According to Vergine et al.(2015), alkalinity influences the pH values obtained during the anaerobic process, which in turn affects both the TVFA production and the VFA composition. In addition, VFA-producing microorganism can function in a wide range of pH, depending on

the type of waste used as substrate. Appels et al. (2008) reported that using wasted activated sludge at low pH values produced mainly acetic and butyric acids and at pH near 8.0 produced mainly propionic acid. On the other hand, Lee et al. (2014) used dairy wastewater as substrate to produce VFA and obtained higher propionic production at pH near 4.0, whereas acetic and butyric acids were favored at pH 6.0. Wang et al. also demonstrated that the optimum pH for acetic and butyric acids formation was 6, with a low propionic acid concentration in the mixture, using food waste as substrate for VFA production.

The total solids (TS) content inside the reactor also influences the bioprocess efficiency, either in terms of VFA production or, ultimately, in methane generation (Liotta et al., 2015). In literature, some authors reported that biological systems operated successfully at "wet conditions" and produced considerable amounts VFA. Marouani et al (2002) reported the accumulation of high amounts of VFA in a batch digester treating food and vegetable wastes at 8 % of TS content, and Bouallagui et al (2005) reported a larger and faster VFA production at "wet conditions" (system operated between 8 and 18% of TS), due to the high biodegradability of food and vegetal wastes. On the other hand, some authors refer that high TS content decreases the methane formation due to limitations in mass transfer, decreasing the performance of the system (Le Hyaric et al., 2012). Fernández et al. (2008), reported the influence of TS content on the start-up and performance of process of dry anaerobic digestion of food wastes. According to this author, when TS concentration increased from 20% to 30%, COD removal of the process decreased from 80.7 % to 69.1 % and the methane yield at 30 % of TS content was 17 % less than the one determined at 20 % of TS content. For all these reasons, studying the effects of operating conditions on acidogenic fermentation is of crucial importance, namely for the control of VFA mixture composition and for the stabilization of the bioreactor performance.

6.3 Results and Discussion

In order to evaluate the environmental conditions that maximize the VFA production from OFMSW, 12 anaerobic batch assays were performed with three replicates, during 24 days. Three total solids content in the reactor (5, 8 and 10 % TS) and four external alkalinity additions (0, 10, 30 and 50 $gCaCO_3\ L^{-1}$) were tested. These conditions to produce high VFA concentration were defined based on preliminary studies and results obtained with different

solid wastes (Silva et al., 2013). Table 6.1 summarizes the main results obtained for the performance of the reactors during different operating conditions. These results are important, since they may way furnish valuable information on the dynamics of the anaerobic process.

Table 6-1: Parameters determined in the batch assays: initial TCOD and sCOD concentrations and maximum sCOD and VFA concentrations measured, maximum degree of solubilization ($DS_{máx}$) and maximum degree of acidification ($DA_{máx}$), maximum odd-to-even ratio of VFA and maximum VFA amount in sCOD (effluent quality) in all assays.

%ST	Alkalinity ($gCaCO_3\ L^{-1}$)	$TCOD_{in}$ ($gCOD\ L^{-1}$)	$sCOD_{in}$ ($gCOD\ L^{-1}$)	$sCOD_{max}$ ($gCOD\ L^{-1}$)	$[VFA]_{max}$ ($gVFA\text{-}COD\ L^{-1}$)	DS_{max} (%)	DA_{max} (%)	Odd-to-even$_{max}$	Effluent Quality (% of VFA)
5.00	0.00	33.09 ±2.57	19.85 ±1.66	32.14 ±1.46	4.95 ±0.49	92.50 ±1.84	14.95 ±1.38	0.77 ±0.046	72.03 ±0.72
	10.00	40.61 ±1.33	12.53 ±0.75	36.04 ±2.98	16.64 ±1.50	83.73 ±1.67	40.98 ±3.41	1.10 ±0.009	65.47 ±1.51
	30.00	41.44 ±1.02	7.49 ±2.45	37.89 ±2.9	20.35 ±1.63	89.55 ±1.78	49.10 ±2.39	1.05 ±0.013	87.25 ±1.63
	50.00	39.42 ±1.36	12.01 ±2.31	33.42 ±3.11	30.59 ±1.77	78.13 ±1.55	77.59 ±2.75	1.03 ±0.019	83.55 ±2.59
8.00	0.00	57.80 ±3.06	14.68 ±0.99	44.51 ±0.88	11.97 ±1.2	69.17 ±1.38	20.71 ±2.18	0.98 ±0.009	67.67 ±0.86
	10.00	55.60 ±2.00	20.63 ±0.99	46.54 ±1.66	18.06 ±1.63	74.10 ±1.47	32.49 ±3.09	2.78 ±0.012	54.59 ±1.05
	30.00	56.60 ±2.55	15.57 ±0.83	44.42 ±0.66	28.17 ±2.25	70.32 ±1.40	49.76 ±3.2	0.99 ±0.002	98.96 ±1.19
	50.00	56.20 ±1.43	11.30 ±0.53	42.67 ±0.94	34.46 ±2.41	69.88 ±1.39	61.31 ±3.53	2.02 ±0.004	97.41 ±0.97
10.00	0.00	71.40 ±1.87	34.7 ±1.46	68.28 ±2.65	10.29 ±1.03	91.50 ±1.72	14.41 ±1.44	1.18 ±0.011	22.38 ±0.43
	10.00	70.90 ±2.92	19.75 ±1.34	64.26 ±1.05	16.62 ±1.50	87.02 ±1.83	23.43 ±2.11	0.53 ±0.009	50.90 ±0.57
	30.00	69.27 ±1.80	22.75 ±1.21	68.95 ±1.82	26.57 ±2.13	99.32 ±1.88	38.36 ±3.09	0.96 ±0.003	75.16 ±1.45
	50.00	66.33 ±1.64	19.88 ±1.64	65.96 ±1.99	26.26 ±1.84	99.20 ±1.87	39.59 ±2.79	1.08 ±0.006	80.25 ±1.61

6.3.1 pH and COD conversion

The pH profiles obtained during the acidogenic fermentation of OFMSW for all assays are shown in Fig. 6.1. As expected in an acidification process without pH control, pH decreases

during the initial phase of the process, reaching values between 4 and 6.5, depending on the operational conditions, during which most of the VFA production occurred as well as other intermediates that dissociate and produce protons.

In this study, different external alkalinity additions were performed without pH control, since not only this methodology applied to the acidification process has not been thoroughly investigated, but also would prevent the addition of chemicals for the process control, thus contributing to the reduction of the operational costs. In conclusion, this method would be more cost-effective in comparison with the self-adjusting pH methodology.

In addition, higher external alkalinity additions in the reactors (30 $gCaCO_3\ L^{-1}$ and 50 $gCaCO_3\ L^{-1}$) were also investigated in order to increase the final pH to be obtained and favor the production of odd-equivalent VFA, especially propionic acid, that normally is favored at higher pH range (Gameiro et al., 2015). This type of VFA is the preferred carbon source to produce PHA with improved characteristics (such as P(HB-co-HV)) in a later phase. So, the optimization of the acidogenic process should take into account the final purpose of VFA application prior to industrial exploitation.

Figure 6.1 shows that, for all assays, pH decreases sharply in the beginning of the first 24 hours of inoculation followed by stabilization until the end of the fermentation process. It is clear that both initial and final pH values increased with the increase of the alkalinity added regardless of the total solid inside the reactors, and the minimum pH ranged mostly between 4 and 6. From all the experiments, it can be observed that, in three of the assays (5% TS and alkalinity of 30 and 50 $gCaCO_3\ L^{-1}$ and 8% TS and alkalinity of 50 $gCaCO_3\ L^{-1}$), pH was always higher than 6, and in two cases (5% TS) final pH was around 8, which would favor the production of propionic acid. This result agrees with a previous study of Appels et al. (2008), who investigated, in experiments conducted at controlled pH values, using wasted activated sludge at low pH values, were produced mainly acetic and butyric acids and at pH near 8.0 was produced mainly propionic acid. It is also observed in the Fig. 6.1, that in batch assays without alkalinity added or moderate alkalinity addition (10 $gCaCO_3\ L^{-1}$), it was obtained the lowest pH values, varying between 4.0 and 5.5, which could prevent propionic acid formation.

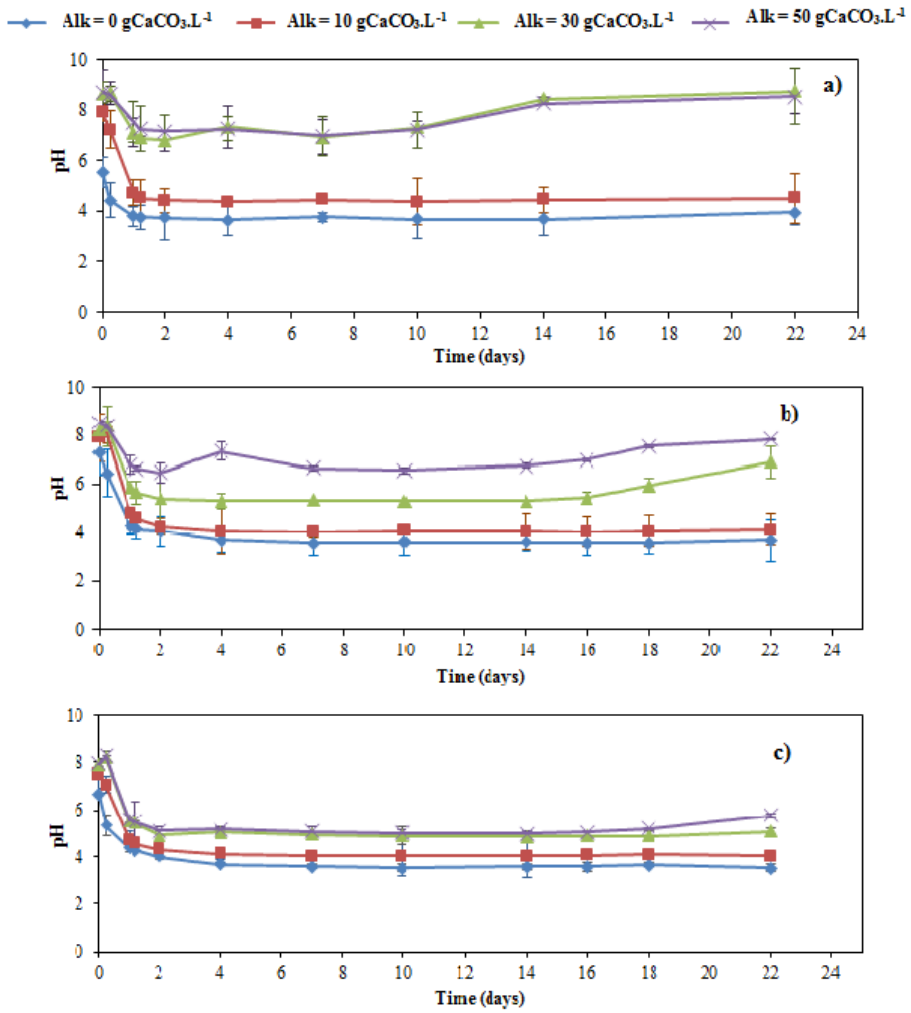

Figure 6-1: pH profile during the operation time for all assays: (a) TS content of 5%, (b) TS content of 8 %, (c) TS content of 10 %; error bars represent standard deviations of triplicate determinations.

The daily variation of sCOD during all the experiments are shown in Figure 6.2 (a – c). Similar observation can be seen for the evolution with time of the soluble COD for all batch reactors, with a significant increase in the beginning of the reactors operation (first 4 days), reflecting the role of the first step of this process, referred as disintegration and hydrolysis of particulate organic matter to soluble compounds. However, although there is a general increase of the sCOD for all reactors in the first 4 days, the assays with 10% TS presented a different behavior from the other two sets of assays 5% and 8% TS). The highest increase of the sCOD was performed later on, between day 4 and 7. The maximum sCOD obtained in the experiments was 68.95g L^{-1}, for the assays with 10% TS, followed by the assays with 8 % TS (46.54g L^{-1}) and the assays with 5% TS (37.89 g COD L^{-1}). According to the

performance of the bioreactors, it is clear that alkalinity addition and TS content inside reactors will affect significantly the first step (disintegration and hydrolysis), and consequently, the second step (acidification) of the fermentation process.

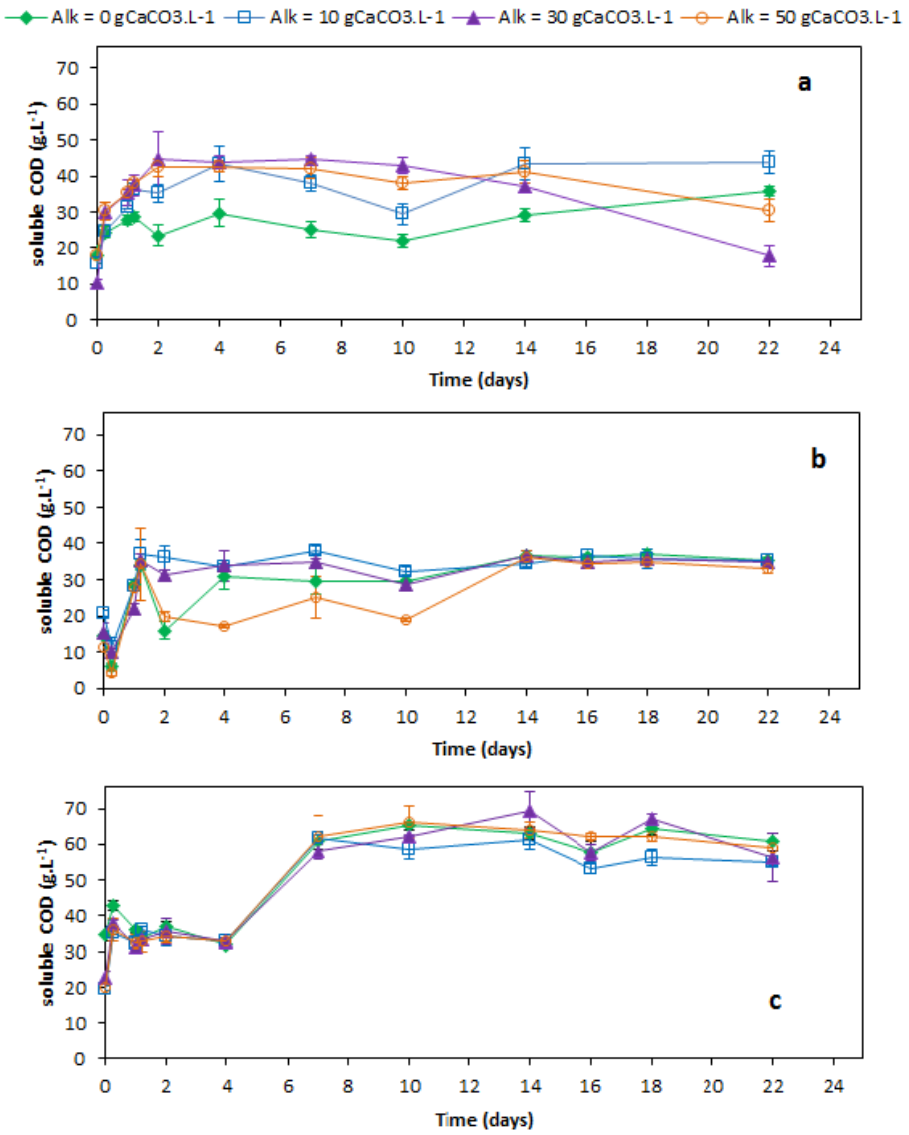

Figure 6-2: Soluble COD concentration during time for the conditions under study: (a) TS content of 5%, (b) TS content of 8 %, (c) TS content of 10 %; error bars represent standard deviations of triplicate determinations.

6.3.2 Solubilization of particulate organic matter

In the anaerobic digestion of wastes with high amount of particulate organic matter, the rate-limiting step is normally the hydrolysis step, contrary to what happens with simpler wastes, where the methane production by methanogenic microbial communities is the rate-limiting

step (Gavala et al., 2003). In the hydrolysis step, the conversion of insoluble particulate organic matter into a soluble fraction has a crucial importance in the subsequent VFA production by the acidogenic bacteria (Xue et al., 2015).

To evaluate the extension of the hydrolysis step in the OFMSW anaerobic fermentation, the evolution with time of the SCOD concentration was determined and the solubilized amounts with their standard deviations for all assays were determined and are presented in fig.6.2.

It can be seen in figure 6.3 that, in all experiments, the profile for the solubilization of organic matter (reflected in the values of COD solubilized) for each TS content is affected by the initial alkalinity concentration added. Considering the profiles obtained with the two lowest TS content studied (fig. 6.3a and 6.3b), the decrease of the particulate material (measured as the increase of the solubilized COD) promotes a faster solubilization because the solubilized COD reached a maximum value in the period up to 4 days. For the highest TS reactor content (10 %), the maximum value of solubilized COD was obtained later on, up to 8 days of fermentation in all assays (fig. 6.3c), which may be a reflection of a partial inhibition of the hydrolytic bacteria due to high solids content inside the reactor and possible mass transfer limitations.

The maximum values for the solubilization degree (DS) in each assay were determined according to equation 15 and are presented in table 6.1. The increase in TS content led to a decrease of the solubilization degree of the particulate organic matter, decreasing the average values of DS from 80-90 % in the assays with 5 % TS to 70 - 74 % in the assays with 8 % ST. The influence of the alkalinity addition is different in the three sets of experiments. For the assays with the lowest solids content, an increase on alkalinity did not favor the solubilization of the organic matter, being the assay with no alkalinity addition the one with the highest solubilization degree (92.5 %). For the intermediate TS content, the DS was not affected by the increase on the alkalinity, reaching 70 - 74 %. For the assays with the highest TS content (10 %), although most of the solubilization step occurred after 4 days of operation, it was obtained the highest values for DS, varying from 87.02 to 99.32 %, increasing the DS with the increase of alkalinity addition.

In this study the C/N ratio was not controlled but was determined for each assay, at the beginning of the experiment. The carbon and nitrogen concentrations were determined from the same sample and the values obtained varied from 17 to 28 $g_{carbon}/g_{nitrogen}$, with an average

value of 22.7 ± 5.46 $g_{carbon}/g_{nitrogen}$. The values determined are in the same range as the ones obtained by Fdez.-Güelfo et al.(2011) which used industrial OFMSW as a substrate, or by Romano and Zhang (2008) which used onion juice and aerobic wastewater sludge for anaerobic co-digestion or reviewed by Li et al. (2011), which considered that C/N ratio as optimum for the anaerobic digestion process of solid waste.

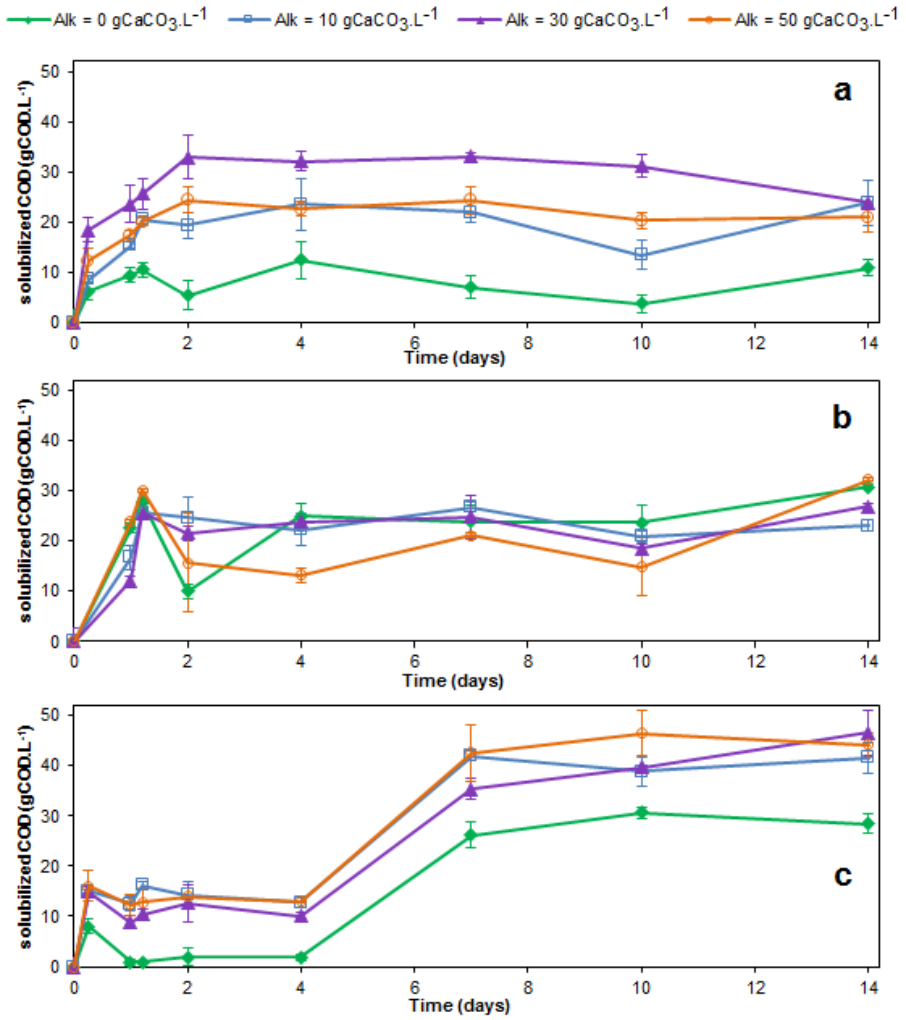

Figure 6-3: Solubilized COD profiles during time for the conditions under study: (a) TS content of 5%, (b) TS content of 8 %, (c) TS content of 10 %; error bars represent standard deviations of triplicate determinations.

