PhD Thesis

UPC – Program on Environmental Engineering

SEWAGE SLUDGE ANAEROBIC DIGESTION
Study of synergies and operational strategies of co-digestion

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Als meus pares,

M^a Carmen i Manolo

“No es la especie más fuerte la que sobrevive, ni la más inteligente, si no la que responde mejor al cambio”

Charles Darwin

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TABLE OF CONTENTS

INDEX OF FIGURES

INDEX OF TABLES

INDEX OF FIGURES

INDEX OF TABLES

LIST OF ABBREVIATIONS

ABP	anaerobic by-products
ACD	anaerobic contact digester
AD	anaerobic digestion
A_F	area of the floor
A_R	Area of the roof
ASBR	anaerobic sequential batch reactor
A_W	area of the walls
BNR	biological nutrients removal
BOD	Biochemical oxygen demand
C	carbon
CGY	crude glycerine
CI	confidence interval
CH_4	methane
CHP	combined heat and power (engine)
C/N	carbon nitrogen ratio
Co	cost
CO_2	carbon dioxide
COD	chemical oxygen demand
Cp	specific heat value
CST	centrifugal settling test
CSTR	continuous stirred tank reactor
CTMS	chlorotrimethylsilane
DAF	dissolved air flotation
DAS	data acquisition system
EEc	electric energy consumption
EEp	electric energy production
EE_S	electric energy supplied
EF	energy flow
EPS	extracellular polymers substances
FAME	fatty acid methyl ester
FID	flame ionization detector
F/M	food to microorganisms ratio
FOG	fat, oil and grease
F_S	sludge flow
FVW	fruit vegetable waste
FW	food waste
GC	gas chromatograph
GW	grease waste
H	hydrogen
H_2	hydrogen gas
HRT	hydraulic retention time
IA	intermediary alkalinity

IC_{50}	half maximal inhibitory concentration
In	inoculum
IP	Inerter content
IS	inorganic solids
ITQ	inclined tubular digester
LCFA	long chain fatty acids
LCV	lower calorific value
M	mesophilic
MP	methane potential
MPS	mean particulate size
MSW	municipal solid waste
MY_{VS}	Methane yield on VS basis
N	nitrogen
NH_4^+-N	ammonium nitrogen
$NO_3^{-}N$	nitrate
$NO_2^{-}N$	nitrite
O	oxygen
OFMSW	organic fraction of municipal solid waste
OLR	organic loading rate
P	period
PA	partial alkalinity
$P_{calCH4:}$	heating value of methane
PH_2	hydrogen partial pressure
PN/A	nitritiation/anammox
PO_4^{-3}-P	phosphate
PS	primary sludge
P_w	power
q_L	heat flow needed to maintaining the anaerobic reactor’s T
q_T	heat flow needed to increase the sludge temperature
R_m	maximum slope
S	sulphur
SAT	specific activity test
SOFC	solid oxide fuel cell
SO_4^{-2}-S	sulphate
SRB	sulphate reducing bacteria
SRT	sludge retention time
SS	sewage sludge
t	time
T	temperature
T_A	average monthly environmental temperature
TCD	thermal conductivity detector
T_D	operating temperature of the digester
Te:	thermophilic

TE	thermal energy
TE_C	thermal energy consumption
TEp	thermal energy production
TEs	thermal energy supplied
T_F	temperature of the floor
TKN	total Kjeldahl nitrogen
TMP	theoretical methane potential
TN	total nitrogen
TP	total phosphorous
TPAD	two-phased anaerobic digestion
TS	total solids
T_S	temperature of the sludge
TSS	total suspended solids
t_{wd}	the hours that the digester is producing biogas
t_{we}	the working hours per month of the CHP engine
V	volume
VFA	volatile fatty acids
VFT	vacuum filtration test
VMP	volumetric methane production
VS	volatile solids
UASB	upflow anaerobic sludge blanket
U_F	heat transfer coefficients floor
U_R	heat transfer coefficients roof
U_W	heat transfer coefficients of the walls
W	weight
WAS	waste activated sludge
WW	wastewater
WWTPs	wastewater treatment plants
$Y_{CH4:}$	methane production
$\eta_{E:}$	electric efficiency of the CHP engine
η_T:	thermal efficiency of the CHP engine
ρ:	sludge density
λ:	lag phase
α	coefficient between MP/TMP

RESUM

Les Estacions de Depuració d'Aigües Residuals (EDARs) es caracteritzen per la seva elevada eficiència en l'eliminació de la matèria orgànica i nutrients, però també per el seu elevat consum energètic. Actualment, i degut a l' escassetat de recursos, les estratègies de reducció del consum energètic s'han convertit en una eina important per gestionar les EDARs de manera més sostenible.

En aquesta tesi, la co-digestió anaeròbia de fangs d'EDAR amb residus orgànics de diferent orígens i composicions, s'ha proposat com una estratègia atractiva per apropar-se a un escenari de tractament d'aigües residuals autosostenible energèticament, per mitjà de la millora de la producció de biogàs. L'estudi de l'estratègia de co-digestió es va centrar en l'efecte de la composició dels co-substrats (lípids mitjançant l'adició de **residus greixosos** procedents de la unitat de flotació per aire dissolt -DAF- de l'EDAR, alcohols mitjançant l'adició de **glicerina crua** d'una industria de producció de biodiesel, i una composició mixta aportada per la **fracció orgànica de residus municipals** -FORM-), i l'efecte de la temperatura d'operació (mesòfil i termòfil) en el rendiment general del procés de digestió anaeròbia dels fangs d'EDAR.

Es van portar a terme experiments de co-digestió en continuo per avaluar el rendiment en la producció de metà i l'estabilitat del procés. La producció de metà es va incrementar entre 2,3 i 2,2 vegades en comparació amb la mono-digestió dels fangs d'EDAR quan l'adició del residu greixós representava el 26% i el 27% de la DQO d'entrada, durant la digestió mesòfila i termòfila respectivament. L'adició de la glicerina crua va incrementar la producció de metà 2,5 vegades durant la digestió mesòfila, mentre que el procés en condicions termòfiles va mostrar una elevada inestabilitat, principalment deguda a l'acumulació d'àcids grassos volàtils. L'adició de FORM va incrementar la producció de metà entre 3 i 5 vegades més que la mono-digestió dels fangs d' EDAR, quan l'adició de FORM va ser del 51% i el 33% de la DQO d'entrada, a temperatures de operació mesòfiles i termòfiles respectivament.

L'adaptació de la biomassa mitjançant increments lents de la dosis del residu greixós, va demostrar ser una bona estratègia per incrementar la degradació dels lípids i reduir l'efecte inhibitori dels àcids greixosos de cadena llarga. La co-digestió termòfila va presentar una menor tolerància als àcids greixosos de cadena llarga que la digestió mesòfila, i per tant, el temps d' adaptació de la biomassa termòfila va ser superior.

La co-digestió anaeròbia termòfila dels fangs amb la glicerina crua es va mostrar molt inestable degut al pH extrem que presentava la glicerina i la seva ràpida descomposició en àcids grassos volàtils. Per una altra banda, la co-digestió mesòfila va mostrar un bon rendiment, concloent que dosis addicionals de glicerina per damunt de 2% v/v no mostraren una millora en el rendiment de metà. En aquest cas, es pot dir que l'estratègia d'optimització basada en la relació C/N cal que sigui modulada per altres factors com les característiques de

la glicerina crua (especialment el pH i l'alcalinitat total) i la temperatura d' operació.

La FORM es va presentar com un co-substrat adequat per a treballar en els dos rangs de temperatures, mesòfiles i termòfiles. Els resultats obtinguts suggereixen que l'adició de la FORM podria ser una estratègia vàlida per fomentar l'activitat de les poblacions termòfiles oxidats d'àcids grassos saturats, i les poblacions metanogèniques acetoclàstiques. Així mateix, es va demostrar que els assajos d'activitat específics són una eina viable per explicar i gestionar les respostes del sistema, especialment quan els paràmetres de control convencionals no es van mostren adequats per explicar el rendiment del reactor.

Els resultats obtinguts han demostrat que la co-digestió es un bon enfocament per optimitzar el balanç energètic del les EDARs. Però dependent de la composició i la temperatura dels residus orgànics, cal aplicar diverses estratègies operatives per a que el procés es dugui a terme de manera estable, evitant episodis inhibitoris.

Els resultats obtinguts amb les estratègies de co-digestió de fangs d'EDAR estudiades en aquesta tesi doctoral, demostren que la co-digestió dels fangs amb diferents residus orgànics, és una alternativa atractiva per aconseguir l'autosuficiència energètica de les EDARs, i fins i tot, que les EDARs es converteixin en instal·lacions productores netes d'energia.