6.3.3 Acidogenic potential of OFMSW

6.3.3.1 Performance of acidogenic fermentation

The efficiency of the acidogenic fermentation of OFMSW was affected by both operational parameters (alkalinity addition and TS content in the reactor). In addition, the type of

substrate used in this work had also a great influence on the two main response parameters under study (the type and the amount of VFA produced) and also on the pH reached at the end of the experiments, as also reported by Singh et al. (2015) and Jankowska et al. (2015).

The variation of the TVFA concentration at different conditions regarding added alkalinity concentration and TS content in the reactor during the fermentation time are shown in Fig.6.4. The profile of TVFA concentration in all reactors, in the beginning of the experiments exhibit a sharp increase, with a similar trend as it was verified for sCOD. Organic matter degradation in the initial phase of the fermentation process led to the accumulation of soluble compounds, which were further transformed in VFA and other simple compounds, causing high VFA concentrations. All reactors achieved maximum TVFA productions in the first days of fermentation, being the reactors with the highest alkalinity additions (30 and 50 g $CaCO_3$ L^{-1}) the ones which achieved the highest VFA concentration. A decrease in TVFA produced was observed when the highest TS content was applied (10%), as represented in Fig. 6.3c. Fernández et al. (2013) also reported that the efficiency of the acidogenic process decreases at higher TS concentrations.

The results regarding the maximum DA obtained as well as the maximum VFA concentration achieved in each assay are presented in Table 6.1 and the TVFA concentrations during time are represented in Figure 6.4 (a – c). In all assays, the increase in the initial alkalinity concentration led to an increase of both the total amount of VFA produced and the DA values obtained. DA reached its maximum value of 77.59 % when was tested the highest alkalinity concentration (50 $gCaCO_3.L^{-1}$) and the lowest TS concentration (5 %). The lowest values of DA were always achieved when no alkalinity was added, independently of the TS content in the reactors, which can be explained by the type of waste under study, which is rich in highly biodegradable organic matter (Capela et al., 2008).

The increase on DA due to the increase on alkalinity concentration has a linear behavior for each TS content inside reactors (Fig. 6.5a), with high determination coefficients between 0.88 and 0.98. The lower the TS reactor content, the more quickly DA increases with the addition of alkalinity and this enhancement nearly doubled for the increase of TS from 5 to 10 % of TS.

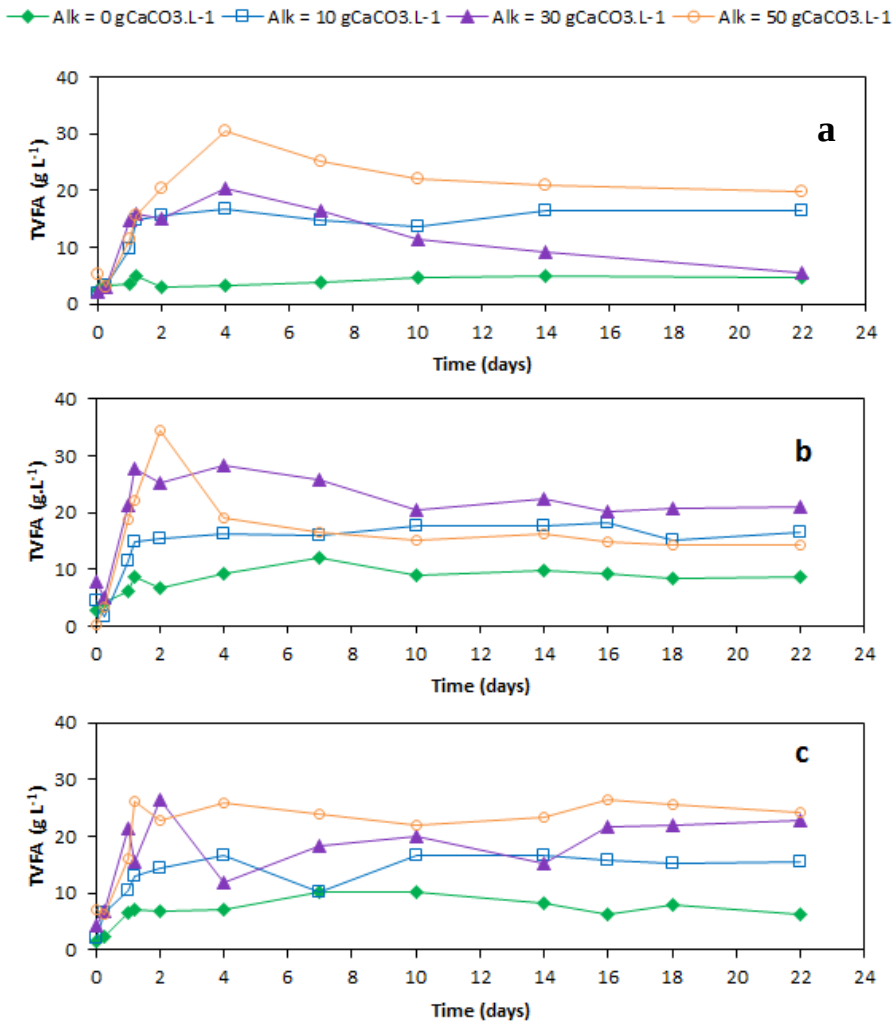

Figure 6-4: Daily variation of TVFA concentration during time for the conditions under study: (a) TS content of 5%, (b) TS content of 8 %, (c) TS content of 10 %.

The influence of DA due to the increase on TS content inside the reactors has also a linear behavior, presenting a decrease for each alkalinity concentration added. These trends are presented in figure 5.5b, where three sets of experiments (10, 30 and 50 $gCaCO_3$ L^{-1}) presented good determination coefficients, with R^2 between 0.595 and 0.982. The increase on TS content led mostly to a decrease on DA and this behavior is more pronounced when the highest alkalinity concentration was tested (50 $gCaCO_3$ L^{-1}). The TS content inside the reactors may directly affect the mass transfer phenomena and, consequently, the biotransformation of organic molecules (amino acids, fatty acids or sugars) into VFA.

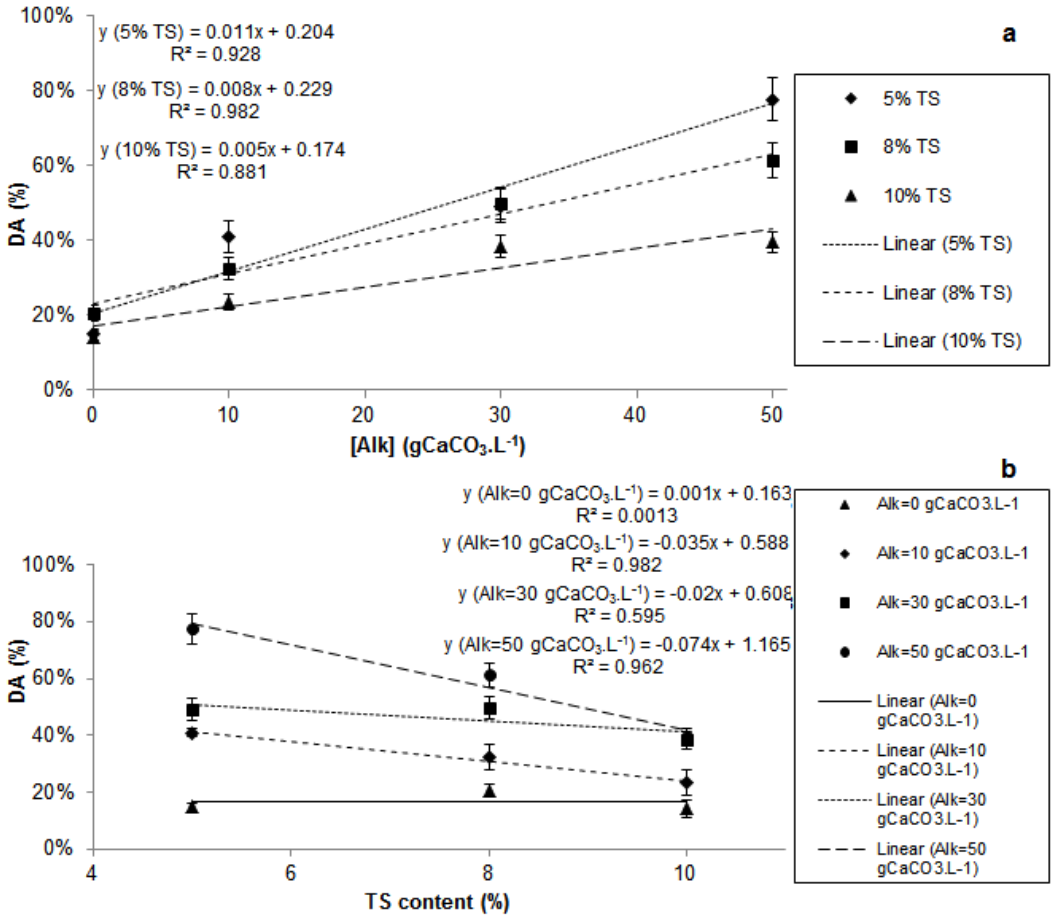

Figure 6-5: (a) DA and (b) VFA concentration versus alkalinity concentration added in different assays with 5, 8 and 10 % of TS content and respective equations obtained from the linear adjustment; error bars represent standard deviations of triplicate determinations.

6.3.3.2 VFA production and effluent quality

The composition of the VFA mixtures obtained in all assays is detailed in Fig. 6.6. The individual amounts of *i*-But, *i*-Val, *n*-Val and *n*-Cap acids were very low in all experiments, so they are represented as "others" in Figure 6.6. The predominant VFA species during the experiments, independently on the environmental conditions applied, were *n*-But, acetic and propionic acids, corresponding to more than 60 % (w/w) of the VFA mixture for all assays. The increase on the initial alkalinity led to an increase on the amount of *n*-butyric acid produced, increasing from approximately 40 % in assays without initial alkalinity added to up to 64 % for the highest alkalinity addition (50 $gCaCO_3$ L^{-1}). This trend was previously described by Dogan et al. (2008), where the increase on pH values led to an increase on the *n*-But concentration, using also OFMSW as substrate for acidogenic fermentation.

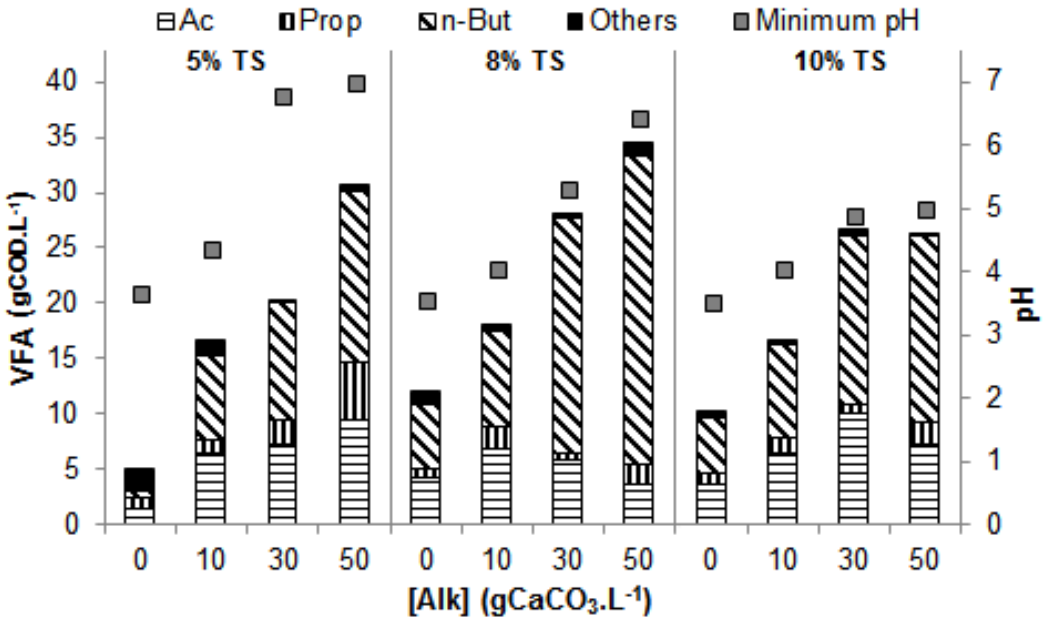

Figure 6-6: Minimum pH values and VFA composition obtained at maximum VFA concentration point, testing different conditions of TS content in the reactor and alkalinity concentrations added

Acetic acid followed the same pattern as *n*-But, increasing its concentration with the increase on alkalinity addition, although maintaining the acetic acid fraction in the mixture around 31.4 % ± 4.6 (w/w) independently on the applied conditions. The increase of acetic acid concentration with the increase of alkalinity (corresponding to an increase of final pH reached) in food wastes acidification processes was also reported by Dahiya et al. (2015), where a similar VFA profile was obtained. These results for the trend of VFA production could be attributed to carbohydrate, soluble proteins and lipids degradation, as reported by Jankowska et al. (2015), where OFMSW can be included because it contains high percentage of carbohydrates.

The propionic acid also increased with the increase of alkalinity and it can be related with the pH values reached inside reactors at the end of the experiments. The experimental conditions that favored the production of propionic acid show pH values higher than 6.5, corroborating other studies, in which it is referred a range of higher pH values to enhance propionic acid (Albuquerque et al., 2007). In studies using different organic wastes, the formation of propionic acid was also favored in a slightly higher range of pH values: between 6.8 and 7.1 for the acidification of olive oil mill wastewater (Gameiro et al., 2015) and between 5 and 7 for the acidification of cheese whey wastewater (Silva et al., 2013).

Considering the potential valorization of the acidified waste obtained, the control and manipulation of the operational conditions in the acidogenic phase can lead to a suitable VFA profile, to be used for the production of either PHA (Albuquerque et al., 2007) or other added-value products, such as bioenergy or as preferred carbon source in biological nutrient removal processes (Lee et al., 2014). Taking into account the use of VFA for further production of PHA, the composition of the mixture is a parameter with a significant importance, besides the total amount of acids obtained. Hence, for PHA production, the more suitable conditions that produce an acidified effluent with an odd-to-even ratio higher than 1 (which means that in this work the propionic acid concentration is higher than the sum of acetic and *n*-But acids) are the experiments with an intermediate TS content (8 %) and alkalinity higher than 10 $gCaCO_3\ L^{-1}$. Low (5 %) and high (10 %) TS content in the reactor did not favor the formation of propionic acid when compared with the acetic and *n*-But acids formation. On the other hand, acetic and *n*-But acids were produced in much higher amounts than propionic acid when the highest TS content was tested, being the above mentioned conditions suitable for the production of an acidified effluent that can be used, for example, as a carbon source in anaerobic biomethane and biohydrogen production.

With respect to effluent quality, the maximum values in terms of VFA fraction in the soluble COD are presented in Table 5.2. Generally, the increase on alkalinity, led to an increase on the amount of VFA present in the effluent. These values are related with the amount of initial TCOD that was solubilized and converted into VFA and tended to be higher (75 – 99 %) at higher alkalinities (30 and 50 $gCaCO_3\ L^{-1}$). The increase on solids content inside the reactor led to an acidified effluent with lower VFA content (20 – 80 %).

6.3.3.3 Dependence of acidogenic fermentation on pH

Generally, the addition of external alkalinity has a crucial importance in biological systems that treat highly biodegradable organic wastes as it is the case of OFMSW, once it avoids the sudden drop in pH, acting as a buffer effect. However, depending on the substrate, high alkalinity concentration may affect the VFA synthesis, once the pH range for acidogenic microorganisms is between 5 and 6 (Yu et al., 2002). In addition, when a low biodegradable substrate is used, the presence of high buffer effect may prevent the achievement of pH values that promote the acidification process (Malina and Pohland, 1992). Hence, the

increase on the buffer capacity, when a highly biodegradable waste is used in the acidification process, could promote the VFA formation, once it prevents a sudden drop of the pH values, maintaining the pH range favorable for the activity of acidogenic bacteria and preventing some inhibition problems.

In this study, the minimum pH value achieved varied between 3.5, obtained for the assay with the highest TS content (10%) and no external alkalinity added, and 7.0, in the opposite direction at the lowest TS content (5%) and highest alkalinity added (50 $gCaCO_3\ L^{-1}$). According to figure 4.3, the assays with no alkalinity added or with just 10 $gCaCO_3\ L^{-1}$ showed the lowest pH values with minimum pH in the range of 3.5 to 4.4 when compared with the assays with high alkalinity concentrations. At these low pH values it was observed a lower VFA production, so alkalinity addition to the system was a key factor to avoid a sharp drop in the pH, hence preventing inhibition of the activity of acidogenic microorganisms. Most researchers reported acidic pH as favorable for acidogenic bacteria as described by (Silva et al., 2013) that obtained higher VFA productions from OFMSW at pH 5.6 and Fang and Liu, (2002) which reported that the optimum pH for acidogenic fermentation was in the range 5.0 -6.5. However, other researchers, such as Chen et al. (2013) obtained maximum VFA concentration using food waste at pH 8.0 and Liu et al. (2012) reported that the alkaline pH seems to be beneficial for the degradation of soluble proteins and inhibiting the activity of methanogens. In conclusion, it is known that acidogenic bacteria are able to resist to variations on the environmental conditions, remaining active in a wide pH range (Wu et al., 2007). In the assays where pH was above 5, it was observed higher acidification degrees (DA) between 50 % and 78 %, showing that higher pH values had a positive effect on the OFMSW acidification. The assay with the lowest TS content (5 %) and highest alkalinity concentration (50 $gCaCO_3.L^{-1}$) reached the highest DA (78%), i.e. a higher conversion of the soluble fraction of COD into VFA. It was also observed that the increase on alkalinity can also help to stabilize the pH to higher values, thus improving the hydrolysis phase as reported by Kim et al. (2003) and consequently the VFA formation from organic solid wastes.

6.3.4 Statistical Analysis of acidogenic fermentation of OFMSW

The results obtained in the acidogenic fermentation of OFMSW in terms of total VFA production, degree of acidification (DA) and quality of the effluent obtained in terms of VFA fraction were modeled with response surfaces. This model can depict the effects of both TS reactor content and alkalinity addition on VFA production and composition and so evaluate the importance of each parameter, including linear, squared and interaction effects. Figure 6.7 shows the response surface plots and the contour plots of the polynomial quadratic equations fitted to the experimental data and Table 6.2 presents the regression coefficients and the parameters that evaluate the quality of the fitting.