RESUMEN

Las Estaciones de Depuración de Aguas Residuales (EDARs) se caracterizan por su elevada eficiencia en la eliminación de materia orgánica y nutrientes, pero también por su elevado consumo energético. Actualmente, y debido a la escasez de recursos, las estrategias de reducción del consumo energético se han convertido en una importante herramienta para una gestión más sostenible de las EDARs.

En esta tesis, la co-digestión anaerobia de lodos de EDAR con residuos orgánicos de distinto orígenes y composiciones, ha sido propuesta como una estrategia atractiva para acercarse a un escenario de tratamiento de aguas residuales autosostenibles energéticamente, por medio de la mejora de la producción de biogás. El estudio de la estrategia de co-digestión se centró en el efecto de la composición de los co-sustratos (lípidos mediante la adición del **residuo graso** procedente de la unidad de flotación por aire disuelto – DAF- de la EDAR; alcoholes mediante la adición de **glicerina cruda** de una industria de producción de biodiesel, y una composición mixta aportada por la **fracción orgánica de residuos municipales** –FORM-), y el efecto de la temperatura de operación (mesofílico y termofílico) en el rendimiento general del proceso de digestión anaerobia de los lodos de EDAR.

Se llevaron a cabo experimentos de co-digestión en continuo para evaluar el rendimiento de metano y la estabilidad del proceso. La producción de metano incrementó entre 2,3 y 2,2 veces en comparación con la mono-digestión de los lodos de EDAR cuando la adición del residuo graso representó el 26% y el 27% de la DQO de entrada, durante la digestión mesófila y termófila respectivamente. La adición de la glicerina cruda incrementó la producción de metano 2,5 veces durante la digestión mesófila, mientras que el proceso en condiciones termófilas mostró una elevada inestabilidad principalmente debido a la acumulación de ácidos grasos volátiles. La adición de FORM incrementó la producción de metano de 3 a 5 veces más que la mono-digestión de los lodos de EDAR, cuando la adición de FORM fue del 51% y el 33% de la DQO de entrada, en temperaturas de operación mesófilas y termófilas respectivamente.

La adaptación de la biomasa mediante incrementos lentos de la dosis de residuo graso, se demostró como una buena estrategia para incrementar la degradación de grasa y reducir el efecto inhibitorio de los ácidos grasos de cadena larga. La co-digestión termófila mostró una menor tolerancia a los ácidos grasos de cadena larga que la digestión mesófila, y por lo tanto, el tiempo de adaptación de la biomasa termófila fue superior.

La co-digestión anaerobia termófila de los lodos con la glicerina cruda se mostró muy inestable debido al pH extremo de la glicerina y su fácil descomposición en ácidos grasos volátiles. Por otra parte, la co-digestión mesófila presentó un buen rendimiento, concluyendo que dosis adicionales de glicerina cruda por encima de 2% v/v no mejoran en el rendimiento de metano. En este caso, se puede

concluir que la estrategia de optimización basada en la relación C/N debe ser modulada por otros factores como las características de la glicerina cruda (especialmente el pH y la alcalinidad total) y la temperatura de operación.

La FORM se presentó como un co-sustrato adecuado para trabajar a rangos de temperaturas mesófilas y termófilas. Los resultados obtenidos sugieren que la adición de la FORM podría ser una estrategia adecuada para fomentar la actividad de las poblaciones termófilas oxidantes de ácidos grasos saturados, y las metanogénicas acetoclásticas. Además, los ensayos de actividad específicas demostraron ser una herramienta viable para explicar y gestionar las respuestas del sistema, especialmente cuando los parámetros de control convencionales no fueron suficientes para explicar el rendimiento del rector.

Los resultados obtenidos han demostrado que la co-digestión es una estrategia adecuado para optimizar el balance energético del tratamiento de las aguas residuales. No obstante, dependiendo de la composición y temperatura de los residuos orgánicos, es necesario poner en práctica diversas estrategias para llevar a cabo un proceso estable, evitando episodios inhibitorios.

Los resultados obtenidos con las estrategias de co-digestión de lodos de EDAR estudiadas en esta tesis doctoral, muestran que la co-digestión de los lodos con distintos residuos orgánicos, es una alternativa atractiva para conseguir la autosuficiencia energética de las EDARs, e incluso, que se conviertan en instalaciones productoras netas de energía.

ABSTRACT

Wastewater treatment plants (WWTPs) are characterized by their high organic matter and nutrient removal efficiency, but also by their high energy consumption. In the current context where resources are increasingly scarce, all feasible strategies to save energy emerge as an important issue for the sustainable management of WWTPs.

In this thesis, sewage sludge (SS) anaerobic co-digestion with available organic wastes, coming from different sources and having different compositions, was proposed as an interesting strategy to approach an energy self-sufficient scenario for wastewater treatment by means of an improved biogas production. The studied co-digestion strategies were focused on the effect of the co-substrates composition –lipids, by adding **grease waste** (GW) from the dissolved air unit of the WWTP; alcohols, by adding **crude glycerol** (CGY) from a biodiesel facility; and of mixed composition, when adding the **organic fraction of municipal solid waste** (OFMSW) – and the effect of operational temperatures –mesophilic and thermophilic– on the general performance of the SS anaerobic digesters.

This approach was evaluated in terms of methane yield and the stability of the process throughout different continuous co-digestion trials. Methane productivity increased 2.3 and 2.2 times in comparison with sewage sludge mono-digestion when the GW added amounted to 26% and 27% of the COD inlet under mesophilic and thermophilic temperature conditions, respectively. The addition of GGY showed a 2.5 increase in methane productivity under mesophilic temperature, while in thermophilic range the co-digestion showed great instability mainly due to volatile fatty acids accumulation. Addition of the OFMSW showed a 3 to 5 times increase in methane productivity compared to SS mono-digestion, when the OFMSW added amounted respectively to 51% and 33% of the COD inlet under mesophilic and thermophilic conditions respectively..

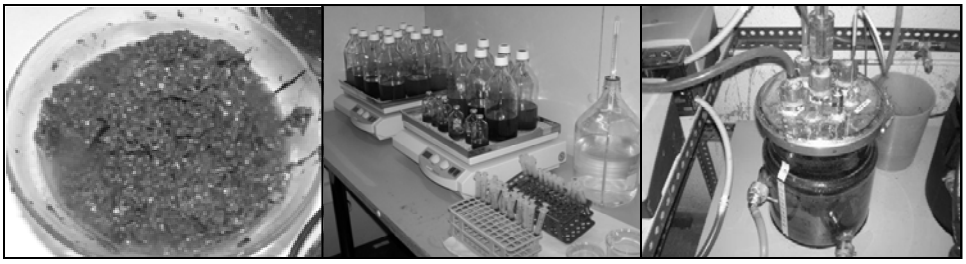
The biomass acclimatization brought about by a slow increase of the influent dose of **GW**, could be a good strategy to increase fat degradation and reduce the inhibitory effect of LCFAs. Thermophilic co-digestion showed a lesser tolerance to LCFAs than mesophilic, and therefore, the adaptation time to grease waste was longer.

Thermophilic anaerobic co-digestion of SS with **CGY** proved to be very unstable due to the extreme pH of CGY and its fast decomposition into volatile fatty acids. On the other hand, mesophilic co-digestion showed a good performance, concluding that doses above 2% v/v of **CGY** did not bring about an improvement on the methane yield. In this case, it can be concluded that the optimization strategy based on the C/N ratio must be modulated by other factors such as the characteristics of crude glycerol (particularly its pH and total alkalinity) and the operational temperature.

The **OFMSW** was proved to be a suitable co-substrate both under mesophilic and thermophilic temperature conditions. Results suggest that the addition of the **OFMSW** could be an adequate strategy to promote the activity of thermophilic saturated fatty acid oxidizers and acetoclastics methanogenic bacteria. In addition, the evolution of specific activities was assessed and used as a feasible tool to explain and manage the response of the system, especially when conventional control parameters were not enough to explain the performance of the reactor.

Within the obtained results, it has been demonstrated that co-digestion is a suitable approach to optimize the energy balance of a WWTP. But, depending on the composition of the organic waste and the temperature range of operation, different operational strategies should be put into practice to find the most stable process, and avoid inhibitory episodes.

Based on the results obtained with the strategies studied in this PhD thesis, sewage sludge co-digestion with different organic wastes could be expected to represent an attractive alternative to attain energy self-sufficient wastewater treatment operations, and perhaps even net energy producing WWTPs



CHAPTER 1

CONTEXT, OBJECTIVES AND THESIS OUTLINE

This chapter introduces the importance of optimizing the energy balance in a Waste Water Treatment Plant through the energy valorisation of sewage sludge, its main waste, via anaerobic digestion process, and its optimization applying co-digestion strategies with other organic wastes. Finally, this chapter gets into the objectives and general outline of the thesis.