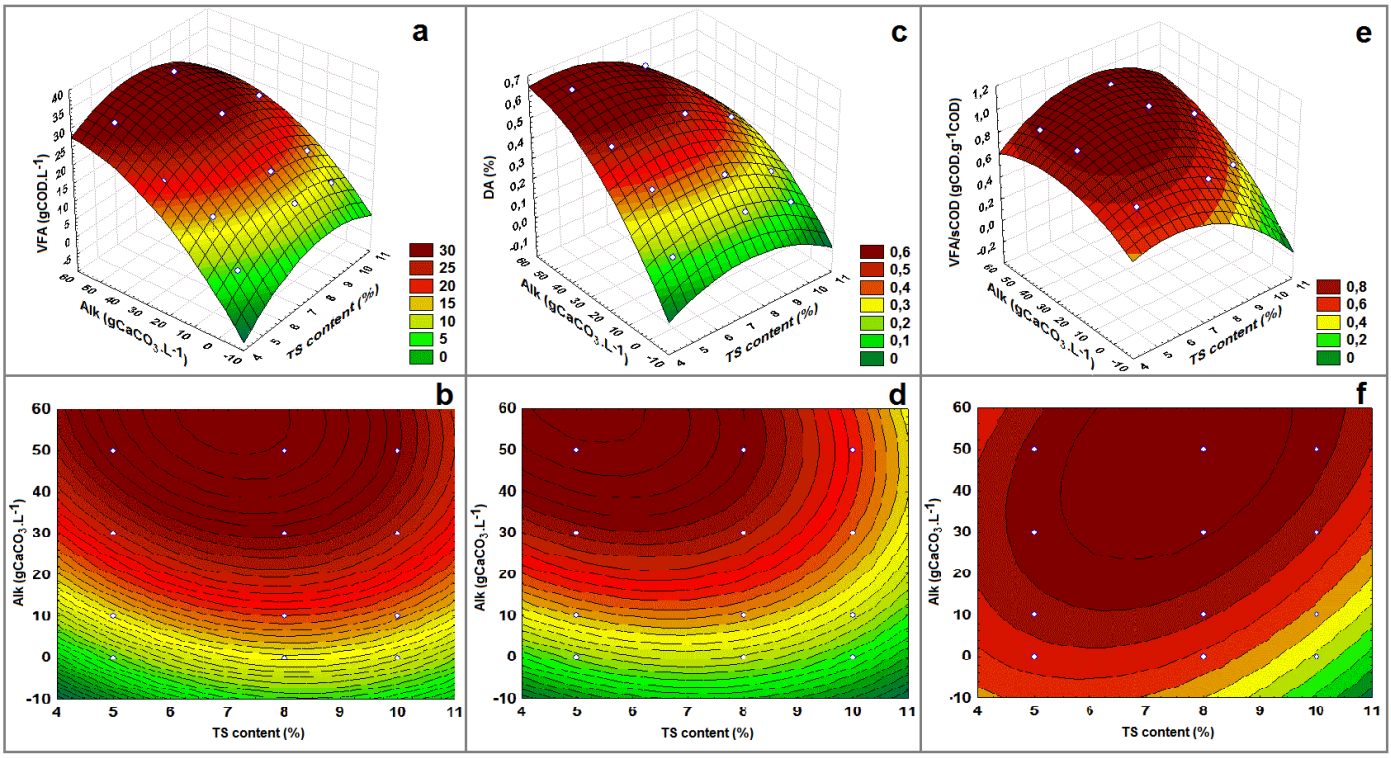

Figure 6-7: Response surface plots and contour plots calculated from batch fermentation of OFMSW: (a) and (b) total maximum VFA concentration (gCOD L^{-1}); (c) and (d) Degree of acidification (%); (e) and (f) effluent quality in terms of VFA (gCOD g^{-1}COD), versus alkalinity addition ($gCaCO_3$ L^{-1}) and TS content (%) in reactors.

The response surface plot represented in Fig. 6.7a and the contour plot in figure 6.7b illustrate the dependence of the total VFA produced, calculated as COD equivalents, on TS reactor content and alkalinity concentration. As it can be observed, there is an increase on the VFA production with the increase of TS reactor content and alkalinity concentration simultaneously, reaching the highest value of 34.46 g VFA-COD L^{-1} at an intermediate TS

reactor content of 8 % and at the highest alkalinity concentration (50 $gCaCO_3.L^{-1}$). Considering the surface plot, when no alkalinity concentration was added, the increase on

TS reactor content did not significantly affect the VFA production. However, the increase on alkalinity concentration from 10 to 30 $gCaCO_3$ L^{-1} and from 30 to 50 $gCaCO_3$ L^{-1}, considering the same TS reactor content, led to a significant enhancement on the amount of VFA produced. On the other hand, the increase on TS reactor content from 5 to 8 % considering the same alkalinity concentration added led to an increase on VFA production, reaching the maximum value at 8 % TS content, being this effect more pronounced at higher alkalinities. At TS reactor content higher or lower than 8 %, the VFA formation decreases.

Table 6-2: Fitting coefficients and evaluation of regressions

Regression coefficients	VFA	DA	VFA/sCOD
β_0	-31.61	-0.32	-0.19
β_1	10.78	0.16	0.29
β_2	0.91	$1.86x10^{-2}$	$3.7x10^{-3}$
$\beta_{1,1}$	-0.66	$-1.13x10^{-2}$	$-2.4x10^{-2}$
$\beta_{1,2}$	$-2.4x10^{-2}$	$-7.0x10^{-4}$	$1.5x10^{-3}$
$\beta_{2,2}$	$-6.1x10^{-3}$	$-1.0x10^{-4}$	$-2.0x10^{-4}$
R^2	0.83	0.82	0.55
ANOVA (p-value)	$1.29x10^{-4}$	$1.69x10^{-4}$	$1.13x10^{-2}$
Standard Error of Estimate	3.66	$7.1x10^{-2}$	0.14

Table 6-2 presents not only the regression coefficients obtained from the multiple regressions of total VFA production, degree of acidification and the quality of the effluent in terms of VFA, but also the evaluation of regression criteria and the standard errors of the estimated parameters. For VFA production, the value of R^2 was 0.83, which indicates a good correlation between values, and the very low *p*-value obtained ($1.29x10^{-4}$) shows that the data have a good correlation, indicating that the two predictor variables studied (TS reactor content and alkalinity addition) provide information on the total VFA production, with a low standard error of the estimate.

From the linear ($\beta 1$ and $\beta 2$) and quadratic ($\beta 1,1$ and $\beta 2,2$) coefficients, it can be concluded that TS reactor content has the highest effect on VFA production, once both coefficients ($\beta 1$ = 10.78 and $\beta 1,1$ = - 0.66) related to VFA production are about ten times higher than the coefficients related with alkalinity addition ($\beta 2$ = 0.91 and $\beta 2,2$ = $-6.1x10^{-3}$).

The degree of acidification (DA) is one of the most important parameters to evaluate acidogenic systems and it expresses the liquid VFA production from initial biodegradable

COD, representing the global yield of the acidogenic process. Fig. 6-7c and 6-7d represent the dependence of DA on TS reactor content and alkalinity addition. The surface plot obtained for DA has a similar determination coefficient ($R^2 = 0.82$) when compared with the surface plot obtained for VFA production, representing also a lower scatter around the regression surface, and a very low *p*-value ($1.69x10^{-4}$), indicating a good relationship between the two predictor variables and the response variable (acidification degree). The surface response (Fig. 6-7c) and the contour plot (Fig. 6.7d) show clearly the suitable conditions to obtain the maximum DA values: high alkalinity concentrations and low TS reactor content, being the opposite conditions very disfavored for a good performance of the acidogenic process (in terms of DA).

Considering the surfaces obtained, TS content presents also a higher effect on DA than alkalinity, being this observation confirmed by the linear and quadratic coefficients determined: the values for linear (β_1) and quadratic ($\beta_{1,1}$) coefficients related with TS content present higher values (0.16 for linear coefficient and $-1.13x10^{-2}$ for quadratic coefficient) than those obtained for alkalinity addition ($1.86x10^{-2}$ for linear coefficient and $-1.0x10^{-4}$ for quadratic coefficient). In practice, the increase on TS reactor content affects DA in a greater extension than the alkalinity, being this effect more evident at higher alkalinities and lower TS reactor contents, which are the suitable conditions to achieve higher DA values. Hence, the maximum DA (78 %) was achieved at an alkalinity concentration of 50 $gCaCO_3\ L^{-1}$ and a TS reactor content of 5 %, with a trend to increase with the increment on the alkalinity concentration. This behavior, regarding the dependence on alkalinity, is correlated with the type of substrate under study and its very high biodegradability (Silva et al., 2013), thus being the acidification of OFMSW favored by high alkalinity concentrations.

Taking into account the studied operational parameters, the surface response curves for VFA production and DA demonstrate that TS content in the reactor is the most important parameter, being more expressive for VFA production than for DA (yield of VFA produced). According to the results presented, DA, as the most relevant parameter in acidogenic fermentation, is influenced by both factors under study. The maximum values for VFA production (34.46 $gCOD.L^{-1}$) and DA (77.59 %) were obtained at different values for TS content (see Table 4.1), but at the same alkalinity concentration (50 $gCaCO_3\ L^{-1}$). Hence, these results confirm that the manipulation of the operational conditions improves VFA

production and allows an effective recovery of VFA from the anaerobic acidification of OFMSW.

Fig. 6-7e and 6-7f represent the dependence of the effluent quality, in terms of VFA fraction, on TS reactor content and alkalinity addition. Although the surface response for effluent quality has the lowest correlation coefficient (R^2 = 0.55) and the highest *p*-value ($1.13x10^{-2}$), when compared with the response curves for VFA concentration (Fig. 6-7a and 6-7b) and DA (Fig. 6-7c and 6-7d), it also indicates a significant relationship between the two predictors and the response variable, but with a higher scatter around the regression surface than what was observed for the other two response variables studied. The maximum value for the fraction of VFA in the treated effluent (c.a. 99%) was obtained when an alkalinity concentration higher than 30 $gCaCO_3$ L^{-1} was added to the system, combined with an intermediate TS content (between 6 and 9%). The values for the linear (β_1 and β_2) and quadratic ($\beta_{1,1}$ and $\beta_{2,2}$) coefficients were low but it is clear that the TS content inside the reactor has a greater influence in the effluent quality than the alkalinity concentration added. The linear coefficient (β_1 = 0.29) and the quadratic coefficient ($\beta_{1,1}$ = $-2.42x10^{-2}$) related with TS content are much higher than the correspondent linear (β_2 = $3.7x10^{-3}$) and quadratic ($\beta_{2,2}$ = $-2.0x10^{-4}$) coefficients related to alkalinity concentration added. The surface plot shows this dependence of effluent quality in terms of TS content and the increase on TS content led to a sharp drop on VFA concentration in the treated effluent, also observed in both surfaces for VFA production (Fig. 6-7a) and DA (Fig. 6-7c), being this behavior more pronounced when low alkalinity concentrations were tested.

For all the studied response variables, and considering the test significance of the regression (ANOVA), the *p*-values obtained are below 0.05, meaning that experimental behaviors were well described by the computed models, attesting the adequacy of the regressions determined with a confidence level of 95 %, thus ensuring the quality of the surfaces obtained by both the standard error and the *p*-value determined. In general, VFA concentration, DA and the quality of the effluent in terms of VFA were favored at higher alkalinities, above 30 $gCaCO_3$ L^{-1}, and low TS content inside the reactor. High total VFA production and high effluent quality in terms of VFA are favored at 8% of TS content, while DA is favored when lower TS content (5 %) was tested.

6.4 Conclusions

This work section assessed the influence of both TS content in the reactor and initial alkalinity on the hydrolytic-acidogenic fermentation of OFMSW, in order to produce a mixture of VFA suitable for the production of added-value products, such as PHA, methane or other materials. The following conclusions can be drawn:

The production of VFA in an anaerobic acidogenic process can be included in an integrated system for the management of solid organic wastes, such as OFMSW, where the pollution load reduction is combined with the generation of highly valuable marketable products.

The first step of anaerobic acidification process, which combines the disintegration and hydrolysis of OFMSW to soluble substrates, was fast and occurred up to 8 days, reaching higher rates for all experiments (70 – 99 %) which is in the agreement with the easily biodegradable waste under study.

When considering the two sequential steps in the acidification process, the alkalinity addition was crucial for having higher acidification degrees for all sets of experiments.

The increase on the initial alkalinity concentration was beneficial for all the response variables: total VFA production (maximum of 34.46 gCOD L^{-1}), the conversion of tCOD into VFA (maximum DA of 77.59 %), and effluent quality in terms of VFA (maximum 98.96 %).

TS content is the predictor parameter which presented the highest effect on all response variables studied (VFA production, degree of acidification and effluent quality in terms of VFA), confirmed by the higher values for linear and quadratic coefficients related with TS content when compared with coefficients related with alkalinity, obtained in the fitting of surface response curves to the experimental data.

The statistical approach performed show that the operational conditions can be adjusted in order to obtain specific compositions of the VFA mixture, according to the subsequent usage for these metabolites produced in the acidogenic fermentation of OFMSW. Higher odd-to-even VFA ratios were obtained at higher alkalinity addition which was crucial for PHA production.

7. CSTR operation for the acidogenic fermentation of OFMSW: Effect of operational parameters

7.1 Introduction

In previous chapters, it was studied the effect of some parameters (TS reactor content and alkalinity concentration) in batch acidogenic fermentation assays of several food wastes and other organic residues, for the production of added-value products, such as VFA. In those conditions, OFMSW was one of the substrates with very high biochemical acidification potential, besides being actually one of the solid residues in Cape Verde islands with the highest production, which causes high environmental problems.

Hence, in chapter 6, it was studied the acidogenic potential of OFMSW at different TS reactor content and alkalinity concentration. In those assays, the highest conversion of the organic matter into VFA (up to 78%) and the highest quality in the acidified effluent in terms of VFA (99%) was achieved in the digestion assay of OFMSW with a total solids content inside the reactor of 5 % TS and an alkalinity concentration of 10 g $CaCO_3$ L^{-1}. Hence, the aim of this chapter was to study the acidogenic potential of OFMSW performed in CSTR reactors in a long-term basis. The choice of a CSTR system was based in the fact that this technology has been tested successfully for acidification processes, being suitable systems to degrade the liquid fraction of organic municipal waste (Held et al., 2002). According to Jiang et al. (2012), CSTR is among the most used type of reactor for acidogenic processes due to the promising results at low pH conditions and the potentially inhibition of the methanogens activity. In addition, the microorganisms in a CSTR system are suspended in the digester content through intermittent or continuous mixing, where this complete mixing offers good substrate/inoculum contact with slight mass transfer resistance (Mao et al., 2015). In this sense, the operation of the CSTR reactor lasted for approximately six months, varying the organic load and alkalinity concentration applied, in order to convert most of the organic matter fed to the reactor into VFA.

During the operation phase of this reactor, several acidified effluents were collected and further used as raw materials (substrates) in the PHA production process, either in the biomass enrichment of PHA accumulating microorganisms step or in the PHA production and accumulation step.

7.2 Results and Discussion

The anaerobic reactor CSTR-type used in this study was fed with just OFMSW, in order to evaluate the fermentation process performance at a long-term operation time, with the final goal to obtain high VFA production to further be used as substrate. In this work, the CSTR was initially fed continuously, but due to the continuous obstruction on the feed inlet, the operation mode was modified and the reactor was operated in a semi-continuous mode, as it was described in chapter 3, section 3.3.2. In the semi-continuous mode of operation, the OFMSW acidification potential was tested, and the CSTR performance evaluated, with pH being monitored at different experimental conditions of organic loading rate and alkalinity applied to the system. The HRT was maintained constant during all experiment (2.5 days), in order to achieve a long-term stable operation.

After a start-up period, in Stage I, the reactor was operated at a loading rate of 3.0 gCOD L^{-1} d^{-1} and a constant high alkalinity of 5.0 g $CaCO_3$ L^{-1}. Stage II corresponded to an increase of the loading rate to 4.0 gCOD L^{-1} d^{-1} and a decrease of the alkalinity to 3.5 g $CaCO_3$ L^{-1}, in order to obtain more favorable conditions for acidogenic fermentation. Stage III corresponded to another increase on the organic loading rate to 5.0 gCOD L^{-1} d^{-1} and a decrease of the alkalinity to 2.5 g $CaCO_3$ L^{-1}. Stage IV corresponded to another increase on the organic loading rate to 6.0 gCOD L^{-1} d^{-1} maintaining the same alkalinity (2.5 g $CaCO_3$ L^{-1}). Stage V corresponded to another increase on the organic loading rate to 6.5 gCOD L^{-1} d^{-1} and another decrease of the alkalinity to 2.0 g $CaCO_3$ L^{-1}, to further evaluate the reactor capacity in terms of acidification. In the last stage, Stage VI, the organic loading rate was decreased to 6.0 gCOD L^{-1} d^{-1}, maintaining the lowest alkalinity (2.0 g $CaCO_3$ L^{-1}).

During all experiment, several acidified effluents were collected and preserved for further analysis, in order for them to be used in a later stage as feedstock for culture selection and PHA accumulation in aerobic steps of the OFMSW valorization process.

7.2.1 Solubilization and acidification efficiencies

In this study, acidogenic fermentation of OFMSW in a CSTR reactor was investigated with the increase with time of the organic loading rate (OLR) and the decrease of alkalinity (Alk), as represented in Fig. 3-8, in order to maximize the VFA accumulation, and optimize the

operational conditions. The system was operated at a constant HRT of 2.5 days without any external pH control. In order to evaluate the performance and the efficiency of the process, the CSTR effluent was extensively characterized. The physical–chemical parameters such as pH, Alk, sCOD, TCOD and VFA were measured for both input (feed) and output (effluent) streams during the all experimental operation period. The average results of the process performance, such as the degree of acidification (DA), the odd-to-even VFA ratio produced, the ratio between VFA concentration e sCOD in the effluent output, for the different stages of operation are summarized in Table 7-1.

Table 7-1: Summary of the experimental results obtained in the CSTR operation

Operation		Parameters						
Stage	Days	OLR	Alk	sCOD	TVFA	VFA/sCOD Out (%)	Odd-to-Even VFA ratio	DA (%)
Stage I	8– 35	3.0	5.0	3.38 ± 0.03	1.78 ± 0.32	33.42 ± 1.21	0.30 ± 0.10	23.53 ±1.04
Stage II	36 – 60	4.0	3.5	4.99 ± 0.04	3.86 ± 0.24	58.99 ± 0.27	0.51 ± 0.24	38.47 ±0.19
Stage III	61– 90	5.0	2.5	8.21 ± 0.16	5.80 ± 0.12	61.46 ± 0.19	0.55 ± 0.17	46.37 ±0.19
Stage IV	91 – 130	6.0	2.5	12.28 ± 1.00	7.52 ± 0.14	65.51 ± 0.22	0.44 ± 0.13	58.89 ±0.17
Stage V	131– 160	6.5	2.0	10.80 ± 1.81	5.73 ± 0.12	53.07 ± 0.15	0.32± 0.13	36.79 ±0.16
Stage VI	161– 180	6.0	2.0	10.34 ± 0.27	7.52 ± 0.14	61.89 ± 0.12	0.30 ± 0.12	27.85 ±0.16

OLR – (gCOD $L^{-1}d^{-1}$)
Alk – (g $CaCO_3L^{-1}$)
sCOD – (g L^{-1})
TVFA – (g COD L^{-1})

In Figure 7-1 (a) it is described the effect of OLR and alkalinity applied to the system on the pH of the acidified effluent (output), monitored during 180 days of operation. The results obtained revealed that the output pH values varied significantly with the increase of OLR and the decrease of alkalinity added.

During Stage I, pH values remained very low (3.5 to 4.0), which resulted in a low acidification potential (23.5% in average). These low pH values observed for the lowest OLR (3.0 gCOD $L^{-1}d^{-1}$) and the highest alkalinity concentration added (5.0 $gCaCO_3L^{-1}$), corresponding to Stage I, showed that these conditions were not favorable to the process, leading most probably to low hydrolysis and consequently low acidification.

The increase of the OLR from 3.0 to 4.0 gCOD $L^{-1}d^{-1}$ and the decrease of alkalinity from 5.0 to 3.5 $gCaCO_3L^{-1}$ (Stage II) at 36th day of operation led to a rapid pH increase to values higher than 7, in the beginning of this period. After that, the pH decreased to values near 6

at 61 days of experience and was maintained around that value until the end of this stage. These results confirmed that the operational conditions in this stage were more favorable for the fermentation process, in terms of either the hydrolysis or the acidification steps, leading to an increase in the acidification degree (38.5% in average). In stage III, with another increase on the OLR and a decrease in the alkalinity, pH remained near 6, although with another increase in the acidification degree (46.4% in average) as show in Fig. 7.1(a).