1.1 CONTEXT

Treatment of industrial and municipal wastewaters prior to being discharged to the environment was one the first environmental challenges modern societies faced years ago. In this context, Wastewater Treatment Plants (WWTPs) have been built for more than 50 years. Nowadays, there are around 3,000 WWTPs in Spain, even their number increasing in recent years due to the application of Directive 91/271/EEC decreeing all member states to treat wastewaters before discharging them to the environment.

WWTPs are characterized by their high organic matter and nutrients removal efficiencies, but also by their high energy consumption. In Spain, each year close to 3,000 hm^3 of wastewater is treated, entailing 1% of the total national energy consumption (IDAE, 2015). Moreover, the energy consumption cost amounts to around 30% of the total operational costs of WWTPs.

An increase in energy consumption for wastewater treatment is expected due to higher than ever demands on the quality of treated water. Therefore, technological treads must align themselves so as to reduce this expected increase with higher energy efficiencies and greater energy recoveries from the sludge generated as waste during the process. In this sense, opportunities in research fields relate to the development of operational conditions leading to optimize those processes which could improve their energy balance. Two research lines are of interest for their relevance in energy savings, and their role in policies committed to the promotion of renewable energies and the fight against climate change: such as the recovery or removal of nutrients using energy efficient technologies and sewage sludge (SS) energy recovery maximization by means of anaerobic process optimization.

SS is the main waste generated in a WWTP and it is characterized by high concentrations of organic matter, nutrients, heavy metals and pathogens. Therefore, it is necessary to apply a treatment prior to its valorisation or final disposition. SS management costs are usually high, reaching up to 50% of the total WWTP operational costs (Rulkens *et al.*, 2007).

In the European Union close to 7,000,000 tons of the fresh SS are generated annually, and 14% of this production is produced in Spain (EUROSTAT, 2015). Figure 1.1a shows SS production rates for different EU countries in 2010, with Germany, Spain, and the UK being the leading SS producers. Figure 1.1b shows the evolution of its production from 2002 to 2010. As it can be seen, sewage sludge production in the EU remains quite stable, with a slight decrease in production on some countries.

Gómez *et al*. (2006) showed that the energy potential of the sewage sludge produced in Spain, considering its valorisation via anaerobia digestion and its combustion in CHP engines, is of 0.49 TWh per year, equivalent to 0.15% of the net electrical energy production for Spain.

In this context, anaerobic digestion has gained prominence as a technology capable of stabilizing SS, and simultaneously producing renewable energy in the form of biogas. This biogas produced could supply from 40% to 70% of the electrical energy demand of a WWTP, depending on its design and operation (Shizas and Bagley, 2004). Different strategies have been investigated in the last years to increase biogas production in order to attain energy self-sufficiency in WWTPs. Among others, SS pre-treatments and co-digestion with others substrates are the two strategies that have received more attention.

Anaerobic co-digestion is defined as the digestion of a mixture of different organic wastes with different origins and compositions in order to make the most of their composition's complementary characteristics. An increase in biogas production is not the sole benefit of this strategy, the use of co-substrates can also qualitatively and quantitatively attenuate the seasonal production of the sewage sludge and each of the organic wastes used, unifying management methodologies and sharing treatment facilities to make a more efficient use of all shared equipment, thus reducing overall operational costs (Alatriste-Mondragón *et al*., 2006). The selection of substrates and their composition, and the proportion of each one in the feed mixture, is the key to success as it is necessary to obtain the best chemical composition to optimize the activity of the biomass involved in the anaerobic process (carbon-nitrogen ratio, pH, alkalinity, etc.); to avoid the inhibition of different components (ammonia, volatile fatty acids, intermediate products, etc.); to optimize methane production (Mata-Alvarez *et al*., 2014); and improve the dewaterability of the final effluent (Pérez-Elvira *et al*., 2006).

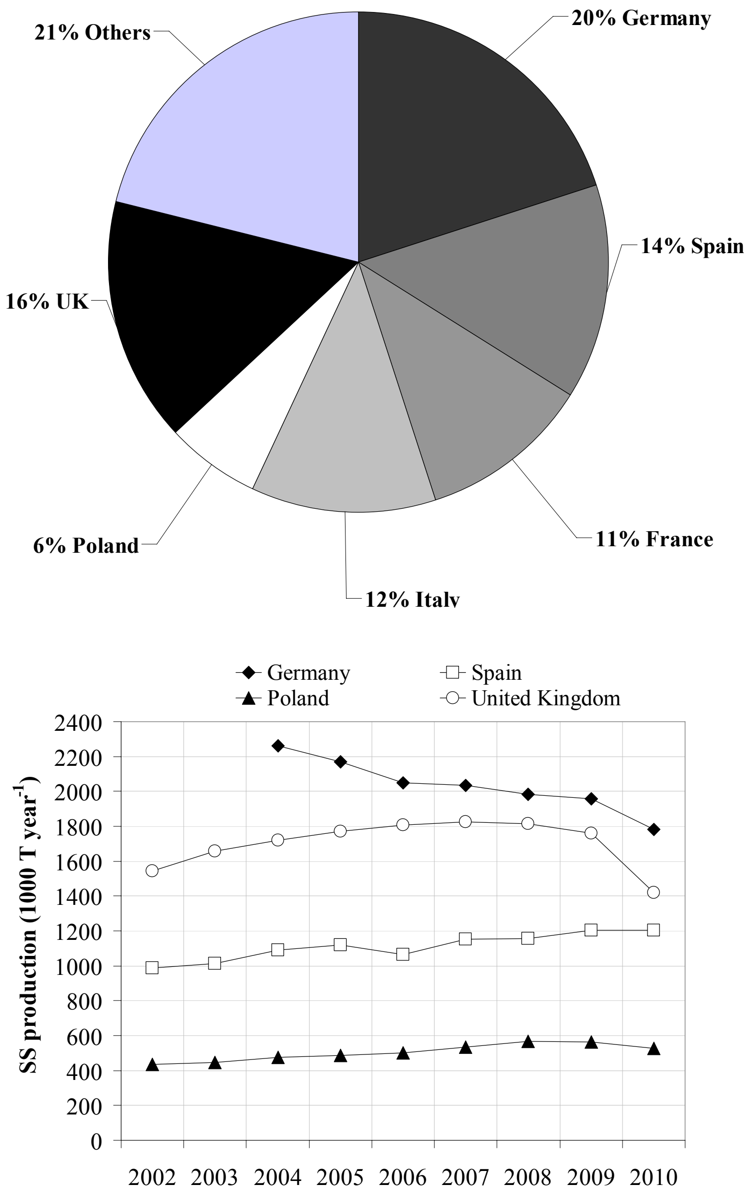


Figure 1. 1. *a) Sewage sludge (SS) production in 2010 of different European countries; b) Evolution of sewage sludge production of different European countries from 2002-2010.*

1.2. OBJECTIVES

The main objective of the present work is to study and develop different operational strategies to optimize the anaerobic co-digestion of sewage sludge with organic wastes from various origins and compositions. In order to achieve the general objective, the following specific objectives have been set:

i. To consider the anaerobic digestion process of sewage sludges as a source of energy in WWTPs, aiming to determine the impact of different strategies to boost energy recovery from said sewage sludge anaerobic digestion.

ii. To evaluate the state of the art of sewage sludge co-digestion, identifying the main advantages and drawbacks of co-digestion in the WWTPs, aiming to develop adequate operational strategies depending on the typology of the organic substrate.

iii. To characterize three different organic wastes as co-substrates –grease waste from the air flotation units, crude glycerol, and the organic fraction of municipal solids wastes–, so as to determine their respective methane yields and identify the main limitations of their use as a co-substrate for sewage sludge anaerobic digestion.

iv. To determine the best mixture of sewage sludge and co-substrate, and the operational conditions to maximize the methane yield and to avoid inhibitory or instability episodes.

v. To assess the effect of these different co-substrates mixtures on the specific biomass activity involved in the anaerobic digestion process.

vi. To compare the sewage sludge process in mesophilic and thermophilic temperatures, as well as the dewaterability properties of the digestates produced in each of the operational conditions tested.

1.3. THESIS OULTLINE

In order to attain the main objective of this thesis, as well as the specific ones, this work is divided in 9 chapters. Below, a description of the content of each chapter is presented.

A short introduction focusing the topic, the objectives and the thesis outline is described in **Chapter 1**.

An analysis of the significance of anaerobic digestion in different WWTPs as a source of renewable energy, and the evaluation of different strategies, like co-digestion, aiming to increase energy production is shown in **Chapter 2**. This chapter is included as a baseline to provide a context for the current energy management of WWTPs and their potential to improve their energy balance so as to fulfil the paradigm shift 'from waste to resource'.