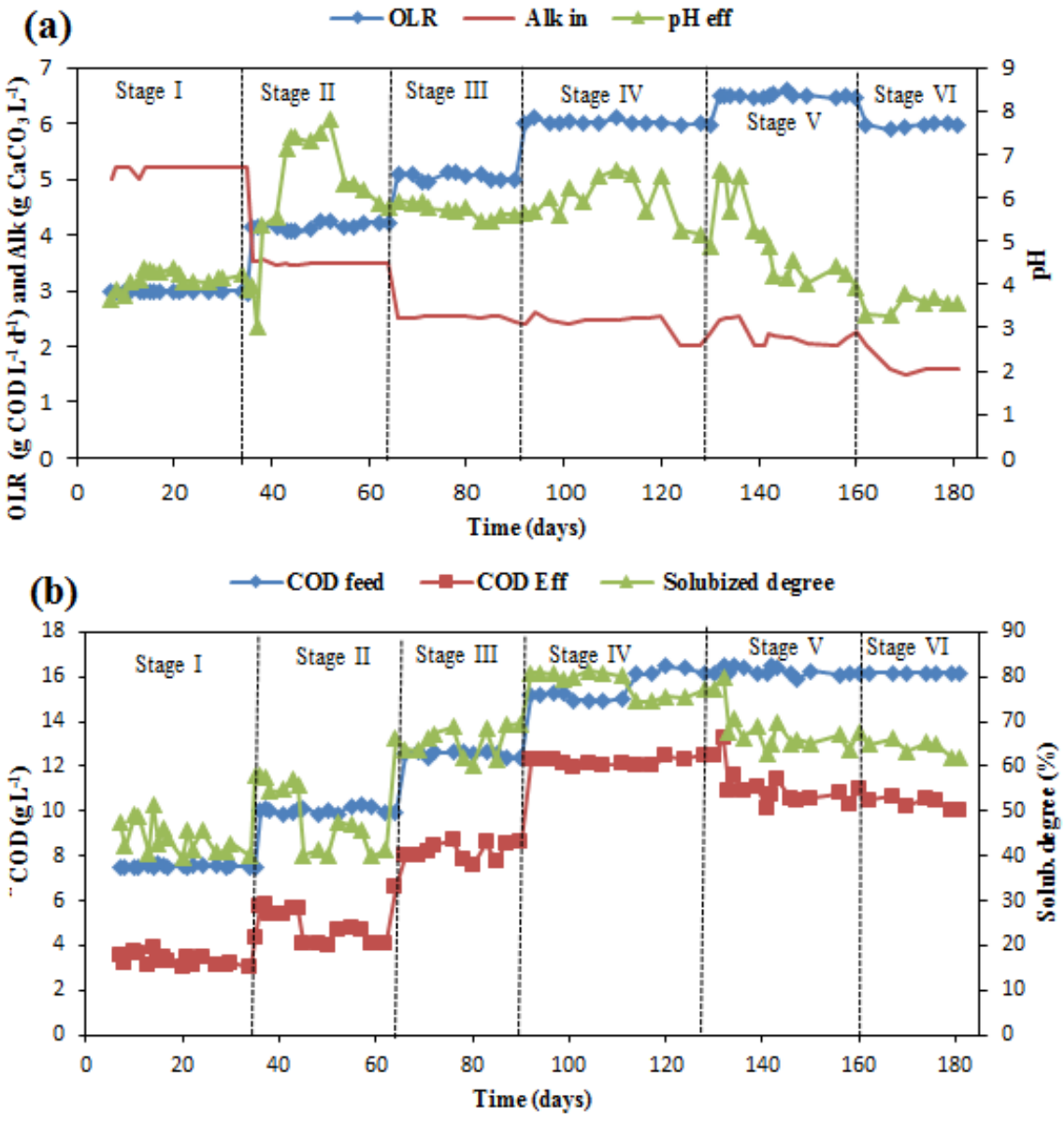

Figure 7-1: Performance of the CSTR reactor processing OFMSW under different OLR and Alk conditions: (a) pH and Alk evolution; (b) TCOD of the feed, solubilized COD and solubilized degree in the fermented effluent.

In Stage IV, the OLR was again increased from 5.0 to 6.0 gCOD L^{-1} d^{-1} but in this phase maintaining the Alk at 2.5 $gCaCO_3$ L^{-1}. In this stage, pH was not kept constant as in the previous stage, but s varied between 6.5 and 5.0, showing a similar behavior to the one verified in stage II, with an increase in the beginning followed by a decrease to the lowest values in the end of the period. However, in this stage it was obtained the highest value of the acidification degree (58.9% in average) (see Table 7.1), indicating favorable conditions for acidogenic fermentation.

From the 130th day to the 160th day (corresponding to Stage V), the OLR was again increased (6.5 gCOD L^{-1}) and the alkalinity decreased (2.0 $gCaCO_3$ L^{-1}), in order to achieve the maximum acidogenic capacity of this system. It can be observed in Fig. 7.1(a) that pH showed the same behavior as in previous stage, with an increase in the beginning followed by a decrease to values around 4 (lower than the ones observed in previous stage). As a result, these conditions affected adversely the acidification process, which caused a decrease on the acidification degree (36.8% in average), showing that it was achieved the maximum hydrolysis/acidification activity in the previous stage (stage IV).

At stage VI, the OLR was than decreased to 6.0 gCOD L^{-1} d^{-1}, maintaining the Alk in the lowest value (2.0 $gCaCO_3$ L^{-1}). In these conditions pH also have a similar behavior than the ones verified in others stages, showing an increase in the beginning followed by a decrease, reaching in this stage the lowest values lower than 4.0, which were not favorable to the hydrolysis/acidification process, as reported by the acidification degree (27.9 % in average). These results are consistent with previous studies reporting that both pH and the behavior of the acidogenic fermentation process are affected by alkalinity and total solids in the reactor (Virgine et al., 2015; Gameiro et al., 2016) and by OLR (Lim et al., 2008; Zhang et al., 2015).

Fig. 7-1(b) shows the variations of the COD in the feed (in terms of TCOD), the sCOD of the effluent (COD eff) and solubilization degree (SD) determined dividing the sCOD eff by TCOD in the feed, at different OLR and Alk applied to the system. As observed in Fig. 7-1(b), the OLR and Alk applied to the system have both a direct effect on the production of soluble organic compounds from the substrate, i.e. on the hydrolysis/solubilization process of complex organic matter present in OFMSW. Hence, it can be observed in Fig. 7-1(b) that sCOD in the effluent followed the same trend as the TCOD in the feed up to Stage IV. The

highest sCOD values were obtained in Stage IV, where pH ranged between 6.5 and 5.0, with consequently higher SD values (75 to 80 %), and high acidification degree (58.9% in average). In general, for all the experimental conditions tested, the SD was higher than 40 %, value suggested by other authors for the activity of acidogenic bacteria, which use the organic matter present in the waste for VFA production of (Yu et al., 2002; Castelló et al., 2009; Wu et al., 20016).

Comparing the ratio of VFA produced to sCOD in the effluent, which represents the quality of the effluent in terms of VFA (amount of VFA present in the total of soluble compounds), it was observed the highest value in Stage IV, where the pH was not that low (5.0 and 6.5). Values of DA in this stage were also the highest (58.89 %), as presented in Table 7-1. At pH values lower than 4, the VFA/sCOD ratios and DA tended to be relatively low (as observed in stage I), or DA low (as observed in stage VI), indicating that strong acidic conditions inhibited not only the activity of methanogenic microorganisms, but also the activity of hydrolytic and acidogenic bacteria, hence decreasing the accumulation of VFA. Besides VFA, the most common products observed in these type of fermentations are alcohols (Ren et al., 2007). In this study, alcohols were not quantified, not only due to some limitations in the experimental conditions, but also to the fact that only VFA were the intermediates needed as substrates for further production of value-added products (PHA).

The results obtained in the distinct phases of the CSTR operation indicated that the particulate complex organic matter present in the feed to the system was effectively solubilized at the operational conditions tested. In fact, the fermentation/acidification process was favored for higher OLR and lower alkalinity, and the highest performance was obtained in Stage IV, at an OLR of 6.0 g COD L^{-1} d^{-1} and a low alkalinity of 2.5 $gCaCO_3$ L^{-1}, since the alkalinity needs for these systems are normally lower than for methanogenic systems. . This result is similar to the one obtained by Aslanzadeh et al. (2014), when compared single and two-stage anaerobic digestion processes of food processing industry wastes and OFMSW.

7.2.2 VFA production and composition

It is shown in Figure 7-2 that VFA concentrations in the effluent (presented as COD equivalents) are a function of the OLR and Alk applied during the entire fermentation period

of 180 days, in the CSTR system under study. In general, VFA increased with the increase of the OLR and the decrease of alkalinity applied to the system, being the maximum achieved in Stage IV. Acetic, propionic, *n*-butyric, and *n*-valeric acids were the most abundant fermentation products in the VFA mixture analyzed, with the peak concentrations reached between 90th and 130th day (Stage IV) of approximately 2507, 1764, 3640 and 983 mg VFA-COD L^{-1}, of each specie, respectively, corresponding to 8893 mg VFA-COD L^{-1} as TVFA. Small amounts of *i*-butyric, *i*-valeric and *n*-caproic acids were also observed.

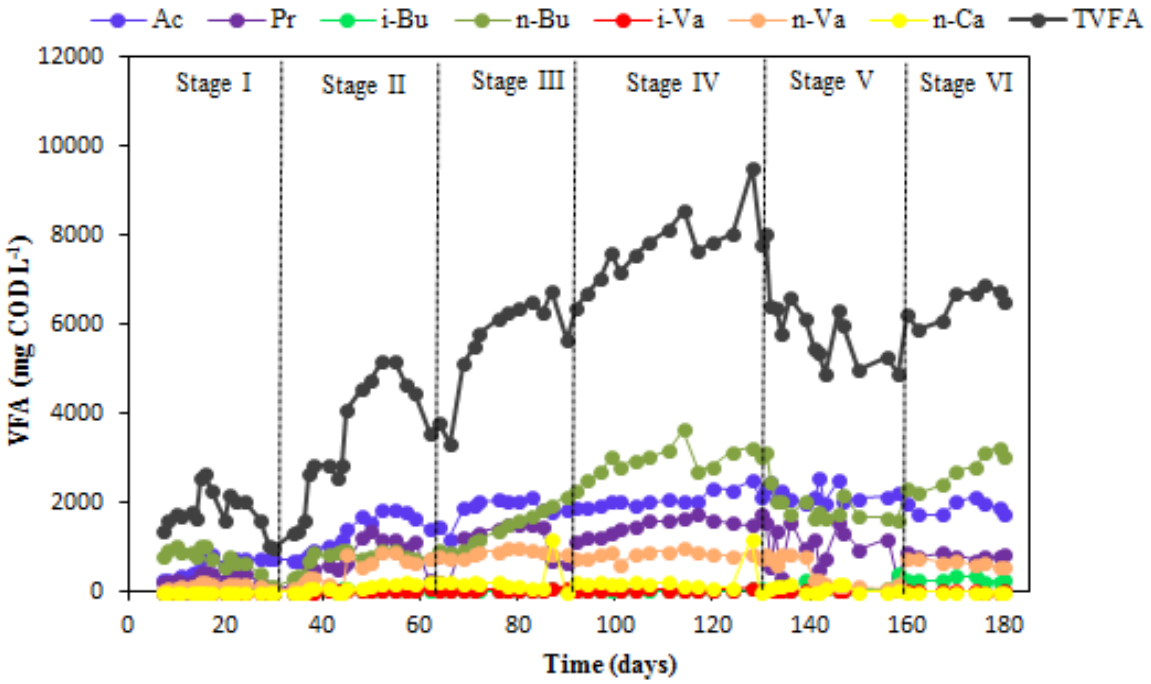

Figure 7-2: Evolutions of individual VFA species and TVFA concentrations, expressed in mg COD L^{-1}, at different CSTR operational stages.

In the initial stage of the CSTR operation (from day 7 to day 35), all VFA species were produced at a low concentration. For this period (Stage I), maximum TVFA production reached 2559 mg VFA-COD L^{-1} at 15th day of fermentation, presenting an average value of 1780 mg VFA-COD L^{-1}. H-Ac and *n*H-Bu were the main dominant species produced, with concentrations of 842 and 1040 mg VFA-COD L^{-1}, respectively. During this period, where the lowest OLR of 3.0 g COD L^{-1} and the highest Alk of 5.0 g $CaCO_3$ L^{-1} were applied, DA and the odd-to-even VFA ratio were relatively low, with values of 23.5% and 0.30 respectively (values presented in detail in Table 7.1). The TVFA concentration increased after day 35, at the same time as the OLR increased to 4.0 g COD L^{-1} and Alk decreased to

3.5 g $CaCO_3$ L^{-1} (Stage II). The same behavior was observed during the next stages (III and IV), with the increase in TVFA with the increase in OLR and decrease of Alk. First it was reached an average of 5800 mg VFA-COD L^{-1} at 5.0 g COD L^{-1} and 2.5 g $CaCO_3$ L^{-1} (Stage III), and after it was reached an average of 7520 mg VFA-COD L^{-1} at to 6.0 g COD L^{-1} and 2.5 g $CaCO_3$ L^{-1} (Stage IV). Afterwards, the further increase in the OLR to 6.5 g COD L^{-1} and decrease of Alk to 2.0 g $CaCO_3$ L^{-1}(Stage V), decreased the TVFA to 5730 mg VFA-COD L^{-1}, confirming that the maximum acidogenic potential was achieved in the previous stage (Stage IV).

The highest VFA concentration in all experiment was obtained at day 125, in stage IV, reaching 9525 mg VFA-COD L^{-1}. From day 130 to day 160, the VFA concentrations decreased sharply at the highest OLR added (6.5 g COD L^{-1} d^{-1}) and the lowest Alk (2.0 g $CaCO_3$ L^{-1}), with also a decrease in pH to around 4, indicating that these operational conditions are not favorable to obtain high acidification potential. These results indicate again that the acidification process is very sensitive to high OLR combined with low Alk.

At the end of the experimental operation (from day 161 to day 180), when OLR was decreased from 6.5 to 6.0 g COD L^{-1} d^{-1}, but maintaining the low alkalinity addition (2.0 g $CaCO_3$ L^{-1}), it was observed an improvement on the process performance, with the TVFA concentration increased to approximately 6517 mg VFA-COD L^{-1}.

Comparing the results obtained in Stages IV and VI, where the same OLR was applied (6.0 g COD L^{-1} d^{-1}), but with different alkalinities added, , it is clear that Stage IV shows better results regarding the VFA accumulation than Stage VI, showing again the direct effect on the combination of the OLR and alkalinity on the acidification process.

With respect to the quality of the acidified effluents produced in the experiment, it was observed that, in Stages I, II and III, the acetic acid was the predominant specie, followed by propionic, *n*-butyric and *n*-valeric, in this order of importance. At Stage IV, it was verified a change in the dominant specie, being now the *n*-butyric acid, followed by acetic, propionic and *n*-valeric, in this order of importance. This is in accordance with literature, which refers that, for higher loading rates, the accumulation of hydrogen causes a change in the production of VFA to species with higher carbon content in detriment of species with lower carbon content. In Stage V, where the operational values for the pair OLR-Alk were not very favorable to the hydrolysis/acidogenic process, the system showed some instability, with

changes in the predominant VFA species. The effluent quality in terms of FVA in Stage VI, were similar to the one obtained in Stage IV, although with lower amounts, due also to the values attributed to the pair OLR-Alk.

In order to obtain a better visualization on the effect of the OLR and Alk to the process performance, Fig. 7-3 (a) and (b) shows the VFA mixture composition as a function of the OLR applied, expressed as absolute concentrations of each specie (Fig. 7-3a) and as a fraction of each specie in the TVFA mixture (Fig. 7-3b).). Besides the amount of TVFA produced, the composition of the VFA mixture obtained in the acidogenic fermentation of wastes is very important, regarding the effective valorization of the acidic streams obtained either into bioenergy (biogas) or into added-value products, such as PHA, a type of biodegradable polymer. With respect to the waste valorization into PHA, the different VFA species are converted to PHA by aerobic PHA accumulating bacteria, depending the polymer produced on the amounts and quality of the VFA mixture obtained during the acidification process. Acidified effluents which are rich in acetic and butyric acids promote the synthesis of hydroxybutyrate (HB) monomers and, on the other hand, acidified effluents with high propionic acid concentration tend to increase the hydroxyvalerate (HV) monomer content in the polymer (Lemos et al., 2006; Dionisi et al., 2004; Takabatake et al., 2000).

During the acidogenic fermentation, it was observed that the increase on the OLR and the decrease on Alk resulted in an increase in the amount of VFA produced, with the exception of the highest OLR tested (6.5 g COD L^{-1}), when the operational values attributed to the pair OLR-Alk were not favorable, reaching during the process very low pH values. Comparing the results of Stage IV and VI for the same OLR, but different alkalinity, it was observed for the period with lower alkalinity, a small reduction on the TVFA production around 13%, but a big change in the process performance (53% reduction on DA) and on the quality of the treated effluent (reduction of 35% in the odd-to-even VFA ratio and 32% on the VFA/sCOD ratio).

Results obtained at OLR of 6 g COD L^{-1} d^{-1} and Alk of 2.5 g $CaCO_3$ L^{-1} exhibited the highest value for the absolute accumulation of VFA (Fig. 7-3 (a)). This high accumulation of VFA was observed for pH values ranging from 5.0 to 6.5, as already presented in Fig 7-1 (a), values also reported in several studies as favorable for VFA accumulation (Capela et al., 2008; Silva et al., 2013; Gameiro et al., 2015; Zhang et al., 2015). H-Ac, Pr, *n*H-Bu and *n*H-

Va acids were the most common fermentation products during all experimental stages. It was also observed a very small contribution of VFA products, such as *i*H-Va and *n*H-Ca acids. The VFA species predominant in this experiment suggest that the substrate used in the CSTR acidification tests is rich in proteins, carbohydrates and lipids, due to the high accumulation of H-Ac, H-Pr and *n*H-Bu acids (Horiuchi et al., 2002). The contribution of the H-Va acid to the VFA mixture is mainly due to the degradation of the proteins present in the complex waste used in these assays (McInerney, 1988).

The relative composition of VFA in the mixture had a notorious change during the experiment as can be observed in Fig. 7-3-(b). In general, the relative predominant species during all experiment were H-Ac acid, with a contribution to the TVFA of 25-40%, or *n*H-Bu acid, with a similar contribution to the TFVA of 25-45%, being H-Ac with higher contribution to the TVFA in Stages II, III and V, and *n*H-Bu to the Stages I, IV and VI. With respect to the other two predominant VFA species, H-Pr contributed with 15-20% to the VFA mixture and *n*H-Va contributed with a lower amount of 10-15%.

In addition to these results, the majority of the COD of the effluent (output) is composed by VFA, ranging from 53-65.5%, with the exception of the effluent obtained in Stage I (33.4%), as reported in Table 7.1. With respect to odd-to-even VFA ratio, this parameter ranged from 0.3 to 0.55, with the highest values for the outputs of Stages II, III and IV (0.44-0.55). For the higher values of this parameter contributed the higher concentrations of propionic acid. The presence of this acid is very important for the production of a PHA with more interesting mechanical properties (higher content in HV monomer).

The operational condition with the highest VFA production (Stage IV), presents a relative amount of each species of 27%, 19%, 38% and 11%, corresponding to H-Ac, H-Pr, *n*H-Bu and *n*H-Va acids, respectively. Thus, these four most fermentable organic products account for 95 % of total VFAs, which is regard as positively for the waste valorization into PHA biosynthesis. So, considering all data, the results obtained in stage IV (as presented in Table 7-1) for the acidified effluent, present the highest VFA quantity and very good quality regarding not only the VFA species, but also the percentage of VFA in the soluble COD. This acidified effluent was collected and preserved at 4 ^{o}C for further use as a feedstock for mixed-aerobic cultures enrichment for biopolymer production (PHA) and batch PHA accumulation tests.