A literature survey about sewage sludge anaerobic co-digestion is presented in **Chapter 3**. A critical evaluation of the main advantages and risks connected with WWTPs, and the identification of some gaps needing to be addressed, are both included. This information was the basis to identify and to develop the operation strategies for each of the organic substrates tested.

In **Chapter 4**, the feasibility of mesophilic sewage sludge co-digestion with trapped grease waste from a WWTP dissolved air flotation unit, has been analyzed, both in batch and continuous experiments. In addition, a complete characterization of several grease wastes, sampled from different WWTPs, was carried out. Grease wastes are characterized by their inhibitory effect related with the presence of long chain fatty acids (LCFA). In this chapter, a slow increase in grease waste, put into practice as an operational strategy favouring biomass acclimation to fat-rich co-substrate, has proven to increase long chain fatty acid degradation reducing the latter's inhibitory effect.

Chapter 5 shows the results of thermophilic sewage sludge co-digestion with trapped grease wastes, the strategy of a slow increase of lipid-rich material being also proven as effective. The thermophilic biomass showed a lower ability to adapt itself than the mesophilic biomass, as well as the need of lengthier adaptation times. A comparative analysis of LCFA degradation in mesophilic and thermophilic conditions was carried out. The results showed that thermophilic biomass has a lower LCFA degradation capacity than the mesophilic biomass. In addition, thermophilic effluents showed worse dewaterability properties after grease waste co-digestion, whereas mesophilic effluents showed better properties.

Results from the thermophilic and mesophilic sewage sludge co-digestion with crude glycerol are presented in **Chapter 6**. Although glycerol is an interesting co-substrate due to its high biodegradable organic matter content and its high biogas potential, a strict control strategy is required to limit the risk of organic overloading. These results showed that as operational strategy, SS-glycerol varies from mesophilic to thermophilic digestion. Thermophilic conditions are more sensible to glycerol characteristics –that is, extreme pH and alkalinity–, than mesophilic ones. The carbon-nitrogen ratio control is shown as an adequate strategy to optimize this operation, avoiding worsening of the specific activity biomass and the dewatering properties of the anaerobic sludge.

Mesophilic sewage sludge with the organic fraction of municipal solid wastes (OFMSW) co-digestion was analyzed in **Chapter 7**. Several OFMSW collected from different source-separated OFMSW were analyzed. A strategy to mechanically reduce the particulates size was analyzed with the aim to increase the methane yield. In addition, the evolution of specific activities was assessed and used as a feasible tool to explain and manage the system response, especially when conventional control parameters were deemed useless.

In **Chapter 8**, thermophilic sewage sludge anaerobic co-digestion with OFMSW experiments are shown. The hypothesis that the feed composition has a strong influence in the specific substrate activity was verified in this chapter, showing that the addition of OFMSW is an adequate strategy to increase the activity of the specific thermophilic biomass.

This thesis establishes a better knowledge about sewage sludge co-digestion in mesophilic and thermophilic temperature conditions, with substrates of different origins and compositions, and provides different operational strategies to optimize sewage sludge anaerobic co-digestion applications. Major conclusions and suggestions for further research are presented in **Chapter 9**.

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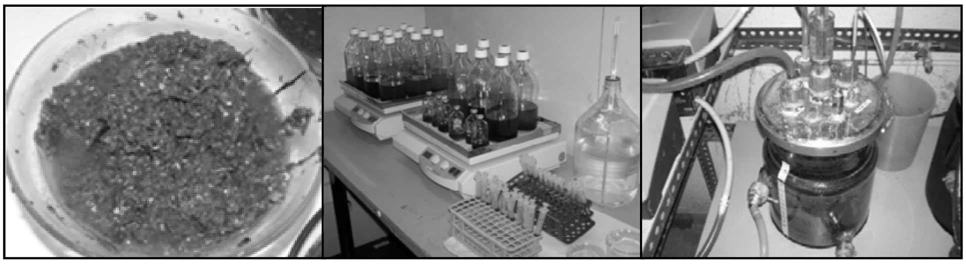

CHAPTER 2

SIGNIFICANCE OF ANAEROBIC DIGESTION AS A SOURCE OF CLEAN ENERGY IN WASTEWATER TREATMENT PLANTS

Nowadays, energy consumption is one of the major concerns of wastewater treatment plants (WWTPs). Time ago, anaerobic digestion was usually implemented for sewage sludge stabilisation but energy recovery optimization has recently gained importance. The energy balance of five WWTPs located in Catalonia revealed that depending on the configuration of the plant and its operation, between 39% and 76% of the total electric energy consumed in the WWTP could be supplied by the biogas produced. In the second part of this work, a carbon, nitrogen and sulphur flux analysis was carried out, together with an energy content evaluation for each stream in the WWTP. Results showed that 37% of the carbon found in the raw wastewater was removed during the active sludge process and 24% was transformed into biogas. The remaining carbon was found in the anaerobic dewatered sludge (22%) and in the treated water (19%). As a result, 34% of the initial energy was recovered in the form of biogas.

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2.1. INTRODUCTION

Wastewater treatment plants (WWTPs) play an important role in environmental protection. In past decades, all efforts were focussed on obtaining high quality water and today mature technology based on biological removal of organic matter and nutrients is available. In particular, these technologies are highly effective but also not very efficient in terms of energy consumption. The activated sludge system is the most common biological treatment in WWTPs due to its good performance and its high effectiveness, but it also uses up high amounts of energy; representing over 40% of the total electric energy required to operate a sewage plant (Guo *et al.*, 2001, Tsagarakis and Papadogiannis, 2006). The amount of energy consumed in WWTPs depends on various factors such as the size of the plant, its design and operation, the composition of the wastewater (WW), urban WW/industrial WW ratio, the quality required for the discharge, as well as other local factors; but it is generally considered to add up 108,000 to 216,000 $\text{kJ}\cdot\text{inhabitant}^{-1}$ equivalents per year (Kolisch *et al.*, 2009).

The new challenge of WWTPs is to minimize energy consumption maintaining the quality of the discharged water (Chae and Kang, 2013). Energy optimization could be obtained reducing the electric energy consumption of the different units of the WWTP, but also recovering energy from the WW (Frijns *et al.*, 2013). The solids found in raw WW contain nearly 3.2 $\text{kJ}\cdot\text{g}_{\text{TS}}^{-1}$ and the average energy requirements to treat raw WW is 0.35 $\text{kJ}\cdot\text{g}_{\text{TS}}^{-1}$, this means that the total energy available could exceed the electricity requirements for the treatment process (Shizas and Bagley, 2004); suggesting that WWTPs could become net producers of renewable energy if an appropriate energy recovery technology is applied in combination with a new conceptual design for the treatment of WW (Verstraete *et al.*, 2009, Jenicek *et al.*, 2013). But nowadays this is still far from feasible.

In this context, anaerobic digestion (AD) is a well developed and robust technology commonly used to recover energy from organic streams. AD is a biological process able to transform organic compounds into biogas, a mixture of CH_4, and CO_2. The lower heating value of biogas depends on the percentage of methane which is usually found to be roughly around 23,400 $\text{kJ}\cdot\text{Nm}^{-3}$ (Chan *et al.*, 2009).

AD is not frequently used to treat WW due to the low organic matter concentration and the low temperature characteristics of raw WW, resulting in low treatment efficiencies and in the accumulation of suspended solids (Jenicek *et al.*, 2013). However, AD is often implemented in the sewage sludge (SS) treatment line aiming to SS stabilization and energy recovery in the form of biogas. Biogas can be used as fuel for a burner used to maintain the reactor's temperature, to fuel a combined heat and power (CHP) engine, or once upgraded, injected in the gas grid or as fuel for road transportation (Chan *et al.*, 2009).

Anaerobic digestion optimization is a common practise to increase the energy self-sufficiency of WWTPs; in this regard SS pre-treatments aiming to improve the biodegradability of SS and consequently increase biogas production are a common practice, though a carefully energy balance is required (Pérez-Elvira *et al.*, 2006, Carrère *et al.*, 2010). Besides pre-treatments, co-digestion, in its turn, optimizes anaerobic digestion increasing the organic loading rate (OLR) with other organic wastes with high methanogenic potential showing an extensive increase in biogas production (Alatriste-Mondragón *et al.*, 2006, Silvestre *et al.*, 2011[1], Silvestre *et al.*, 2014[2]). In all cases, the optimization of energy recovery through biogas production enhances WWTPs environmental performance and lowers its carbon foot print (Barber, 2008, Bravo and Ferrer, 2008).