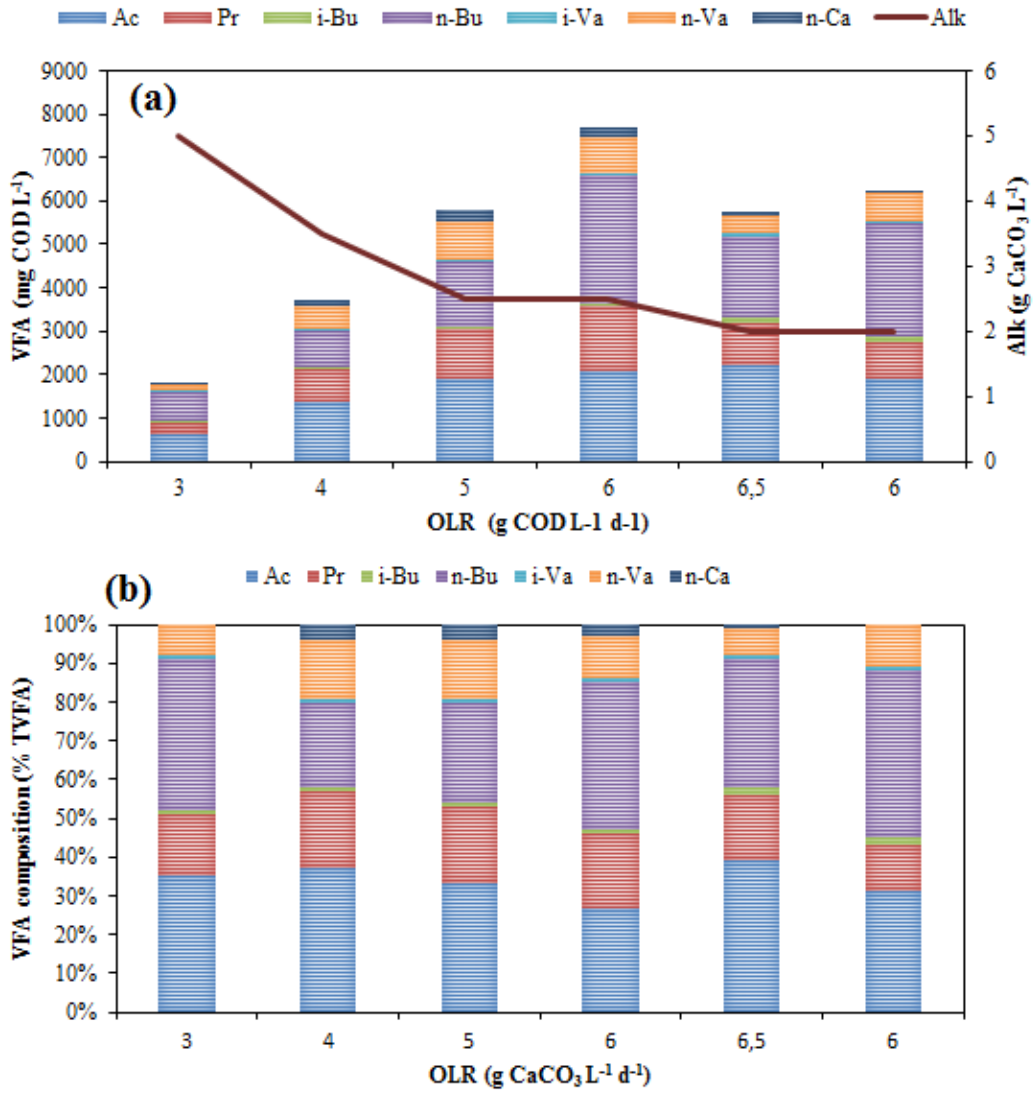

Figure 7-3: Distribution of individual VFA and their percentages to the total VFA (TVFA) with different OLR and alkalinity applied to the CSTR system

7.2.3 Biogas production

Figure 7-4 show biogas and methane production and Figure 7-5 presents biogas composition in terms of carbon dioxide and methane throughout the six experimental stages at different OLR applied. As can be seen in Fig. 7.4, the biogas production tended to increase with the increase OLR, up to Stage V. During the first 31 days (Stage I), the biogas production was negligible. In this period, it was not detected the presence of methane, just carbon dioxide, reflecting the normal performance of an acidogenic CSTR system. After this initial period, and during Stages II III and IV, biogas production did not significantly increase with the increase of OLR, but was kept more or less stable, around 1.5 L d^{-1}, with a methane content

ranging from 10 to 30%, which indicates a hydrolysis/acidification process well stablished, with the correspondent inhibition of the methanogenic activity.

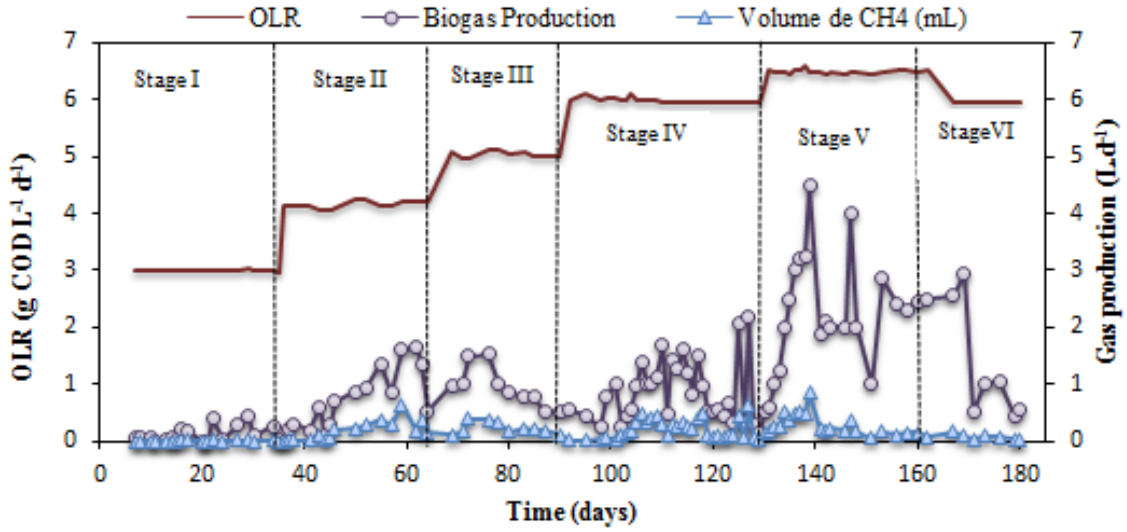

Figure 7-4: Daily biogas and methane productions at different OLR and alkalinity applied during experimental operation of the CSTR system.

With the further increase on the OLR to 6.5 g COD L^{-1} d^{-1} and the decrease in the alkalinity concentration on day 135 (stage V), the biogas production doubled, reaching its maximum value (4.48 L d^{-1}), but the methane content dropped drastically to very low values (lower than 10%). These results confirm what was verified through VFA production, that, even preventing further methanogenic activity, these operational conditions inhibit also the hydrolysis/acidification process. In order to restore a steady performance of the CSTR system, the OLR was decreased to 6.0 g COD L^{-1} d^{-1} and the alkalinity concentration was maintained in a low level (2.0 g $CaCO_3$ L^{-1}). Thereafter, the biogas production gradually decreased to values lower than 1.0 L d^{-1} until the end of the operation.

Methane percentage in biogas (Fig. 7-5) ranged between 3 - 40 % , with an overall average in the percentage of CH_4 in biogas for the stages II, III, IV, V and VI of 19 ± 12.1, 25 ± 7.4, 21.7 ± 9.8, 13.4 ± 7.7 and 5.8 ± 2.0, respectively. Based on these results, it was possible to predict that the optimum operational condition tested was the pair (OLR of 6 g COD L^{-1} d^{-1} and alkalinity of 2.5 g $CaCO_3$ L^{-1}) for the acidogenic fermentation process using OFMSW as substrate, in a CSTR system.

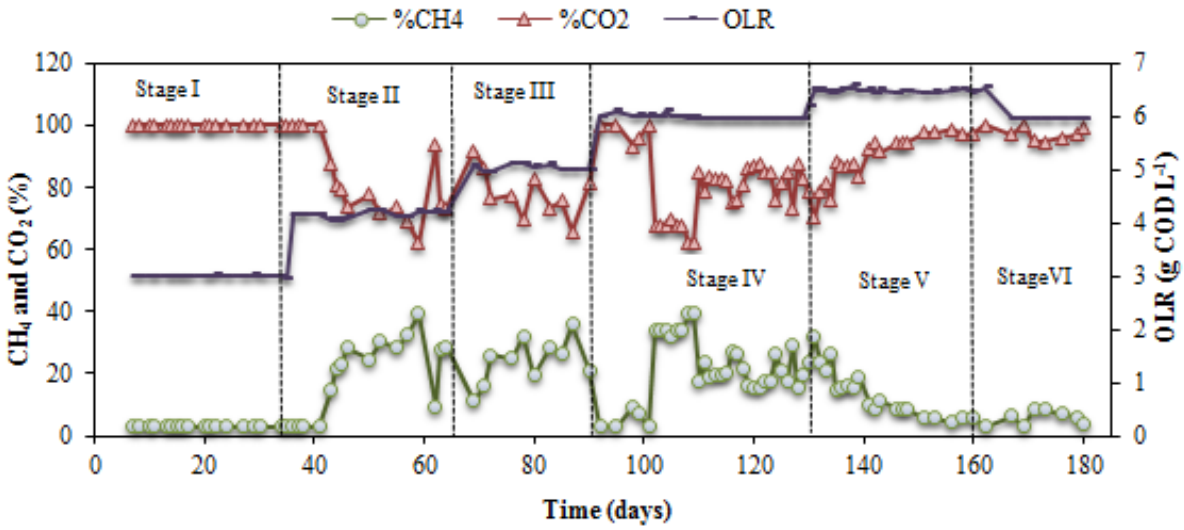

Figure 7-5: Evolution of CH_4 and CO_2 (%) in the biogas with different OLR and alkalinity applied to the CSTR system as a function of time.

Based on data results, it can be concluded that the percentage of methane obtained in this study provide evidence that at the operational conditions of OLR and alkalinity tested, the acidogenic process is technically feasible and efficient.

7.2.4 Effect of the pair OLR and Alkalinity applied for the stability of the acidogenic fermentation process

In order to verify the feasibility of the acidogenic fermentation of OFMSW using a CSTR system, for the operational conditions tested, several parameters regarding VFA production (TVFA and odd-to-even VFA ratio) as function of OLR (Fig. 7-6) and process performance (DA, VFA/sCOD in the effluent and TVFA/Alk ratio) as function of OLR (Fig. 7-7 and 7-8) will be analyzed.

As can be seen in Figure 7-6 the TVFA concentration increased linearly until an OLR up to 6 g COD $L^{-1}d^{-1}$ (from 1.78 ± 0.32 to 7.52 ± 0.14 g COD L^{-1}), and then decreased with the increased of OLR (6.5 g COD L^{-1} d^{-1}) to 5.73 ± 0.12 g COD L^{-1}. It also was observed that the Odd-to-Even ratio of the effluents reduced linearly as the OLR increased from the 5.0 to 6.5 g COD L^{-1} d^{-1}. Several researches have pointed the effect on the quality of effluent under high OLR (Ren et al., 2006, Silva et al., 2013). In addition, in these stages, H-Ac, H-Pr and

*n*H-Bu were the main species of VFA (see Fig.7-3), thus contributing to the deceases and low odd-to-even ratios of VFA (0.55 – 0.32).

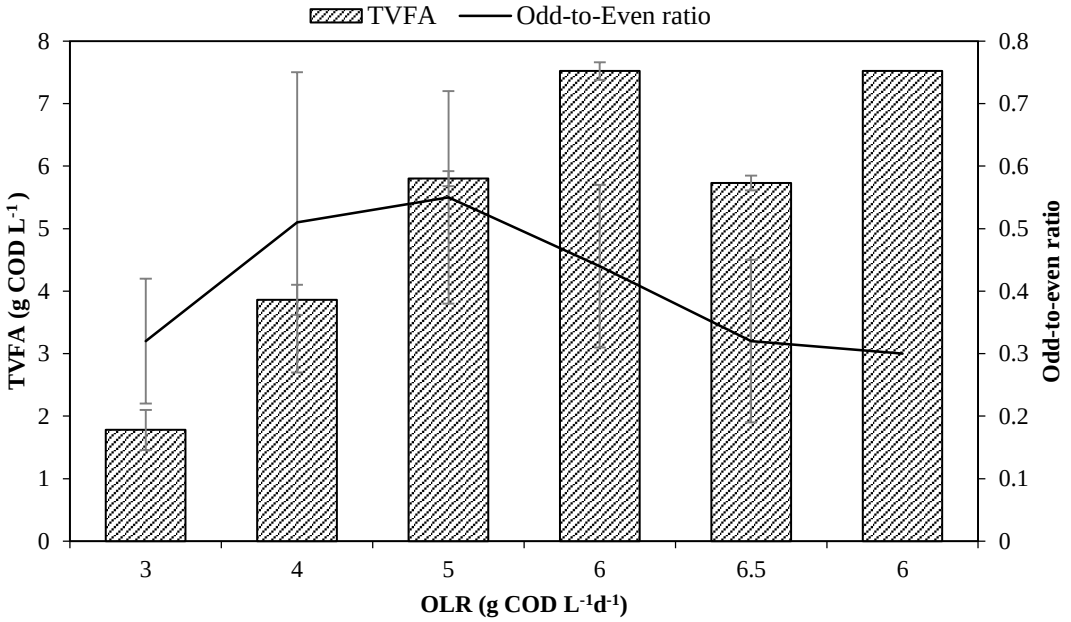

Figure 7-6: Evaluation of TVFA production and Odd-to-Even ratio at different OLR applied. Error bars indicate standard deviation.

The ratio between TVFA produced and alkalinity during all experimental period are represented in Figure 7-7, and is used in this research as a reliable parameter for monitoring anaerobic digestion imbalance.

In this study, the average VFA/Alk at the end of each acidification stage was far more than 0.4, which resulted in the inhibition of the methanogenic activity during most of the experiment.

The VFA/Alk ratios in the first 35 days were lower than 0.4, which is considered a favorable condition for methanogenic activity, although is this study the content of methane in the biogas was negligible, indicating a long period for microbial adaption as also referred by other authors, that this fermentation period was low (Dong et al., 2009). After 35th day, with the increase of the OLR, VFA/Alk ratios raised to very high values, presenting a sharply increase at day 128, reaching values around 5, which shows a good performance and adequate operational values for OLR and Alk (up to Stage IV) to maintain a stable acidification process. The VFA/Alk ratios in stages II, III, IV, V and VI varied from 1-2, 2-3, 3-5, 2-3 and 2-3, respectively. Although all periods had favorable conditions for

predominance of hydrolysis/acidification process, presenting VFA/Alk much higher than 1, other parameters should also be analyzed.

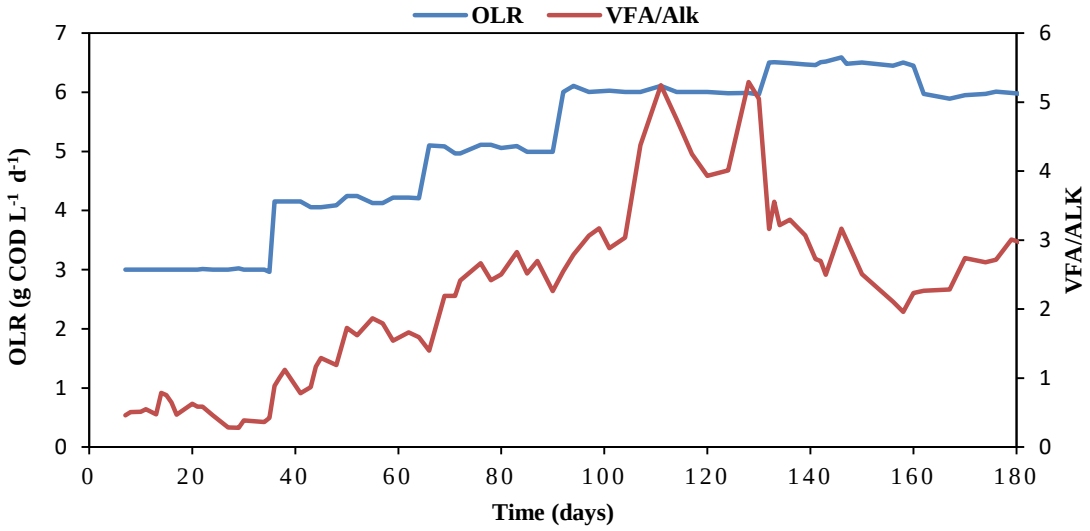

Figure 7-7: Evaluation of OLR and TVF/Alk ratio to the CSTR system as a function of time.

Following the Figure 7-8, it is clear that DA and VFA/sCOD increased gradually under different OLR studied. The total percentage of DA and VFA/sCOD reached the peak value when the OLR was 6.0 g COD L^{-1} d^{-1}. The critical performance of CSTR reactor was achieved at the maximum OLR addition (6.5 g COD L^{-1} d^{-1}). During this period, the percentage of DA and VFA/sCOD decreased from 58.9 % to 36.9 % and from 65.5 % to 53.07 % respectively.

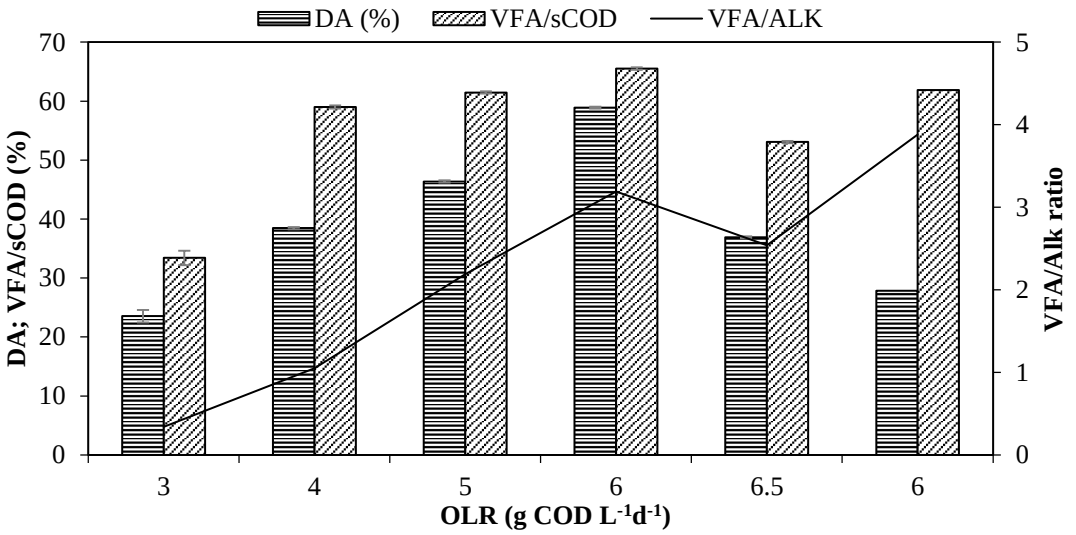

Figure 7-8: Comparative between DA, VFA/sCOD in percentage and evaluation of VFA/Alk ratio according to different OLR applied.

It also observed that, the VFA/Alk ratio presented a notable decrease from 3.19 at OLR 6.5 g COD $L^{-1}d^{-1}$ to 2.5 at maximum OLR (6.5 g COD $L^{-1}d^{-1}$). A rapid increases of VFA/Alk ratio was observed when OLR decreased again to 6.0 g COD $L^{-1}d^{-1}$. The evolution of the parameter shows clearly the effective coupling of the hydrolysis and acidogenesis stages at the OLR increases.

Based on Fig.7-8 and the data presented in Table 7-1, it can be concluded that the CSTR digester worked with a good performance indicated by a long-term stability in acidification process and VFA production with a high buffering capacity. These results again confirmed that the conditions studied were optimal for performance and reactor stability.

7.3 Conclusions

The acidogenic fermentation of OFMSW was studied in a CSTR system, operated in a semi-continuous mode, during approximately 180 days, at six operational conditions including different organic loads applied (3.0 – 6.5 g COD L^{-1}) and different alkalinities (2.0-5.0 g $CaCO_3$ L^{-1}). To evaluate the reactor performance at different operational conditions, it was studied the VFA production, the process efficiency in terms of DA, and the composition of VFA species in the acidified effluent.

The maximum VFA production (7.5 gVFA-COD L^{-1}) was obtained under one of the highest OLR of 6.0 g COD L^{-1} d^{-1} and average Alk of 2.5 g $CaCO_3$ L^{-1}. This condition resulted also in the highest acidification degree (58.9%) and the highest quality of the treated effluent in terms of VFA (65.5%), although with a lower odd-to-even VFA ratio (0.44).

With respect to the acidified effluent composition, the operational condition with an average OLR (5.0 g COD L^{-1} d^{-1}) and an average alkalinity (2.5 g $CaCO_3$ L^{-1}) resulted in the highest odd-to-even VFA ratio (0.55), which is relevant parameter for the waste valorization into PHA with high levels of HV. This condition resulted in a lower TVFA production (5.8 gVFA-COD L^{-1}), a lower acidification degree (46.4%) and a lower quality of the treated effluent in terms of VFA (61.5%).

The pair OLR–Alk is the main parameter, which affects the production, composition and profile of VFA species present in the acidified effluents. The increase on the OLR caused the increase of the VFA production, with H-Ac, H-Pr., *n*H-Bu. and *n*H-Va acids being these

species the predominant in all experiment stages. The relative major predominant species during all experiment were H-Ac acid and nH-Bu acid, with a similar contribution to the TVFA of 25-45%. H-Pr contributed with 15-20% to the VFA mixture and *n*H-Va contributed with a lower amount of 10-15%.

The optimum operating condition for further use of the VFA produced in the CSTR system for PHA production from OFMSW was obtained at an OLR of 6.0 g COD L^{-1} d^{-1} and an alkalinity of 2.5 g $CaCO_3$ L^{-1}.

8. Polyhydroxyalkanoates (PHA) production from acidified OFMSW

8.1 Introduction

The work performed in previous chapter has the aim to study the acidogenic fermentation of OFMSW in a CSTR system, in order to obtain the maximum conversion of the organic matter present in that waste into volatile fatty acids (VFA). In the present chapter, fermented effluents rich in VFA produced under CSTR – acidogenic fermentation (studied in chapter 7) were used as carbon source for microbial PHA production. Thus, SBR reactors were used for the selection and enrichment of PHA accumulating mixed microbial cultures. Details for reactors operating conditions used, were described in section 3.3.3 in Chapter 3.

The SBR reactor was conducted under aerobic dynamic feeding (ADF) process, alternating a period of feast (condition of external substrate excess) with a period of famine (absence of substrate). Hence, the SBR was operated in a cyclic way (F:F) under fully aerobic conditions in order to require the microorganisms to adapt the imposed conditions, either increasing the growth yield or accumulating carbon and energy reserves intracellularly under nutrient-limiting conditions (Dircks et al., 2001). The exposition of transient carbon supply is much used in several studies of mixed cultures in order to maximize the PHA production at low cost (Jiang et al., 2012; Keller et al., 2015; Korkakaki et al., 2016), thus, this strategy are regarded as alternatives to pure cultures, being capable to produce PHAs from renewable resources and without the need for sterile conditions. According to Albuquerque (2009), these systems allows also to obtain microorganisms with high PHA production capacity and storage, and can reach more than 80% of the intracellular polymer content. Furthermore, mixed microbial culture (MMC) process can be used for permanent selection and maintenance of a culture with the highest productivity of PHAs synthesis (Ivanov et al., 2015). In addition, the use of pure cultures has a very high cost due to large demands for sterility and higher requirements for the equipment and control devices in comparison with MMC approaches (Bengtsson et al., 2010).

A remarkable advantage of the use of MMC is its high ability to easily adaptat to complex substrates, including olive oil effluents (Dionisi et al., 2005), food waste (Rhu et al., 2003) and sugar cane molasses (Albuquerque et al., 2007). The production of PHA is versatile and of high socio-economic importance, being the biopolymers with high potential to replace the conventional plastics in the future (Możejko-Ciesielska and Kiewisz, 2016). Thus, studies must be made on the production of biopolymers, in order to reduce the costs of the raw

materials by using sources of low-cost and easy carbon, besides non-harmful isolation and purification of PHA polymers.

The aim of the study in the chapter, was at first conducting mixed microbial culture selection with a fermented OFMSW rich in VFA mixture, in order to evaluate the maximum PHA storing capacity of the enriched culture. Secondly, it was used the fermented OFMSW and the biomass from the SBR culture enrichment assays for batch PHA accumulation, in order to evaluate the accumulation capacity and the profiles on the final PHA accumulation stage. The detection of PHA inside the biomass using Nile Blue staining was also observed.

8.2 Results and Discussion

8.2.1 Enrichment of PHA accumulating microorganisms with fermented OFMSW

The basic setup and operation of the reactors for the enrichment in PHA-accumulating microorganisms followed the SBR strategy described by (Albuquerque et al., 2007). Three reactors (SBR_1, SBR_2 and SBR_3) were operated in different operational conditions using fermented-OFMSW rich in VFA as carbon source. Reactor SBR_2 was sequentially operated in the SBR_1.

Table 1 shown the main characteristics of the fermented OFMSW used as carbon source in the current work. The carbon source that was used contained significant concentrations of VFA, having an amount of H-Ac (2.3 g COD L^{-1}), H-Pr. (1.8 g COD L^{-1}) and H-Bu. acid (2.7 g COD L^{-1}) favorable for the production of PHA with more interesting mechanical properties (higher content in HV monomer) (Dionisi et al., 2005). The effect of fermented OFMSW feeding on the establishment of the "feast and famine" conditions in the SBRs was investigated at three different OLR (1.4, 1.7 and 3.0 g COD L^{-1} d^{-1}) with the same cycle length of 24 h. During the experimental assays, reactors SBR_1, SBR_2 and SBR_3 were operated under the same conditions (pH, HRT and SRT) and different condictions with respect to length of the famine time in comparison with the length of the feast time (F:F ratio) and the C:N ratio (see sub-chapter 3.3.3). The selection trend was monitored by determining the duration of both feast and famine phases achieved, by using the dissolved oxygen (DO) concentration in the selection media. The objective of different operational condition was to determine the impact of reactor operation mode on the selection and

enrichment PHA-accumulation culture with the highest PHA accumulation capacity to be used in further fed-batch assays for PHA accumulation.

Table 8-1: Composition of the fermented OFMSW used as carbon source

Parameters	Units	Concentration
TVFA	gCOD L^{-1}	7.5 ± 5
Acetic	gCOD L^{-1}	2.3
Propionic	gCOD L^{-1}	1.8
Butyric	gCOD L^{-1}	2.7
Valeric	gCOD L^{-1}	0.5
Caproic	gCOD L^{-1}	0.2
VFA/sCOD	(%)	65.5 ± 5.0
TKN	gN L^{-1}	0.9 ± 2.4
pH	---	4.5 – 6.5 ± 0.1
C:N ratios	---	16 – 18.9 ± 0.2

Table 8-2 presents the main results obtained for reactors SBR_1, SBR_2 and SBR_3 at end of PHA-accumulation operational time by the mixed culture. It can be observed that, in the reactors SBR_1 and SBR_3, the feast to famine (F:F) time ratio (0.09 – 0.17 h h^{-1}) is considered within the adequate range (equal or less than 0.33 h h^{-1}), indicating that a positive potential for PHAs-accumulating bacteria has been occurred (Valentino et al., 2014).

Table 8-2: Reactor performance under different enrichments conditions.

Assays	Maximum COD $_{removal}$ (%)	F:F Ratio (h h^{-1})	PHA content (%)	VSS $_{final}$ (g L^{-1})	$-r_{VFA}$ Total	$Y_{PHA/VFA}$	$-q_{VFA}$	q_{PHA}
SBR_1	86 ± 0.2	0.09	53.1	2.1 ± 0.5	6.86	0.07	0.05	0.009
SBR_2	80 ± 0.1	0.82	17.2	7.4 ± 0.4	--	--	--	--
SBR_3	87 ± 0.1	0.17	35.2	3.3 ± 0.4	9.22	0.04	0.09	0.08

n.d - not determined.
PHA – (mol PHA. mol $^{-1}$.VSS)
VFA – (Cmmol VFA L^{-1}).
$Y_{PHA/VFA}$– (Cmol PHA. $Cmol^{-1}$VFA).
-q_{VFA} - (Cmol VFA. C mol^{-1} $VSS_{initial}$ h^{-1}).
q_{PHA} - (Cmol PHA- C mol^{-1} $VSS_{initial}$ h^{-1}).

According to Serafim et al. (2008), the F:F ratio is the key factor for PHA production by mixed microbial cultures (MMC), which comes from the internal growth limitation caused by transient substrate availability. Fewer enzymes are required for PHA accumulation and, therefore, PHA storage can occur at a faster rate, providing the cells with a mean of rapidly consuming the available substrate. After that external carbon is depleted, the internal PHA storage serve as energy source for cell growth and maintenance. Dionisi et al. (2006), also

reported that the optimal F:F ratio for MMC should be less than 0.25 $h.h^{-1}$, in order to avoid the microorganisms selection with rapid growth.

However, contrary to reactors SBR_1 and SBR_3, reactor SBR_2 presents a very high F:F ratio (0.82 h h^{-1}), which is far from been within the range to be considered adequate to keep the culture well selected in terms of PHA accumulating bacteria. Indeed, in reactor SBR_3 it was observed high increase of the biomass inside the reactor, suggesting that at the high OLR applied, the system was controlled by the cell growth phase instead of PHA accumulation phase. Consequently, the PHA storage capacity, measured as PHA content, was much lower in SBR_2 (17.2 %) in comparison to SBR_1 (53.1 %) and SBR_3 (35.2%), showing that feast to famine (F:F) conditions were not established, causing favorable conditions for cell growth (substrate depletion) and not to efficiently select PHA-accumulating organisms.

As can be seen in Table 8-2, in this study, the maximum COD removal over the process of enrichment of PHA accumulating microorganisms varied between 80% and 87%, demonstrating also a good performance of this culture for the removal of the pollutant load from a complex wastewater (acidified OFMSW). Different PHA accumulations in the SBR assays were obtained, indicating that, most of substrate depletion was used for PHA production in the case of reactors SBR_1 (53.1%) and SBR_3 (35.2 %), whereas in reactor SBR_2 most of the substrate was consumed during all experiment for cell growth, obtaining just 17.2% of PHA content.

Figure 8-1(a and b) show the performance of the SBR reactors under study as a function of the applied OLR. During the experiments, all SBR reactors were exposed to transient availability of substrate (feast and famine) repetitively in the dynamic aerobic feeding (DAF) process. The main parameters evaluated are the COD output (COD_{out}), VSS (biomass), COD removal (%) and the maximum level of stored PHA in the biomass content (in % PHA) at the end of feast phase.

In general, all three SBR reactors reached high COD removal, above 80% showing, that under the point of view of OFMSW treatment, this process is favorable (Fig. 8-1 and Table 8.2). Although this process resulted in a high COD removal efficiency at all OLRs studied, it could, eventually affect the selection and enrichment of PHA-storing microorganisms and, also the performance of the PHA accumulation process in a later phase (Campanari et al., 2014). It can be seen in the Fig 8-1a), that the volatile suspended solid (VSS) concentration

in SBR_1 reactor was maintained almost constant, varying from 1.5 ± 0.1 in the beginning to 2.1 ± 0.5 g VSS L^{-1} at day 70th of experimental period, presenting a stable behavior, especially from day 45, when it was reached the maximum removal COD (85%). In this stable period, samples were removed at the end of the feast phase, in order to quantify the PHA-content, in the biomass, which achieved a maximum of 53 % PHA-content with a molar composition HB:HV = 83:17. The transition of the feast to the famine phase was identified from the dissolved oxygen (OD) profile, which showed a decrease in the respiration rate upon VFA depletion.

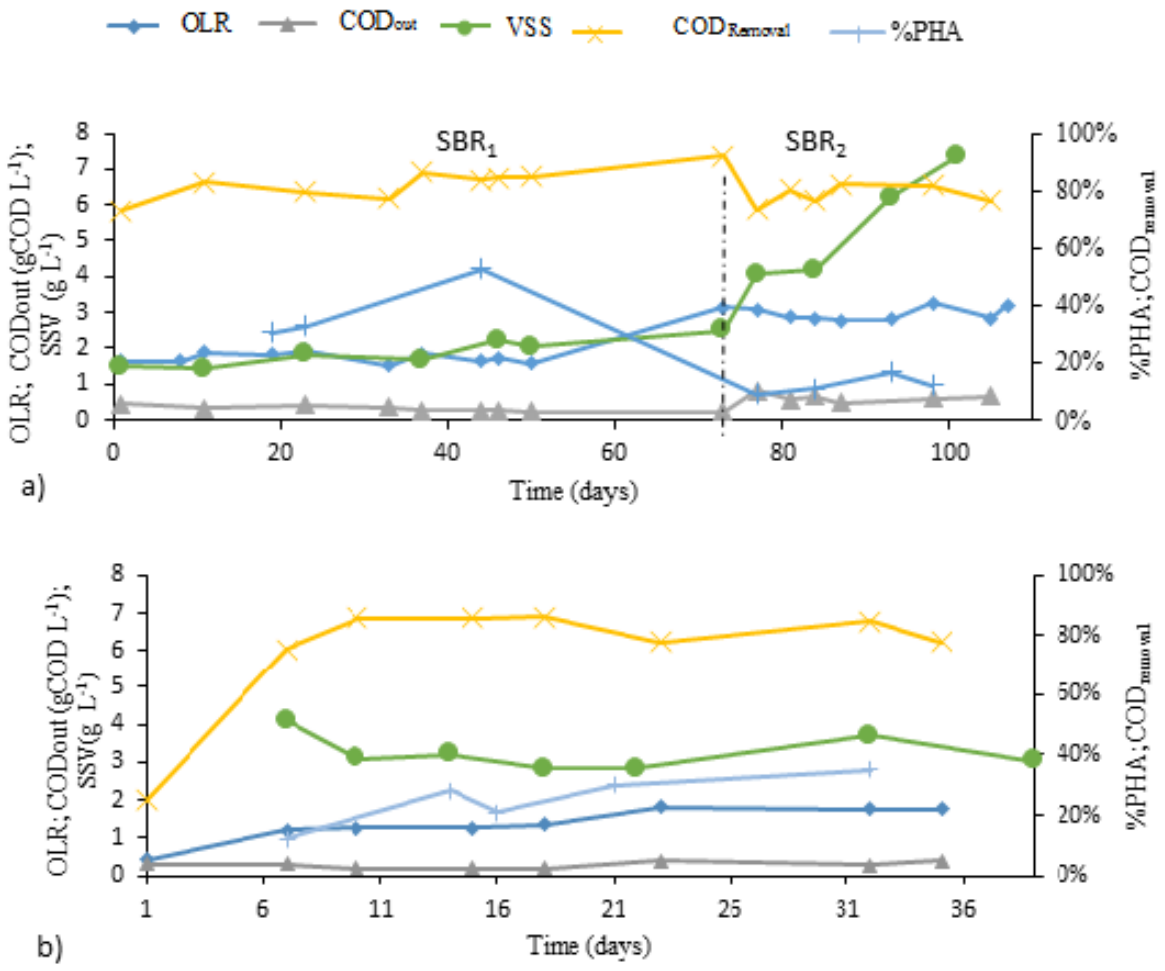

Figure 8-1: Reactor performance over time (variation of OLR, SSV, COD removal and PHA content in the biomass for a) SBR_1 and SBR_2; b) SBR_3

When the OLR was increased from 1.5 to 3.0 g COD L^{-1} d^{-1} (SBR_2), the VSS concentration was linearly increased from 2.1 ± 0.5 g VSS L^{-1} up to around 7.4 ± 0.4 g VSS L^{-1}, most

showing a possible inhibitory effect on microbial ground, especially to the microorganisms with PHA storage capacity. The increase on the OLR significantly affected the F:F ratio which became very high (0.8 h h^{-1}), resulting in the lowest PHA-content in the biomass (17.2 % in mol PHA mol $^{-1}$VSS). A similar trend has been obtained by Dionisi et al. (2006) using different concentrations for the SBR feedstock. The same author verified that an extreme increase on the OLR from 8.5–31.25 g CDO L^{-1} d^{-1}, caused excessive increase in the biomass growth, which affected the normal operation of the reactor, with a biomass formation of aggregates with low sedimentation characteristics (probable excessive production of EPS, extracellular polymeric substances). Beccari et al. (2009) explained this phenomenon based on the different compositions of substrates fed, which probably induced the occurrence of different substrate removal mechanisms. Due to both the low PHA yield obtained in reactor SBR_2 and the high VSS concentration inside the bioreactor, the operation of this reactor became inoperable and it was decided to use the same OLR applied to SBR, for the reactor SBR_3 assays.

Figure 8-1(b) shows the performance of reactor SBR_3 over time. In terms of PHA production, it can be seen that, the during operation time of the reactor, the maximum level of biopolymer accumulated in the feast phase increased from 12% in the beginning up to 35% at day 32th of the experiment, lower than the value obtained in reactor SBR_1. Nevertheless, COD removal in SBR_3 was the highest of the three reactors (87%) (see also Table 8.2), showing also a good performance of the enriched mixed cultures selected for the removal of the pollution load. As it can be seen in Fig 8-1 b), the VSS concentration decreased in the beginning of the experiment until day 21, followed by a linear increase up to day 32 and a small decrease until the end of the experiment, probably due to the adaption of the culture to the new conditions. With respect to PHA production, it was observed a direct relationship with the VSS concentration, that is, an increase on the PHA production with the end of the adaptation period. According to the results obtained in this study, it can be conclude that, the OLR applied for SBR_1 and SBR_3 resulted in a good reactor performance, promoting the enrichment of PHA-accumulating organisms in the reactor content.

Figure 8-2 illustrates the total organic carbon (TOC) consumption, TN and VFA uptake and dissolved oxygen (DO) profile during the feast and famine phases. The transition of feast to the famine phase can be identified by DO profiles, whose concentration profile is indicative

of the rate of the microbial activity, showing a rapid decrease in the respiration rate upon VFA depletion. During this period, the specific oxygen uptake rate was determined, as well as PHA content and COD removal (Table 8-2). Thus, it is possible to identify, though the DO profiles in Fig 8-2, two different phases. Phase I, where it is observed a decrease of the DO concentration to very low values, indicating a fast depletion on the carbon consumed with a rapid decrease in the respiration rate due to VFA depletion (feast phase or first part of the cycle), and phase II, where DO upsurges to close to 100% (final part of the cycle). In phase II, is started with the exhaustion of the carbon source, leading to a sharp increase in the DO concentration (famine phase). In the feast phase, the DO for reactors SBR_1 and SBR_3 after substrate addition was very low, 3.4 % and 11 % respectively, indicating that the DO was used as an electron acceptor in the degradation of the organic matter. TOC and VFA profiles also confirmed this trend (Fig. 8-2).

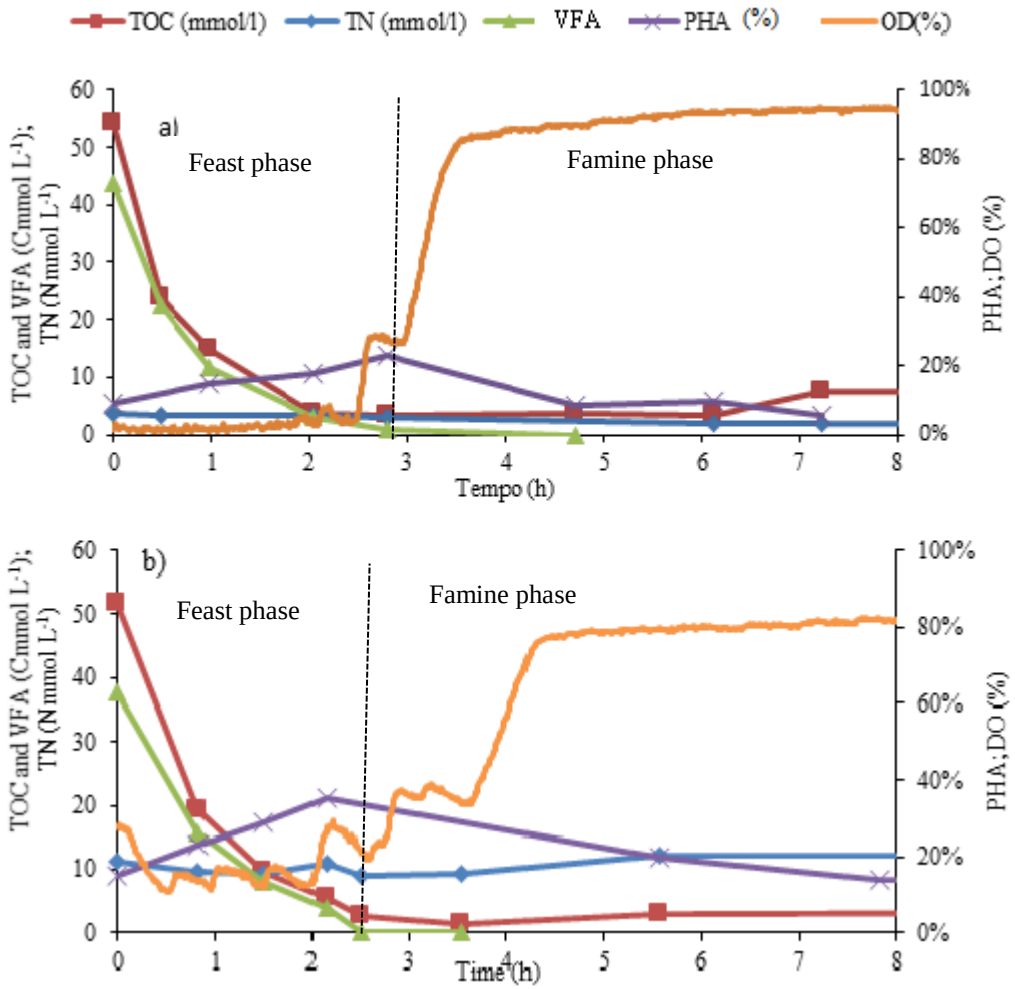

Figure 8-2: Laboratory-scale experimental results in a cycle performed in SBR culture enrichment assays (TOC, VFA, TN, PHA and OD); a): SBR_1; b) SBR_3. The vertical dash line represents the changeover from the feast phase to the famine phase.

During an operational cycle, there was a rapid consumption of the substrate of more than 90% removal of the TOC present at the beginning of the cycle, being 93.2 and 97.1 for SBR_1 and SBR_3 respectively, at the end of the feast phase. It was observed that VFA were available only for 2.5-3 h in the 24 h cyclic operation of the enrichment reactor. VFA were the dominant organic substrate in fermented OFMSW (65.5% of the sCOD was VFA, Table 8.1) and the preferred carbon substrate for PHA production. In the reactor SBR_1 (Fig. 8-3a) VFA were fully consumed at 3:00 h and in reactor SBR_3 (Fig. 8-3b) at 2h and 30 minutes.