The primary energy produced from sewage sludge´s biogas in Europe during 2014 year was $4.97 \cdot 10^{13}$ $kJ \cdot y^{-1}$ (EurObser´vER, 2014). In Spain it has been estimated that the energy contained in SS produced is of around $5.94 \cdot 10^{12}$ $kJ \cdot y^{-1}$ (Gómez *et al.*, 2010) though little ($1.41 \cdot 10^{12}$ $kJ \cdot y^{-1}$) is being nowadays recovered (EurObser´vER, 2014). Catalonia (northeast of Spain) has 340 WWTPs with different size and flow chart configurations for organic matter and nutrient removal. AD is currently implemented in the 26 biggest WWTPs for the treatment of sewage sludge. Most of these plants use biogas to control the temperature of the reactor, the excess being burned in a flare. Only 11 of these plants generate electric energy using CHP engines fuelled with biogas.

To perform an energy balance in a WWTP a necessary first step is to assess the energy content of the different streams, to analyze the energy recovery through biogas, and then estimate the maximum energy self-sufficiency attainable in the WWTP. Besides, it could be helpful to analyze different alternative configurations on the water and sludge lines so as to optimize said energy balance (Garrido *et al.*, 2013, Puchajda and Oleszkiewicz, 2008, Schwarzenback *et al.*, 2008). A calorimetric pump is

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[1] Chapter 4

[2] Chapter 5

the most common technique used to estimate low calorific value (LCV) of different kinds of samples (Shizas and Bagley, 2004) but, being it a device not always available, it is necessary to test simpler methods to estimate LCV. The aim of this study is to determine the role of anaerobic digestion on the energy self-sufficiency of the WWTPs target. The first part of the study assesses the electric and thermal energy balances in the WWTPs, analysing the main factors involved in energy consumption, and also carries out an economic feasibility analysis on the possible inclusion of CHP engines. In the second part, an energy flow analysis and a substance flow analysis of the carbon (C), nitrogen (N) and sulphur (S) present in one of the WWTPs is performed. The use of elemental analysis is also tested to estimate the LCV of every stream in the WWTP.

2.2. MATERIALS AND METHODS

2.2.1 Wastewater treatment plants

Five WWTPs ($WWTP_1$-$WWTP_5$), located in Catalonia (Spain), treating a mixture of domestic WW (60-70%) and industrial WW (30-40%), are analyzed. All WWTPs have a similar flowchart comprising a physical pre-treatment, a primary treatment, a secondary treatment and a sewage sludge line. The main characteristics and operational parameters of the WWTPs are summarized in Table 2.1. The pre-treatment consists of several bar and fine screens with different spacing, and a dissolved air flotation (DAF) unit to remove floatables as greases, and sand. A primary settler removes the suspended solids, and the activated sludge system does the same with the dissolved organic matter. The activated sludge systems of $WWTP_1$, $WWTP_3$ and $WWTP_4$ are designed to operate with low organic loads, between 0.35 and 0.56 $kg_{BOD}\cdot m^{-3}\cdot d^{-1}$ and Food to Microorganism ratio (F/M) between 0.09-0.13 $g\cdot g^{-1}\cdot d^{-1}$, while the remaining two WWTPs ($WWTP_2$ and $WWTP_5$) operated in high load mode, between 0.80-1.24 $kg_{BOD}\cdot m^{-3}\cdot d^{-1}$, and F/M ratio around 0.30 $g\cdot g^{-1}\cdot d^{-1}$. Despite these differences, the BOD and COD removal are similar in the 5 WWTPs, above 90% and between 80 and 87%, respectively. $WWTP_1$ and $WWTP_4$ are designed and operated to remove nutrient (nitrogen and phosphorus) with efficiencies higher than 80%. Accordingly, they have a high sludge retention time (SRT), 18 days and 15 days, respectively. Contrary, $WWTP_{2,\ 3,\ 5}$ are not designed to remove nutrients, with low SRT: 8, 6 and 4 days, respectively. Nevertheless, small amounts of nutrients are also removed by these plants.

Table 2. 1. *Main characteristics of the WWTPs studied*

	Water Line				**Sludge Line**					
WWTP	**Inhabitant equivalents**	**Flow** **($m^3 \cdot d^{-1}$)**	**OLR[1]** **($kg_{BOD} \cdot m^{-3} d^{-1}$)**	**F/M[2]** **($g_{BOD}\ g_{SSV}^{-1} d^{-1}$)**	**Thickening system for PS and WAS**	**Volume AD[3] reactor (m^3)**	**PS[4]:WAS[5]** **(VS basis)**	**HRT_{AD}[6]** **(days)**	**OLR_{AD}[7]** **($kg_{VS}\ m^{-3}\ d^{-1}$)**	**Biogas** **($m^3\ d^{-1}\ m^{-3}$)**
$WWTP_1$	190,380	26,587	0.35	0.13	Settler Centrifugation	7,500	68:32	32	0.78	0.30
$WWTP_2$	358,000	29,086	0.80	0.34	Settler Flotation	7,000	57:32	30	0.86	0.60
$WWTP_3$	121,500	21,765	0.56	0.12	Settler Flotation	8,800	46:54	22	1.02	0.36
$WWTP_4$	204,166	21,074	0.39	0.09	Settler Flotation	8,000	46:54	24	0.86	0.61
$WWTP_5$	451,250	27,873	1.24	0.29	Settler Centrifugation	7,040	58:42	19	1.23	0.87

[1]Organic loading rate (OLR); [2]Food to microorganisms ratio (F/M); [3]Anaerobic digestion (AD); [4] primary sludge (PS); [5]waste activated sludge (WAS); [5]anaerobic digestion; [6]AD hydraulic retention time (HRT_{AD}); [7]AD organic loading rate (OLR_{AD}).

The sewage sludge line consists of a thickener unit, a mesophilic anaerobic digestion unit, and a dewatering unit. In all five plants the primary sludge is thickened by sedimentation while the secondary sludge is thickened by aerated flotation, in WWTP$_2$, WWTP$_3$ and WWTP$_4$, and by centrifugation with the addition of an organic polymer in WWTP$_1$ and WWTP$_5$. The anaerobic digestion process, in all cases, consists of a continuous stirred tank reactor (CSTR) with a digestion volume between 7,000 - 8,800 m^3. The average hydraulic retention time (HRT) applied on the different WWTPs varies between 19-32 days.

Dewatering is performed with a centrifuge in all WWTPs except for WWTP$_3$, where a filtration unit is employed. WW and sewage sludge flow rates, physicochemical characteristics (chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solids (TSS), total nitrogen (TN) and total phosphorus (TP)) of the different streams of the WWTP, operational parameters of the aerobic and anaerobic processes, biogas production, and the electric and thermal energy production (considering the use of a CHP engine) and consumption of the plant were jointly used for the energy and economic balance calculations. The aforementioned data were provided by the plant operators, and correspond to the monthly average of an entire year in operation of the WWTPs.

2.2.2 Energy and economic balance calculations

2.2.2.1 Electric and thermal energy production from biogas

The biogas conversion into electricity and thermal energy was calculated considering a CHP and the monthly biogas production rates in equations 1 and 2, respectively.

$$EE_P = Y_{CH4} \cdot P_{calCH4} \cdot \eta_E \cdot t_{we} \cdot t_{wd}^{-1} \cdot 0.9 \qquad \text{(Eq 1)}$$

$$TE_P = Y_{CH4} \cdot P_{calCH4} \cdot \eta_T \cdot t_{we} \cdot t_{wd}^{-1} \qquad \text{(Eq 2)}$$

where EE_P (kJ·d^{-1}) is the net electric energy production, TE_p (kJ·d^{-1}) is the net thermal energy production, Y_{CH4} is the methane production (m^3·day^{-1}), P_{calCH4} is the heating value of methane (34,020 kJ·m^{-3}), η_E is the electric efficiency of the CHP engine (35%), η_T is the thermal efficiency of the CHP engine (55%), t_{we} are the working hours per month of the CHP engine (666.7 h·month^{-1}, with a total of 8000 h·year^{-1}), t_{wd} are the hours that the digester is producing biogas (considering 24 h per day, and 30 days per month), and 0.9 is a factor taken into account for the CHP engine's self consumption (10% of the total electric energy generated).

2.2.2.2 Electric and thermal energy consumption

All data relative to the electric energy consumption (EE_C) were provided by plant operators and corresponded to the monthly average of electric consumption during an entire year in operation. Thermal energy requirements (TE) for each WWTP correspond to the requirements for heating the raw sludge (q_T - $kJ \cdot d^{-1}$) and maintaining the anaerobic reactor's temperature (q_L - $kJ \cdot d^{-1}$). Heat requirements were calculated on a monthly basis considering the average temperature of the city where the WWTP is located. Thermal energy requirements were calculated according to equations 3 and 4, considering that the reactor shape was cylindrical with a diameter 3 fold higher than its height, with a conical floor, and made of concrete with a wall thickness of 300 mm.

$$q_T = F_S \cdot \rho \cdot (T_D - T_S) \cdot C_p \qquad \text{(Eq 3)}$$

$$q_L = A_W \cdot (T_D - T_A) \cdot U_W + A_F \cdot (T_D - T_F) \cdot U_F + A_R \cdot (T_D - T_A) \cdot U_R \qquad \text{(Eq 4)}$$

where F_S is the sludge flow ($m^3 \cdot d^{-1}$), ρ is the sludge density ($kg \cdot m^{-3}$) , T_D (ºC) is the operating temperature of the digester (35 ºC), T_S (ºC) is the temperature of the sludge which was considered to be 5 ºC higher than the average monthly environmental temperature, and Cp is the specific heat value (4.16 $kJ \cdot {}^{o}C^{-1} \cdot kg^{-1}$). T_A (ºC) is the average monthly environmental temperature; T_F (ºC) is the temperature of the floor which was considered to be 3ºC higher than the average environmental temperature, A_W, A_F and A_R are the areas of the walls (m^2), floor and roof respectively, and U_W, U_F and U_R are the heat transfer coefficients ($kJ \cdot s^{-1} m^{-2} \cdot K^{-1}$) of the walls, floor and roof, respectively. Two different situations were considered – with insulation ($U_W = 7 \cdot 10^{-4}$ $kJ \cdot s^{-1} \cdot m^{-2} \cdot K^{-1}$) and without insulation ($50 \cdot 10^{-4}$ $kJ \cdot s^{-1} m^{-2} \cdot K^{-1}$). The value for U_F was $17 \cdot 10^{-4}$ $kJ \cdot s^{-1} \cdot m^{-2} \cdot K^{-1}$considering that the floor of the digester was below ground level and in contact with dry earth, and the values for U_R were $14 \cdot 10^{-4}$ $kJ \cdot s^{-1} \cdot m^{-2} \cdot K^{-1}$ and 45 10^{-4} $kJ \cdot s^{-1} \cdot m^{-2} \cdot K^{-1}$–with and without insulation respectively–, assuming a cover thickness of 100 mm (Metcalf & Eddy, 2004).

In addition, the thermal energy (TE) required for drying the dewatered sludge was calculated using the monthly flow and total solid concentrations of each WWTP, taking into account that the T_E needed for evaporation and heating is 2,628 kJ·kg^{-1} and assuming that dry sludge has a total solid concentration of 92% (Stasta *et al.*, 2006).

2.2.2.3 Economic feasibility analysis

The aim of this analysis was to assess the economic feasibility of the possible inclusion of a CHP unit fuelled by biogas in the WWTPs analysed, since none of them have one. The features of the CHP engines were selected according to the maximum potential production per month of each WWTP. The investment cost for each CHP unit was estimated using equation 5, where the cost (Co; €) depends on power (P_w; kJ). The equation was based on information provided by different CHP suppliers.

$$Co = 4480.90 \cdot P_w^{0.7383} \qquad \text{(Eq 5)}$$

The payback period of the investment was calculated taking into account that the electric energy generated is sold into the Spanish grid, with an average price of 216 €·kJ^{-1}. A sensitive analysis was carried out to ascertain the influence of the price of electric energy on the payback period.

2.2.3 Energy and substance flow analysis

The energy and the C, N and S flow analysis of WWTP$_2$ was performed taking samples and analysing the elemental composition of the raw wastewater, the treated wastewater, the primary and secondary sludge, and the dewatered digested sludge. The samples were dried at low temperature (60ºC) during 24 h in an oven. The low temperature was selected in order to minimize the loss of volatile compounds. The lower calorific value (LCV; kJ·g_{TS}^{-1}) of the samples was determined analyzing the elemental composition (carbon-C, nitrogen-N, sulphur-S, hydrogen-H and oxygen-O) of the total solids (TS) and inorganic solids (IS) of the different samples and applying the modified Du Long equation (Equation 6) (Tchobanoglous *et al.*, 1993).

$$LCV = 80.56 \cdot C\% + 338.89 \cdot (H\% - O\% \frac{1}{8}) + 22.22 \cdot S\% + 5.56 \cdot N\% \qquad \text{(Eq 6)}$$

The elemental composition (C%, H% and S %) was determined by catalytic oxidation combined with gas chromatography (LECO). N percentage in the TS and in the IS was determined measuring the total Kjeldahl nitrogen (TKN), total ammonia nitrogen (NH_4^+-N), nitrate (NO_3^--N) and nitrite (NO_2^--N) concentrations, according to standards methods (APHA, AWWA, WEF, 1995).

2.3. RESULTS

2.3.1 Analysis of the WWTPs performance

WW flow was found to be similar in all WWTPs with a daily flow between 20,000 and 30,000 $m^3 \cdot d^{-1}$, but also happened to show great differences in WW characteristics. COD, BOD and TN concentrations vary between 571-1,046 $mg_{COD} \cdot L^{-1}$, 296-550 $mg_{BOD} \cdot L^{-1}$ and 41-71 $mg_{TN} \cdot L^{-1}$ respectively. On the other hand, TSS concentrations in the raw WW were similar in all cases, with values between 339-359 $mg_{TSS} \cdot L^{-1}$.

The primary settler performs similarly in all WWTPs, with over 50% in removal efficiency of TSS and a maximum of 80% in the case of $WWTP_1$. COD and BOD removals were between 30-40% and 35% in the five WWTP.

The secondary treatment in all WWTP consisted in an activated sludge process, but with different designs and operational conditions. The activated sludge units in the $WWTP_1$, $WWTP_3$, and $WWTP_4$ operated with a low to medium organic loading rate (OLR) (between 0.35 and 0.56 $kg_{BOD} \cdot m^{-3} \cdot d^{-1}$) and a food to microorganism ratio (F/M) between 0.09 and 0.13 $g_{BOD} \cdot g_{SV}^{-1} \cdot d^{-1}$ (Table 2.1). In the case of $WWTP_2$, and $WWTP_5$ the activated sludge system operated with a higher organic load (0.80-1.24 $kg_{BOD} \cdot m^{-3} \cdot d^{-1}$ respectively) and an F/M ratio of 0.3 $g_{BOD} \cdot g_{SSV}^{-1} \cdot d^{-1}$. Despite these differences, COD and BOD removal efficiencies were similar in all WWTPs, with BOD and COD removal efficiencies over 90% and between 80 - 87% respectively. The HRT applied to the anaerobic digester varied widely between the different WWTPs, being comprised between 19 and 32 days (Table 2.1). The organic loading rate (OLR) was between 0.78-1.23 $kg_{VS} \cdot m^{-3} \cdot d^{-1}$, and VS removal was close to 40% in all anaerobic digesters. Daily biogas productions were between 2,250 and 6,125 $m^3 \cdot d^{-1}$ (0.30-0.87 $m^3 \cdot m^{-3} \cdot d$). $WWTP_5$ produced 3-fold more biogas than $WWTP_1$, probably due to its higher OLR and minor HRT; and $WWTP_2$, –despite its anaerobic reactor working with similar conditions than the one of $WWTP_1$– produced 2-fold more biogas. This shows that biogas production rates not only depend on the operational conditions of the reactor, but also on the sludge thickening system used, on the composition of the sludge (concentration and characteristics of its organic matter) and on the percentage of primary sludge versus WAS. Primary sludge degrades more easily and shows higher methane potential than WAS (Gavala *et al.*, 2003).

2.3.2 Energy balance of the WWTPs

2.3.2.1. Electric energy balance

Table 2.2 shows the electric energy consumption (EE_C) for the five WWTPs and the estimated electric energy production (EE_P) generated with a CHP engine fuelled with the biogas produced in the anaerobic digestion of the sewage sludge. The estimated electric energy supply (EE_S) recovered from the biogas produced in an entire year of operation was between 39% and 76% of the EE_C. Figure 2.1a shows the EE_C and the EE_P ($kJ·m_{ww}^{-3}$) against the BOD of the inflow WW (BOD_{ww}). As can be seen, the EE_P showed a strong lineal correlation with BOD_{WW} (R^2=0.974), the higher the BOD_{ww}, the higher the biogas production, and therefore a higher production of electric energy could be expected. On the contrary, the EE_C doesn't show a clear linear relationship with BOD_{ww} (R^2=0.684). This confirms than the EE_C not only depends on the inflow BOD but on other parameters like the operating conditions of the activated sludge unit, its removal efficiency, etc. On the other hand, the EE_C per unit of BOD removed showed a strong lineal correlation (R^2=0.890) with the F/M ratio applied to the activated sludge system (Figure 2.1b). As expected, when the aerobic reactor is operated with a lower F/M ratio, oxygen requirements are found to be higher and, as a consequence, the electric energy demand increases.