When the VFA were almost completely consumed, the PHA concentration it reached its peak value (1.11 g L^{-1} and 1.16 g L^{-1} for SBR_1 and SBR_3, respectively) at the end of the feast phase (see Fig. 8-2), confirming that VFA is the main substrate for PHA production. After the depletion of VFA, other organic substrates were still present in the enrichment reactor, as indicated by the residual TOC concentration. In addition, during the famine phase, removal of TOC (Fig.8-1) occurred, which supported the competitive growth of non PHA-storing microorganisms present in the culture (Campanari et al., 2014). Nevertheless, the rate of TOC uptake was low in the famine phase, implying that the culture had to endure a long period of famine once VFA were no longer available in the enrichment reactor.

During feast phase, the consumption rate of the organic carbon in terms of TOC was 8.3 and 14.3 Cmmol L^{-1} h^{-1} for SBR_1 and SBR_2, respectively (Table 8-3). The profiles of VFA and PHA (Fig. 8-2) in the enrichment SBR reactor were in agreement with the metabolic behavior of the PHA-accumulating organisms. Simultaneous uptake of VFA and storage of PHA was observed in the feast phase. Meanwhile, in famine phase, consumption of PHA was observed after the depletion of VFA. In conclusion, these observations confirm that ADF process is capable of promoting the enrichment of PHA-accumulating organisms in the mixed culture (MMC).

Additionally, it can also be seen from Fig. 8-2, an increase on the TOC value after the end of the feast phase (7 h in SBR_1 and 5h 30 in SBR_3), while in reactor SBR_1 the feast phase was over at 3 h and in reactor SBR_3 at about 2:30 h of cycle length. The increase on the TOC concentration can be attributed to the presence of particulate organic remaining in the reactor at that time. This maybe possible due to presence of microorganisms which do not have the ability to accumulate intercellular polymer, but can of the complex organic matter of the

feedstock, causing an increase in the organic carbon of the dissolved phase (Campanari et al., 2014).

At the end of the ADF process applied to SBR_1 and SBR_3, residual organic matter and nitrogen were still present, 93-97% of TOC removal and 57-21% of TN removal, becoming available for the following cycle (Albuquerque et al., 2010). Silva et al. (2016) have also reported similar trends.

Table 8-3: TOC and TN consumption during the feast phase.

Assay	TOC consumed (% C. mol)	-rTOC (Cmmol L^{-1} h^{-1})	TN consumed (% Nmol)	-rTN (Nmmol $L^{1}h^{-1}$)
SBR_1	93.2	8.3	57.0	0.19
SBR_3	97.1	14.3	21.2	0.96

8.2.2 Effect of the C:N ratio in the performance of the SBR enrichment

The SBR_1 and SBR_3 were run with similar OLR, 1.7 and 1.4 g COD L^{-1} d^{-1} but different C:N ratio of 14 and 5 (Cmol $Nmol^{-1}$), respectively. Fig. 8-3 shows the profile of the TOC and TN concentration measured since the beginning the feast phase until the end of the famine phase. C:N ratio is an important control parameter for MMC, because it modifies the microorganisms growth conditions during the operation time. Hence, the nitrogen is a key factor for microbial growth. The absence of nitrogen can cause the use of the main substrate by microorganisms for polymer storage in detriment of the growth of microorganisms (Johnson et al., 2010).

Through Fig. 8-3 it can be seen that, there was a greater organic carbon removal than nitrogen, either as rate of consumption or amount consumed (Table 8-3). As it can also be verified (Table 8-3), there was nitrogen consumption in all SBR assays, particularly in the feast phase (57.0 % for SBR_1 and 21.2% in SBR_3).

Comparing the DO profiles (Fig. 8-2 a and b) with different C:N ratios, it can also be observed similar trends for SBR_1 and SBR_3, where an increase in the oxygen concentration coincides with the VFA exhaustion (end of feast phase). However, at SBR_1 (C:N ratio of 14.3 Cmol $Nmol^{-1}$) the DO concentration stayed very low during most of the feast phase, whereas in SBR_3 (with 5.2 Cmol $Nmol^{-1}$of C:N ratio) it stayed in higher values (around 15 % of saturation), showing that, SBR_1 required a longer time for substrate depletion. The TN

removal for SBR_1 was 57 % and 21 % for SBR_3 (Table 8-3), which corresponded to similar amounts of nitrogen depletion.

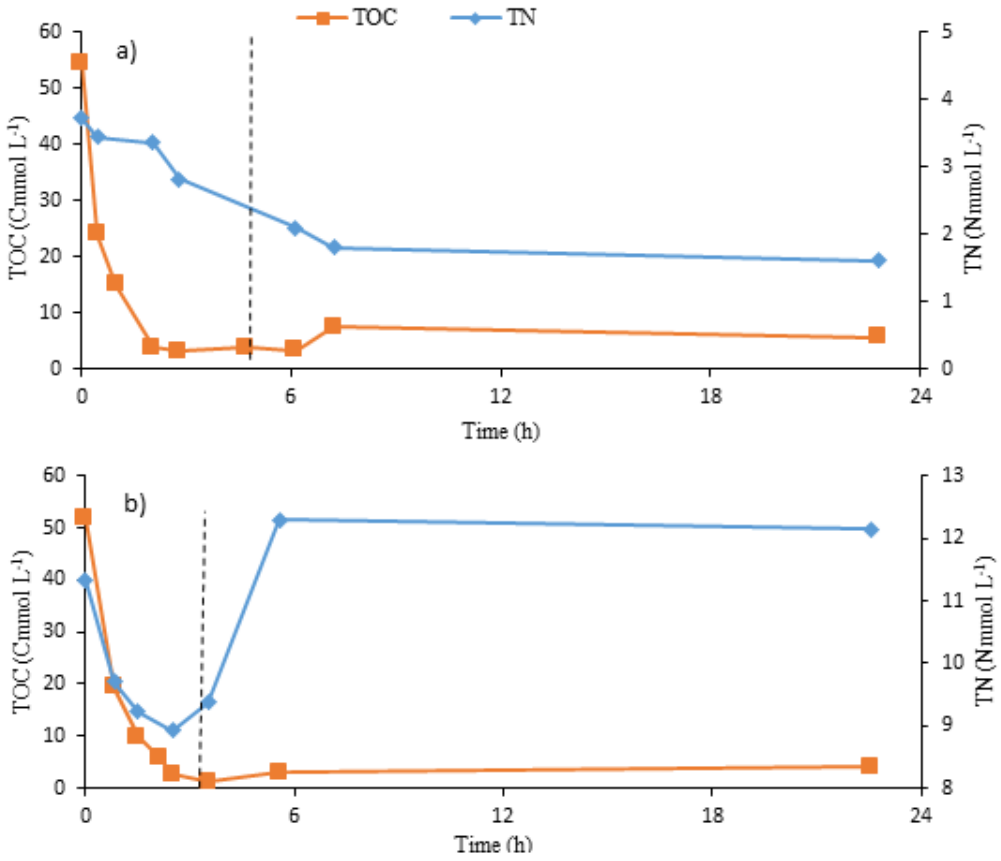

Figure 8-3: Profiles of TOC (Cmmol L^{-1}) and TN (Nmmol L^{-1}) consumption trend) during the SBR assays, a) SBR_1; b) SBR_3. The vertical dash line represents the changeover from the feast phase to the famine phase.

Conversely, in the SBR_1 the TN was not completely depleted by the end of the feast cycle, indicating the occurrence of non-limiting nutrient conditions. Similar phenomenon was observed and described by Albuquerque et al. (2009).

Through these results, it can be conclude that the two different C:N ratio applied not have a determining role in the storage capacity of the culture, probably because in both SBRs there was an excess of nitrogen. Nevertheless, it was observed some trend in the storage capacity as a function of the decrease in the C:N ratio. Thus, a higher PHA production (53.3 % storage capacity) was observed at the highest C:N ratio (reactor SBR_1), although with a lower specific VFA consumption rate (q_{VFA} of 0.05 Cmmol VFA Cmmol^{-1} VSS h^{-1}), suggesting that the substrate degrades slowly not all stored as intracellular polymer (Dionisi et al., 2006). In addition, the specific PHA production rate (q_{PHA}) showed a similar trend, being

negligible at the highest investigated C:N ratio. These results can also be explained due to a high viscosity of the mixed liquor (composed by acidified OFMSW) resulting from microbial activity inhibition (Zhao et al., 2013). In this study, the different PHA production capacity resulted from the SBR operation could be influenced by other parameters, particularly the VFA content, in a slightly higher extension than the C:N ratio addition. Based on the results obtained with the two SBR reactors operated after 70 days (SBR_1) and 35 days (SBR_3), it can conclude that SBR_3 had a better performance than SBR_1 in terms of either PHA concentration (1.16 g PHA L^{-1}) inside the reactor (Table 8-2) and specific PHA production rate (0.08 Cmmol PHA $Cmmol^{-1}$ VSS h^{-1}). Thus, the biomass rich in PHA from SBR_3 was collected and reserved for the batch PHA accumulation assays.

8.2.3 Microscopic observations

At the end of test SBR assays for the enrichment of PHA-accumulating organisms, samples from reactor SBR_3 were collected and examined under microscope, using fluorescence. This technique can give valuable information on biological processes and bacteria characterization and also Gram identification (Jenkins et al., 2003). Nile-blue is among one of the most used dyes for selective staining of PHA inclusions from MMC and is as valuable methodologies to predict intracellular PHA (Serafim et al., 2002). In this study, Nile-blue solution was prepared by dissolving 5mg of Nile blue in 50 mL of ethanol in according to procedure handbook. Figure 8-4 shows microscopic images with and without florescence of the mixed liquor from the enrichment reactor SBR_3.of PHA-accumulating organisms As depicted in the Fig. 8-4 (b), it can be observed granules stored inside the microrganisms present in the samples.

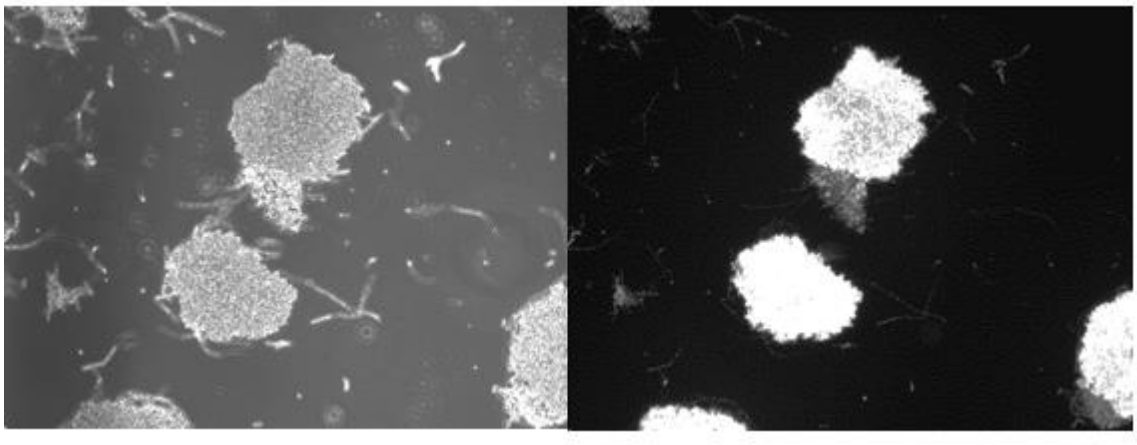

Figure 8-4: Microscopy images of the biomass taken from the cultivation reactor of PHA-accumulating organisms: a) Phase contrast images and b) with Nile-blue fluorescence.

8.2.4 PHA accumulation in batch experiments with acidified OFMSW

Once the culture was enriched with PHA-producing biomass, the biomass can still be used in the accumulation step for PHA production, in order to evaluate the maximum PHA storing capacity of the selected culture produced with the reactor SBR_3, using the same substrate (effluent rich-VFA) from CSTR reactor as carbon source.

PHA assay was operated at the same aeration rate in the reactor SBR with a working volume of 400 mL. During the batch test, the parameters such as pH, VFA and PHA content were monitored. The effect of pH operation parameter is studied due to it has great influence on the production for PHA (Chua et al., 2003). In this study, three pH condition were studied; pH 7.3, pH at 8.0 (uncontrolled) and pH 8.0. The fed-batch test from PHA production was explored in an aerobic condition for 5 h at ambient temperature of 20 – 25 º C, in order to evaluate the maximum PHA accumulating capacity. The polymer content in function of the VFA consumed was also studied. The samples of VFA and PHA were taken during the operation time of the fed-bath and the biomass was converted Cmmol L^{-1}. The main results parameters characterized under fed-batch accumulation assays is depicted in Table 8.4.

Table 8-4: Performance of batch PHA production under various pH conditions.

Batch test	Biomass	pH	Final. PHA (%)	Final PHA concentration	PHA composition (% HB: % HV)	$Y_{VFA/PHA}$	q_{PHA}	$-q_{VFA}$
A1	SBR_3	7.0 - 7.3	25.3	0.83	78:22	0.22	0.24	0.88
A2	SBR_3	in.8.0 [a]	9.0	0.20	85:15	0.21	0.18	0.11
A3	SBR_3	8.0	23.1	0.53	80:20	1.32	0.44	0.06

[a] without pH control
PHA – (Cmol PHA. Cmol $^{-1}$.VSS)
VFA – (Cmmol VFA L^{-1}).
$Y_{PHA/VFA}$– (Cmol PHA. $Cmol^{-1}$VFA).
$-q_{VFA}$ - (Cmol VFA. C mol^{-1} VSS h^{-1}).
q_{PHA} - (Cmol PHA- C mol^{-1} VSS h^{-1}).

As reported in Table 8-4, the maximum PHA at the end of the fed-batch test was equal to 25.3 % correspondent at assays A1 with controlled pH between 7.0 to 7.3, and it obtained high yield PHA over VFA, showing higher microbial activity, as indicated in the -qVFA value (equal to 0.88 Cmol PHA $Cmol^{-1}$VFA). This results is according was obtained by Mohan and Reddy (2013) reporting that a neutral pH could lead to higher PHA accumulation

as compared to pH 6.0. In the assays A2 without pH control, but the initial biomass was acclimatized at pH 8.0, similar at the SBR_3 reactor PHA-enriched, with minimum pH achieved at end of assays was 6.3 resulting a low maximum PHA content (9.0 %), due to a low microbial activity at acidic pH can be toxic effect of non-dissociated VFA used on the PHA-accumulating organisms (Fleit, 1995), due to a non-dissociated VFA could penetrate the cell membrane, enter the in the cell and dissociate to form H^+.

When the pH was controlled (in fed-batch A3), the PHA content increase from 9.0 % to 23.1 %. The PHA production obtained in the fed-batch assays was low than that obtained in SBR tests. The higher PHA content obtained in SBR, PHA-accumulation could be due to the effective establishment of alternate feast and famine conditions. Thus, the alternating feast and famine conditions that help in enriching PHA producing organisms (Dai et al., 2014).

Compared these results achieved in this work, it can conclude that at pH between 7 to 7.3 was more preferred from microorganism to PHA production than pH 8.0. However, in term from q_{PHA} achieved at controlled pH 8 .0 (A3) was higher than that at pH 7.3 (Table 8-4). Thus, in this study, the pH condition affects the concentration of PHA. As can be seen in the Table 8-4, the fraction of HB and HV was similar in the three fed-batch tests. The high fraction of HB obtained in this study was probably due to the greater availability and consumption of H-Ac, and H-Bu acids as compared to H-Pr and H-Va acids (Table 8-1) during the test of PHA production. It is known that H-Ac and H-Bu acids favorited the synthesis of HB while H-Pr and H-Va acids promote the formation of HV (Lee et al., 2014; Gameiro et al., 2016).

Figure 8-5 (A1 – A3) shows the concentration profile of VFA and PHA in the 5-h of PHA accumulation fed-batch assay. As can be seen in Fig. 8-5, simultaneous uptake of VFA and storage of PHA was observed, i.e, the PHA concentration increased concomitantly with the consumption of VFA and were in agreement with the metabolic behavior of the PHA-accumulating organisms. All the batch assays, the peak intracellular PHA content was observed at the end of total depletion of VFA. In the fed-batch A1 (with pH control), the peak intracellular PHA was observed at 2-h and 30 min at the end of the feast phase and it was 25.3 %. In comparison between A2 and A3, it can be observed in A2 (without pH control), microorganisms need more time to use VFA resulted in low intracellular PHA concentrations, where the maximum final PHA content was observed at 5-h and was 9.0 %.

From fed-batch A3 with pH control (pH = 8.0) VFA depletion 4-h and 30 min, and the intracellular PHA peaked was achieved at 4h.

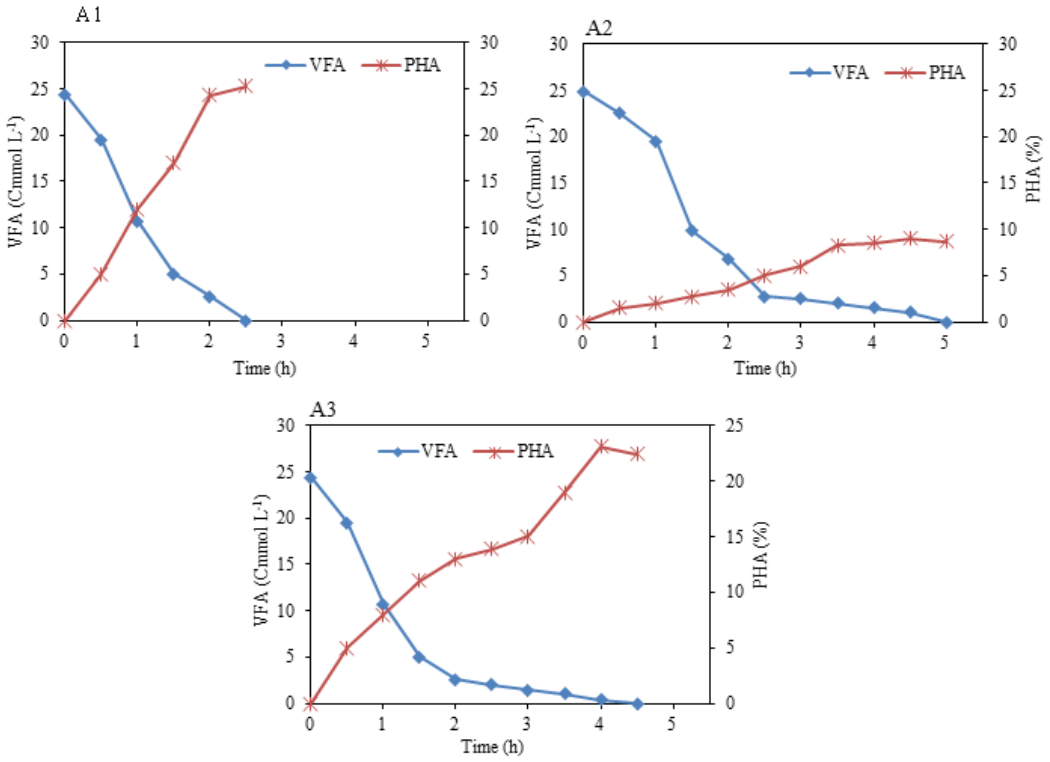

Figure 8-5: PHA profille (%), substrate consumption trend (Cmmol L^{-1}) during the accumulation assays. (A1) Accumulation test with pH control (7.0 – 7.3); (A2) accumulation test without pH control and (A3) accumulation test with pH (8.0).

8.2.5 PHA production and quality: a comparison approach

In this work section it can demonstrated the feasibility of producing PHA from OFMSW effluent rich-VFA. As can been see, OFMSW proved to be a viable source of carbon for the production of VFA and can be used producing PHA starting from mixed microbial culture for low-cost and for economical PHA production. However, there are limited studies to explore this method using OFMSW as a carbon source. Table 8-5 show the performance of the fed-batch accumulation step in this study compared with that of PHA production and composition using a similar feedstock at different conditions applied by several study.

Results obtained in this study indicated that PHA content were in line with previous study reported in the literature for OFMSW-effluent used as substrate for PHA production (Amulya et al., 2015; Chen et al., 2013), although were much low than results reported by Basset (2016), who used OFMSW fermented liquid and permeated nitrogen removal.

According to Basset et al. (2015), the efficiency of PHA accumulation is higher when the liquor and the carbon source are free of nutrients that promote the biomass growth. The different results of PHA content could be derived from the different conditions were established within applied in PHA-accumulation and the composition of carbon source.

Table 8-5: Comparison between OFMSW and other similar food wastes used as substrate for PHA accumulation step

Substrates	Condition	Max. PHA content (%)	PHA composition (% HB: % HV)	Reference
OFMSW	pH control at 7.0 -7.3	25.3	78:22	This study
food waste (FW)	Cycle length reduction to 12 h	23.7	81:29	Amulya et al. (2015)
OFMSW FL	Nitrogen removal via nitrite	52.0	60:40	Basset et al.(2016)
OFMSW	Pretreated leachate	0.48[a]	----	Korkakaki et al. (2016)
Food waste/WAS	---	23.0	76:24	Chen et al. (2015)

FL-fermented liquid.
[a] Calculed gCOD $(gCOD)^{-1}$.

From a quality of biopolymers, PHB is the main composed in PHA production from OFMSW with higher concentrations than PHV (78:22). It is likely that acetic and butyric acids were the main composition of OFMSW-effluent substrate (see Table 8-1), essential for the production of a co-polymer with HB monomers. Since, the PHA composition is strongly dependent on the substrate composition and on the culture bacterial species. In this study, the PHB polymers was better than results achieved by Basset et al. (2016), achieved 60:40 % and Chen et al. (2015) (76:24 %), showing more again, the viability of use the OFMSW–effluent as substrate for the PHA production, leading to a more cost-effective PHA production system and environmentally sustainable.

8.3 Conclusions

The purpose of the study demonstrated the technical feasibility of the PHA production from MMC in SBR which are enriched with OFMSW-effluent by-products. In addition, the high performance of COD conversion into biopolymer and the efficient COD removal makes the proposed process aneffective tool for simultaneous OFMSW treatment and valorization

towards PHA production, and at same time, contributing to the sustainable management of OFMSW, i.e. reduction in the amount of OFMSW to be incinerated and landfills deposition.

In this work, the SBR aerobic mode were operated at OLR and C:N ratios conditions for the selection of PHA-storing microorganisms, followed by a PHA-accumulating in controlled fed-batch bioreactor. Based on the obtained results, the main conclusions from this study are the following:

The methodology used showed a good performance in terms of the PHA production and waste-effluent treatment. During the microbial enrichment of PHA process, all the conditions tested, the COD removal efficiency was more than 80% showing favorable for biotreatment of the effluent.

In in terms of the PHA composition, presence of high HB and HV content supports biodegradability of the PHA obtained production.

The fed-batch PHA accumulation of three different pH studied showed preferential for neutral pH was the most favorable for PHA production, resulting in a PHA content of 25% in 2:30 h, where PHB is the main composed in PHA production from OFMSW with higher concentrations than PHV (88:12).

Finally, the test for enrichments showed that once the conditions is successfully selected, the PHA productivity can be clearly increased, and complex streams such as OFMSW- effluent rich in VFA can be valorized to PHA at low cost production.

9. General conclusions and perspectives of future work

9.1 General Conclusions

The municipal solid waste, particularly organic waste or food waste, is one of the major environmental problems due to the rapid increase in urbanization, industrialization and population. It is known that organic wastes have high content of organic matter in their composition, and when untreated may cause adverse environmental impact, risk to public health and other socio-economic problems. Thus, a potentially promising area of study is the application of suitable technologies to enhance the waste management systems or the development of conversion processes to treat waste and recover added-value products.

In this context, the aim of this work was the study of the biological treatment potential of several organic wastes (OW), applying both aerobic and anaerobic acidification processes, in order to provide different recycling/recovering alternatives and, at the same time, to reduce the need for disposal of the OW into the environment. In addition, it was also studied the use of VFA-rich treated effluents as carbon source to produce PHA.

From the overall results obtained during this work, some of the main conclusions are summarized below:

- From the batch mesophilic assays with four different wastes, namely OFMSW, CG, TW and WAS, it was concluded that the performance of the bioreactor is dependent on the composition of the wastes;
- In the mono-digestion assays, the mono-digestion of TW and OFMSW showed the highest degree of acidification (49.0 % and 41 %, respectively). CG and WAS showed the lowest performance in acidification process (10 % and 6 %, respectively) and the lowest VFA production, with 12.18 $g_{COD}.L^{-1}$ and 0.98 $g_{COD}.L^{-1}$, when CG and WAS were digested, respectively;
- The co-fermentation between CG and TW showed the most efficient VFA production in comparison with the mono digestion of these substrates. The results of this specific study, without additional methanogenic inhibition factors, suggest that anaerobic co-fermentation between these wastes increased the synergies from the substrates; comparatively, the mono-digestion of TW or OFMSW showed higher VFA production than the co-digestion assays previously referred, with higher acidogenic potential;

- The co-fermentation assays using WAS and OFMSW, without pH control, confirmed that the composition of the mixture used as substrate has great influence on bioreactor performance and stability, as well as in the methane ultimate potential. The use of these complex substrates is attractive once they could be treated together, reducing operational costs and creating value from the products obtained;
- From the three first order exponential models used in this study of the co-digestion of WAS and OFMSW, to compare the kinetic constants for hydrolytic and methanogenic steps of AD process, the exponential Curve Factor model was the best model to predict the methane production. Positive linear correlations between the first order kinetic models and the experimental data validate these models, with a confidence interval of 95%, being them a useful tool for reactor design and for the prediction of the process performance and behavior under practical conditions;
- From the acidogenic fermentation of OFMSW, at different TS contents and alkalinity concentrations, it can be concluded that the increase on TS content led to a decrease on the acidification degree whereas the increase on the alkalinity addition led to a higher degree of acidification. Highest degree of acidification (77.59 %) was obtained at the lowest total solids content (5 %) and at the highest alkalinity addition (50 $g_{CaCO3}.L^{-1}$). In terms of the produced VFA, the acidified effluent presenting the highest VFA content (98.96 %) with higher propionic acid concentration, which is a more suitable VFA mixture for the production of high-quality PHA, was obtained at an intermediate total solids content (8 %) and alkalinity addition between 10 to 30 $g_{CaCO3}.L^{-1}$;
- The response surfaces obtained show that all response variables (VFA production, degree of acidification, and effluent quality) presented a higher dependency on total solids content than on initial alkalinity addition;
- The influence of OLR and alkalinity on the VFA production from OFMSW on semi-continuous reactors was evaluated and the maximum VFA contents were obtained at OLR of 6.0 $g_{COD}.L^{-1}.d^{-1}$ and alkalinity of 2.5 $g_{CaCO3}.L^{-1}$, with pH values between 5.0 and 6.0. The main fermentation products were acetic, propionic, butyric and valeric acids, accounting 27 %, 19 %, 38 % and 11 % of the VFA mixture, respectively. Thus, the four most fermentable organic products in sum accounting for 95 % of total VFA mixture, being they promising for PHA valorization;

- The use of VFA-rich effluent to produce PHA was evaluated and the PHA-accumulating bacteria enrichment assays were performed in SBR. The COD removal efficiency was more than 80 % for all the conditions tested, proving the success of the effluent treatment and, at the same time, the selection of the PHA-accumulating microorganisms, with a maximum PHA content of 53 % $mol_{PHA}.mol_{VSS}^{-1}$, obtained in SBR assay with an OLR of 1.7 $g_{COD}.L^{-1}.d^{-1}$ and C:N ratio of 14 $Cmol.Nmol^{-1}$, with a cycle length of 24 h. Increasing the OLR from 1.7 to 3.0 $g_{COD}.L^{-1}.d^{-1}$ resulted in a decrease of performance of SBR, with low PHA content (17.2 %). In terms of PHA concentration and specific PHA production, SBR assay with low C:N ratio (5 $Cmol.Nmol^{-1}$), exhibited better performance;
- In batch tests for PHA production by PHA-enriched biomass, under different pH condition, the maximum PHA was 25.3 %, observed in the assay controlled at 7.0-7.5. The higher PHA content is related to higher microbial activity, as indicated by the $-q_{VFA}$ value (0.88). Thus, a stable mixed microbial culture was selected and it promotes a more sustainable management of OFMSW, as a part of a valorization process, being at the same time a cost-effective process and eco-friendly by-products generation.

9.2 Future work

Based on the results of the present work, some suggestions are presented to contribute an environmentally sustainable management of organic waste and an improvement in the future work.

As the main goal of this study was to optimize and maximize the VFA production by various organic waste, several experimental tests were carried out by aerobic process and anaerobic digestion. It is found that pH and alkalinity values, TS content, temperature, C:N ratio and OLR were important parameter for anaerobic digestion. However, some parameters as the bioreactor dimensions, the presence of nutrients and toxic compounds, must also be studied, once they could affect the performance of bioreactor on the VFA production, affecting all the biological process.

It is known that the VFA potential and, consequently, biomethane potential, were a result of substrate composition as well as biodegradability. The further study on the characteristics of

the organic wastes can be carried out, in order to reduce the cost of process, as well to enhance the optimization of the process, as an innovative way to address organic solid wastes management issues.

The continuous process is a good alternative to evaluate the stability of the process. Thus, applying and enhancing the experimental conditions that allow the evaluation of the operation of OFMSW acidogenic fermentation could promote the acidogenic process and the subsequent recovery and application of the acidified effluents (to be applied in PHA production, for example).

Further investigation on the pilot- scale production of PHA from OFMSW could be pursued to scrutinize the technical aspects (microbial population, dynamics and greater COD reduction) and process economics to eventually transfer the technology from the laboratory to the industry, may can in the future replace the current processes for the production of PHAs or replacement of plastic petrochemical origin.

10. References

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11. Annexes

Annex I - The determination of chemical oxygen demand (COD): preparation of the solutions (closed reflux)

1. Oxidant solution

- Dryer about 25 g of $K_2Cr_2O_7$ at 105 ° C until weight constant.
- Precise weigh 20.432 g of $K_2Cr_2O_7$ and 66.6 g $HgSO_4$, and dilute in 500 mL of distilled water and 167 mL of concentrated sulfuric acid (H_2SO_4) with magnetic stirring for 24h.
- Finally, measure the volume to 2 L with distilled water and conserve in a dark glass bottle.

2. Acid solution

- Precise weigh 23.3 g Ag_2SO_4.
- Dissolve in 2.5 L of concentrated sulfuric acid (H_2SO_4) with stirring magnetic for 24 h and conserve in their own acid bottle.

Annex II - Preparation of these reagents for TCOD determination (open reflux)

1. Reagents.

- Sulphuric acid 96%.
- Digestion reagent: dissolve 33.3 g of mercuric sulphate ($HgSO_4$), 167 mL of sulphuric acid 96% and 600 mL of water. Then dissolve 58.844 g of potassium dichromate (K_2Cr2O_7) primary standard grade, previously dried at 105 ˚C for 2 h, and finally top up the solution with distilled water to 1000 mL.
- Sulphuric acid reagent: dissolve 10 g of silver sulphate ($AgSO_4$) in 1000mL of sulphuri acid 96%. This reagent may be purchased already prepared.
- Potassium dichromate solution 1N: dissolve 49.13 g $K_2Cr_2O_7$ primary standard grade, previously dried at 105 ˚C for 2 h in 500 mL distilled water and 167 mL sulphuric acid 96%. Dissolve, cool to room temperature and dilute to 1000 mL. This reagent may be purchased already prepared.

- Ferroin indicator solution: dissolve 1.485 g 1,10-phenantroline monohydrate and 695 mg $FeSO_4 \cdot 7H_2O$ in distilled water and dilute to 100 mL. This reagent may be purchased already prepared.

2. Ferrous ammonium sulphate (FAS) solution for titration approximately 0.5N

- Dissolve 200 g $(NH_4)_2\ Fe(SO_4) \cdot 6H_2O$ in 500 mL of distilled water and mix with 40 mL of sulphuric acid 96%.
- Cool and dilute to 1000 mL. This solution shall be standardized before use against dichromate as follows: dilute 10 mL $K_2Cr_2O_7$ 1N to about 70 mL of distilled water, add 30 mL sulphuric acid 96% and cool. Titrate with FAS 0.5N using 3 drops of ferroin indicator solution. The concentration of the reducing reagent is calculated using the following equation:

$$NFAS = \frac{10\ mL\ \times 1N}{VFAS\ (mL)} \quad (33)$$

Where: N_{FAS} and V_{FAS} are the concentration (N) and volume (mL) of the reducing agent used

Annex III - Calibration of the chromatographic method for the quantification the VFA

Table 11-1 presents the chemical properties of reagents (VFA) used of standards dilution. Six different feed samples were prepared and the calculation of the standard solution concentration and dilution shown in in Table 11-2 were based on the physic-chemical properties of the reagents used (Table 11-1). Injection of 0.5 µL of each standard in appropriately stabilized chromatograph allowed the identification of each retention times of the analytes and peak associated with each VFA (Table 11-3). The determination of the area of each peak is based on the calculation algorithm by software Microsoft® Excel 2016.

Figure 11-1 shows the calibration curves obtained (minimum method Squares) where it can be verified that for all VFA analyzed and the parameters of linear regressions. Fig. 11-1 shows the graphs of the area of each peak in mV.s according to the volatile organic acid concentration present in each mixed standard used.

Table 11-1: VFA composition of each standard used in this study

Characteristic	**H-Ac**	**H-Pr**	***i*H-But**	***n*H-But**	***i*H-Val**	***n*H-Val**	***n*H-Ca**
M (g mol^{-1})	60.05	74.08	88.11	88.11	102.11	102.11	116.21
ρ (g mL^{-1})	1.05	0.99	0.95	0.96	0.94	0.94	0.95
Purity solution (%)	99.9	99.9	99.8	99.9	99.9	99.8	99.8
Final conc. (g L^{-1})	3.15	2.97	2.91	2.88	2.79	2.79	2.85

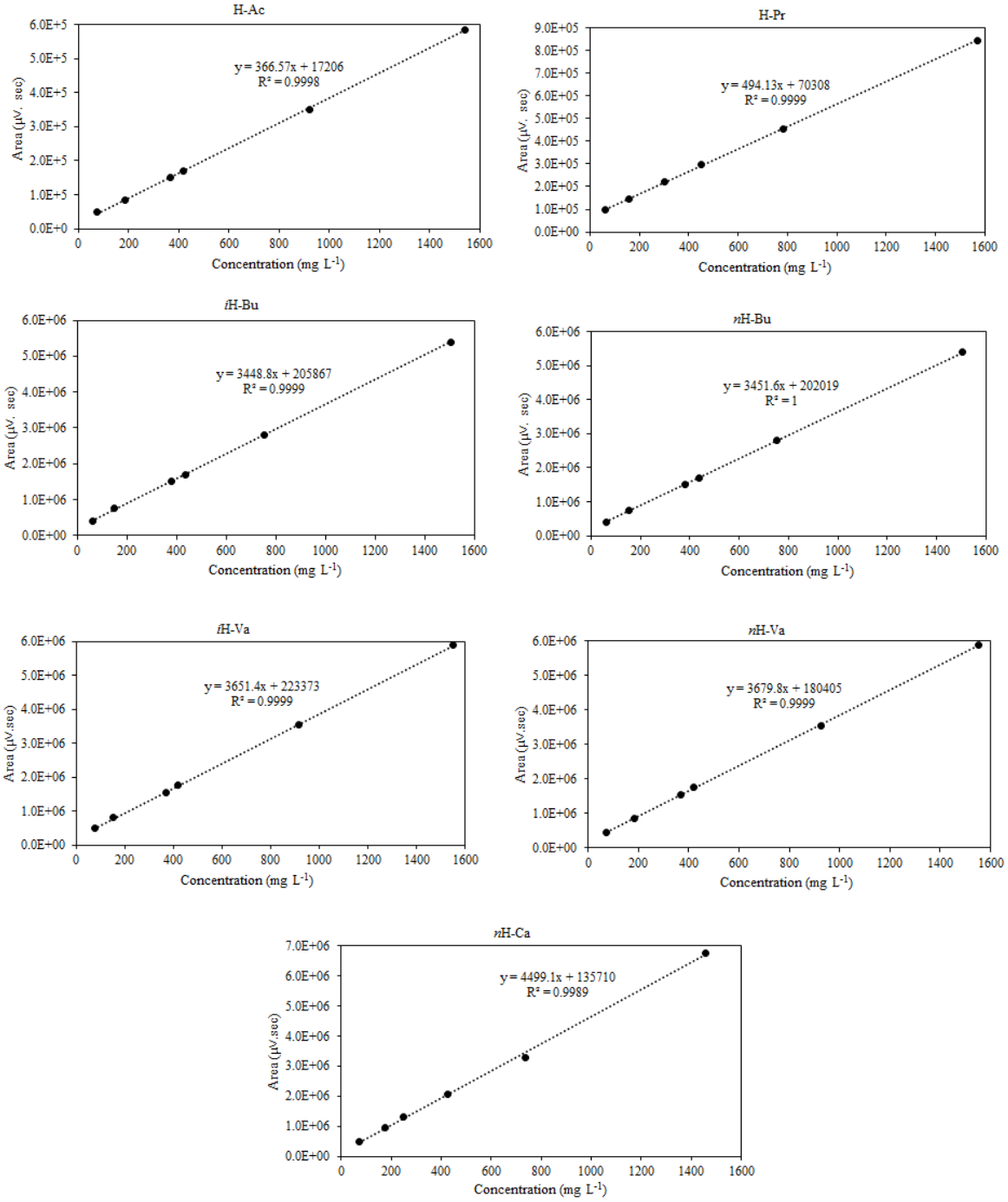

Figure 11-1: Calibration curves for VFA quantification: acetic acid (H-Ac); propionic acid (H-Pr); iso-butiric acid (*i*H-Bu); *n*-butiric acid (*n*H-Bu); iso-valeric acid (*i*H-Va); *n*-valeric acid (*n*H-Va); *n*-caproic acid (*n*H-Ca)

Table 11-2: Individual concentrations for the standards dilution (mg L^{-1}), with 30% of formic acid added.

Diluted standards	H-Ac	H-Pr	*i*H-But	*n*H-But	*i*H-Val	nH-Val	*n*H-Ca
Std 1	64.1	62.7	60.2	60.4	74.0	74.2	73.5
Std 2	184.1	156.8	150.5	152.4	184.1	185.2	173.6
Std 3	367.6	300.9	380.2	382.4	367.6	368.0	247.3
Std 4	472.5	445.5	436.5	432.5	418.5	418.5	427.3
Std 5	838.3	784.2	752.4	754.4	920.2	915.4	736.5
Std 6	1540.4	1568.2	1504.8	1500.5	1541.4	1550.2	1425.6

Table 11-3: Peak retention time associated with each VFA (min).

		H-Ac	H-Pr	*i*H-But	*n*H-But	iH-Val	*n*H-Val	*n*H-Ca
Retention time	average	2.650	3.571	4.455	4.996	6.137	6.993	9.054
	std. dev.	0.072	0.081	0.121	0.127	0.127	0.117	0.099

Annex IV - The calibration curves for monomers (PHA) determination: methodology to PHA quantify.

The determination of the amount of PHA present in the biomass was based on the methods developed by Braunegg et al. (1978) and Comeau et al. (1988). However, slight modifications have been introduced that include pretreating the biomass samples for destruction of cells and release of intracellular hydrolysis of its polymer chains, and subsequent methylation of their monomers. In this way, quantification by gas chromatography (PerkinElmer gas-liquid chromatograph) became feasible.

For calibration was performed with a straight six concentrations prepared from a stock solution containing 3.1 mg mL^{-1} of a standard commercial P (HB-HV) 88% -12% (Sigma Aldrich®). Figure 11-2 shows the calibration curves for quantified HB and HV.

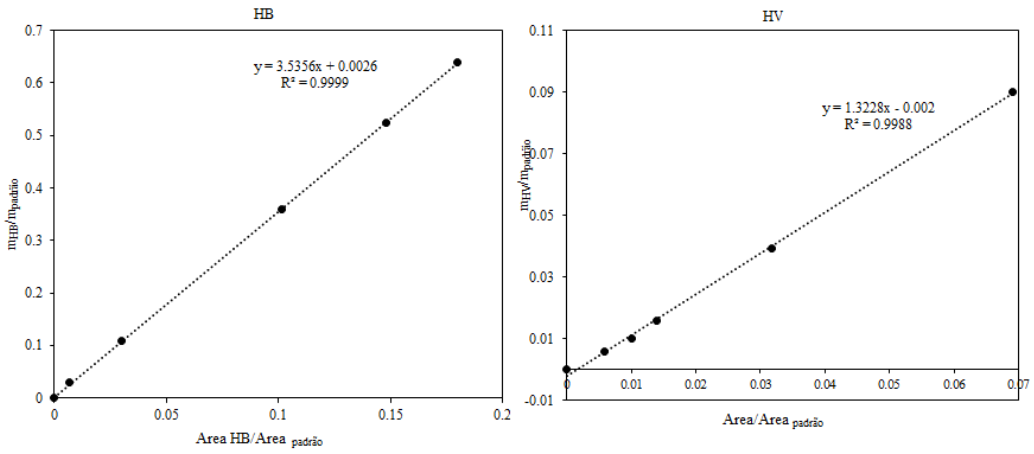

Figure 11-2: Calibration curves for HB and HV quantification