Figure 2c shows the estimated percentage of the EE supplied (EE_S) by the biogas on a monthly basis. In all cases, except for some isolated months, the estimated energy supply was below the energy required, presenting strong variations throughout the year. Several causes could affect the percentage of EE_S by biogas like the changes in WW inflow and its composition (mainly BOD), which in turn modifies the EE_C as well as the EE_P. Changes on the production of sludge in the water line modify the HRT and the OLR applied to the anaerobic digester, and consequently the biogas production. Depending on the WWTP, the months with lower EE_S percentage correspond to the summer months ($WWTP_1$, $WWTP_3$, and $WWTP_4$) or winter months ($WWTP_2$, and $WWTP_5$). Different behaviours of the population and industries discharging WW to the treatment plant (holidays, closure periods, etc.) can explain those differences between WWTPs.

In order to became energetically self-sufficient, the studied WWTPs should increase more than 3-fold their biogas production.

Table 2. 2. *Energy balance of the five wastewater treatment plants (WWTPs)*

Parameters	**units**	**WWTP$_1$**	**WWTP$_2$**	**WWTP$_3$**	**WWTP$_4$**	**WWTP$_5$**
^{1}EE$_C$	kJ d^{-1}	4.24E+07	4.61E+07	4.44E+7	6.78E+07	5.88E+07
^{2}EE$_P$	kJ d^{-1}	1.64E+07	2.89E+07	2.47E+07	3.73E+07	4.46E+07
^{3}EE$_S$	%	39	63	56	55	76
^{4}TE$_C$ (insulated)	kJ d^{-1}	1.71E+07	1.70E+07	2.75E+07	1.94E+07	2.21E+07
Heat Loses (insulated)	%	12	11	8	9	9
TE$_C$ (not insulated)	kJ d^{-1}	2.12E+07	4.13E+07	3.13E+07	2.97E+07	2.59E+07
Heat Loses (not insulated)	%	30	29	21	31	20
^{5}TE$_P$	kJ d^{-1}	2.35E+07	4.13E+07	3.52E+07	5.32E+07	6.37E+07
^{6}TE$_S$ (insulated)	%	111	100	113	179	245
TE$_S$ (not insulated)	%	137	243	128	274	288

^{1}EE$_C$: Electric energy consumption; ^{2}EE$_P$: Electric energy production (estimated from a CHP engine fueled with biogas); ^{3}EE$_S$: Electric energy supplied by biogas; ^{4}TE$_C$: Thermal energy consumption; ^{5}TE$_P$: Thermal energy production (estimated from a CHP engine fueled with biogas); ^{6}TE$_S$: Thermal energy supplied by biogas.

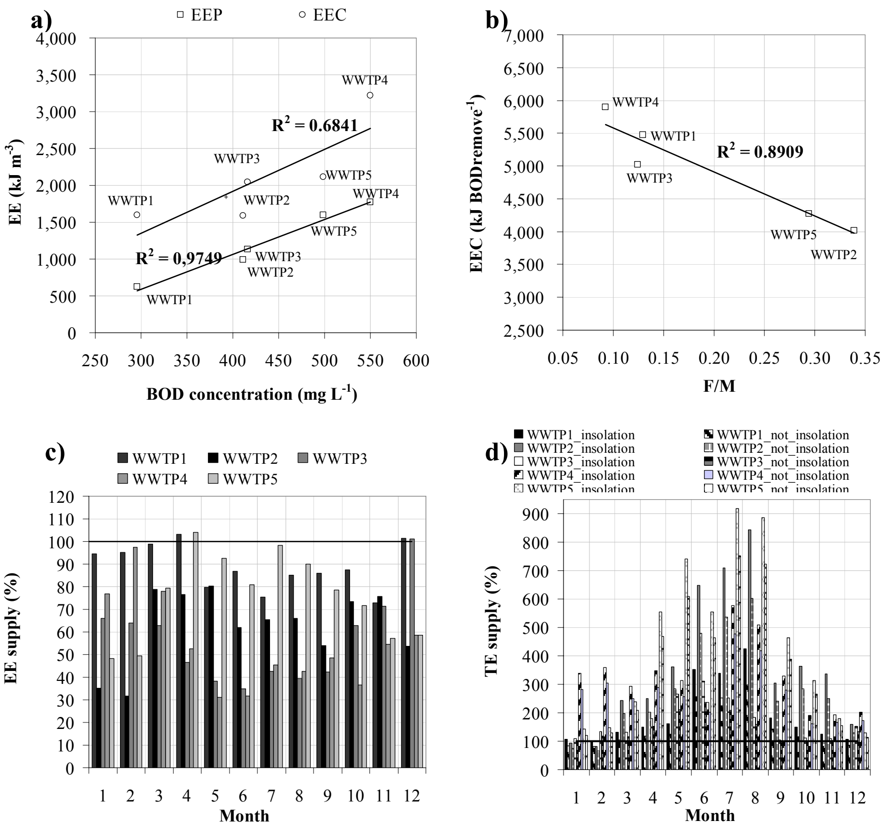

Figure 2. 1. *Energy assessment of the WWTP studied: (a) Electric energy consumption (EEC) and electric energy production (EEP) as a function of the initial BOD; (b) EEC/BODremoval ratio as a function of Food to Microorganism ratio (F/M) in the active sludge system; (c) Electric energy supply (EES) by the CHP fuelled with the produced biogas (monthly basis); (d) Thermal energy supply (TE_s) by the CHP fuelled with the produced biogas (monthly basis) with reactor insulated and not insulated.*

2.3.2.2. Thermal energy balance

In order to check if the estimated thermal energy (TE) recovered in the CHP engine could be enough to fulfil the heat requirement of mesophilic anaerobic reactors, a thermal energy balance was performed in two different situations, with an insulated and a non-insulated reactor (current situation). The TE produced (TE_P) provides enough energy to maintain the temperature of the reactor in both situations (Figure 2.1 d); expect for the winter months (month 1 and month 2 in $WWTP_1$ and $WWTP_2$).

The estimated excess of TE can be used for other purposes in the WWTP, like heating sanitary water or providing part of the energy required for drying the dewatered sludge, thus lowering the economic costs associated with sludge management, and being environmentally and economically advantageous solution (Viklund and Lindkust, 2015). This excess of TE can provided between 26% and 51% of the TE required for drying the SS when the anaerobic reactor is insulated, and between 9 and 46% with a non-insulated one. It is also important to consider –just as for the EE_P, TE_P fluctuations throughout the year (Figure 2.1d).

2.3.3 Economic feasibility analysis

None of the five WWTP had CHP engine, so the installation of such equipment would have an impact in the economic performance of each plant. In order to estimate the payback period, the nominal power for the CHP engines was selected according to the biogas produced in each WWTP (300 kW ($WWTP_1$); 500 kW ($WWTP_2$); 500 kW ($WWTP_3$); 700 kW ($WWTP_4$); 800 kW, ($WWTP_5$)) and the payback period of the CHP motor was calculated taking into account an electric energy yearly average sale price of 216 € kJ^{-1} biogas. The payback period was between 2 and 3 years in all the cases – 1.9, 2.4, 3.0, 2.6, and 2.2 for $WWTP_1$, $WWTP_2$, $WWTP_3$, $WWTP_4$, and $WWTP_5$, respectively. A sensitive analysis of the payback period as a function of the sale price of electric energy was also carried out. As seen in Figure 2.2, the payback period was lower or close to 2 years in all WWTPs analysed when the selling price was ≥216 €·kJ^{-1}. But when the selling price falls down to ≤ 72 €·kJ^{-1}, a sharp increase in the payback period is shown, and the differences reflected between each WWTP become quite significant.

The sensitive analysis showed that the inclusion of a CHP engine in an average WWTP (with a WW flow between 20,000 and 30,000 $m^3 \cdot d^{-1}$ or inhabitant eq. = 121.500 – 451.250), in order to produce electric energy is economically feasible with low payback periods when the price of electric energy is at least 216 €·kJ^{-1}.

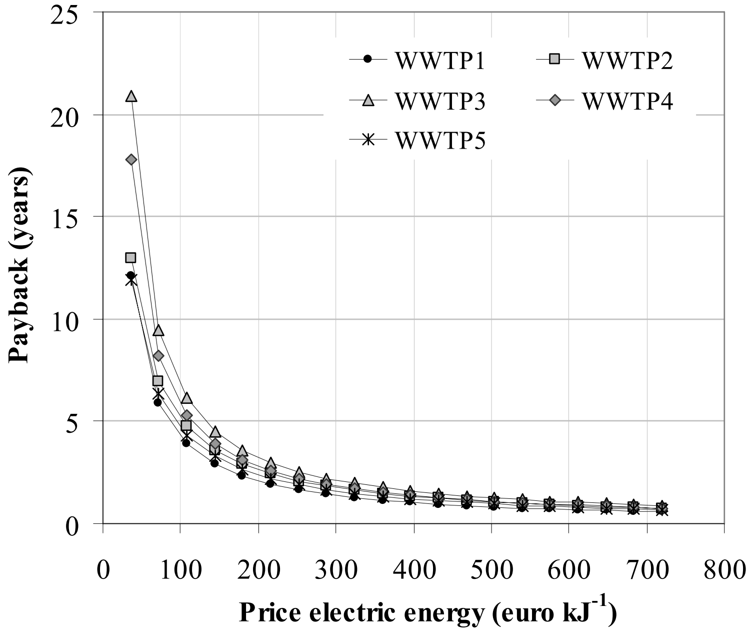

Figure 2. 2. *Payback period for the investment cost of the CHP engine as a function of the price of electric energy*

2.3.4 Energy and substance flow analysis of WWTP$_2$

In order to assess the performance of anaerobic digestion as a technology able to recover the energy content in WW, an energy flow analysis of WWTP$_2$ was performed. Five samples from different streams of the water and sludge lines were taken for this purpose (raw and treated WW, primary, secondary and dewatered digested sludge). Table 2.3 shows the characteristics of each of the streams sampled. As can be seen, the LCV of the TS of raw WW was 6.8 kJ·g_{TS}^{-1}, it being 6 times lower in the TS of the treated wastewater (1.7 kJ·g_{TS}^{-1}). The primary sludge presented the highest energy content (22.8 kJ·g_{TS}^{-1}), followed by the secondary sludge (16.1 kJ·g_{TS}^{-1}) and the dewatered digested sludge (11.95 kJ·g_{TS}^{-1}). These results are found to be in accordance with Shizas and Bagley (2004) – the settleable solids of the WW (primary sludge) have the highest carbon concentration and consequently the highest energy content and biogas potential. Nevertheless, LCV of the WW, the primary and secondary sludges obtained in this study are higher, in comparison, with those values obtained by Shizas and Bagley (2004) –3.2, 15.9 and 12.4 kJ·g_{TS}^{-1}, for raw WW, primary sludge and secondary sludge respectively–, while the energy content of the dewatered digested sludge was similar (12.7 kJ·g_{TS}^{-1}).

The methodology used by Shizas and Bagley (2004) consisted on drying the samples in an oven (103ºC, overnight) and determining the heat of combustion with a calorimetric pump. In a more recent study, Heidrich *et al.* (2011) reported similar LCV of two samples of raw wastewater. In this case LCV was also analyzed with a calorimetric pump, but the samples were dried in an oven or freezer in order to minimize the loss of volatiles. For oven-dried samples the LCV values were 5.96 and 5.23 kJ·g_{TS}^{-1}, and for freeze-dried samples were 10.5 and 6.73 kJ·g_{TS}^{-1}. In the present study, the samples were dried in an oven at low temperature (60ºC), its elemental composition was then analyzed, and LCV was calculated according to equation 6. The LCV of raw waste water was similar to the LCV of the oven-dried samples obtained by Heidrich *et al.* 2011. This suggests that the results obtained with the methodology applied in the present study are in accordance with the results experimentally obtained with a calorific pump. However, the discrepancy in the results obtained in the study of Heidrich *et al.* 2011 point out that the drying method of the sample used before determining the heat of combustion with a calorimetric pump or analysing the elemental composition exerts a large influence on the resulting LCV (Heidrich *et al.*, 2011).

Table 2. 3. *Main characteristics of the different streams of the WWTP$_2$: total solids (TS), volatile solids (VS) and elemental composition of TS*

	TS *(%)*	**VS** *(%)*	**C_{TS}** *(gkg_{TS}^{-1})*	**N_{TS}** *(gkg_{TS}^{-1})*	**S_{TS}** *(gkg_{TS}^{-1})*	**LCV** *(kJ g_{TS}^{-1})*
Wastewater	0.13	0.05	191	51	47	6.8
Treated wastewater	0.09	0.02	57	56	58	1.7
Primary sludge	3.00	1.34	409	69	6	22.8
Secondary sludge	3.04	2.37	382	97	10	16.1
Dewatered digested sludge	23.60	12.72	284	54	11	11.9

C_{TS} - Total Carbon, N_{TS} - Total Nitrogen, S_{TS} - Total Sulphur and LCV - Low Calorific Value of the TS.

Differences on the reported LCV could be also attributable to differences in WW composition, particularly VS/TS ratios. While the WW of present study and those of Heidrich's showed VS/TS ratios between 0.38 and 0.68 (Heidrich *et al.*, 2011), those WW samples analysed by Shizas and Bagley (2004) showed a minor ratio (0.12). Similarly, the

VS/TS ratio in the primary and secondary sludges analysed in the present study were 1 to 3 fold higher than those VS/TS ratios obtained by Shizas and Bagley (2004). Contrarily, the anaerobic sludge presented similar VS/TS ratios and accordingly similar LCV values.

As expected, total carbon concentrations in the TS (C_{TS}) showed a similar pattern than LCV, the samples with higher LCV have also higher C_{TS}. Nitrogen contents of TS (N_{TS}) presented a similar pattern; raw WW has a similar composition than treated WW and the dewatered digested sludge, while primary and secondary sludges showed a higher concentration. As for the sulphur content of TS (S_{TS}), the samples presented different behaviours: raw WW and treated WW presented a similar S_{TS}, while the S_{TS} of the primary sludge was very low, probably because sulphur may be found in the form of soluble sulphates. The S_{TS} of the secondary sludge and the digested sludge is similar and showed low values.

Table 2.4 shows the energy, carbon, nitrogen and sulphur flows in the different streams of the WWTP. As can be seen, raw WW presented a daily energy flow of 2.7 E+8 kJ d^{-1} corresponding to 7,427 kg·d^{-1} of carbon, and a flow of nitrogen and sulphur of 1,967 kg·d^{-1} and 1,815 kg·d^{-1} respectively. The effluents of the WWTP –the treated wastewater– presented an energy flow of 4.3 E+7 kJ·d^{-1} and a carbon, nitrogen and sulphur flow of 1,439 kg·d^{-1}, 1,419 kg·d^{-1} and 1,463 kg·d^{-1} respectively.

Table 2. 4. *Energy flow (EF), total carbon (C_T), total nitrogen (N_T) and total sulphur (S_T) flow of the different streams of the $WWTP_2$.*

	EF (kJ·d^{-1})	**C_T** (kg·d^{-1})	**N_T** (kg·d^{-1})	**S_T** (kg·d^{-1})
Waste Water	2.7 E+08	7,427	1,967	1,815
Treated Water	4.3 E+07	1,439	1,419	1,463
Primary Sludge	1.3 E+08	2,251	380	33
Secondary Sludge	5.1 E+07	1,207	306	31
Dewatered anaerobic Sludge	6.9 E+07	1,632	308	65
Biogas	9.3 E+07	1,839	-	-

The dewatered digested sludge –the other effluent of the WWTP– presents also a significant amount of energy (6.9 E+07 kJ·d^{-1}) and carbon

(1,632 kg·d^{-1}), but low nitrogen and sulphur flows 308 kg·d^{-1} and 65 kg·d^{-1} respectively.

The percentage distribution of the initial content of the energy, carbon, nitrogen and sulphur between the different streams of the WWTP is shown in the Figure 2.3. The primary sludge contained about 48% of the initial energy of the raw WW, while the secondary sludge only contained 19%. The treated WW contained 16% of the initial energy and the remaining 17% is presumably dissipated when oxidizing the soluble organic matter in the aerobic reactor. Therefore, 67% of the initial energy of the WW was transferred to the sludge line, and during the anaerobic treatment 52% of the energy contained in the sludge was transformed into biogas –accounting for 34% of the initial energy content in raw WW–, and the other 48% remained in the anaerobic sludge. Finally, after centrifugation, 26% of the energy content of the raw WW remains in the dewatered sludge. As mentioned in section 2.2, the energy recovered in the biogas, results in a 65% energy self sufficiency for $WWTP_2$. Garrido *et al*. 2013 reported 80% energy self-sufficiencies on a WWTP with similar characteristics, and proposed alternatives to exceed the 100% self sufficiency improving the efficiency of the primary settler and/or the inclusion of the autotrophic nitrogen removal process in the water line.

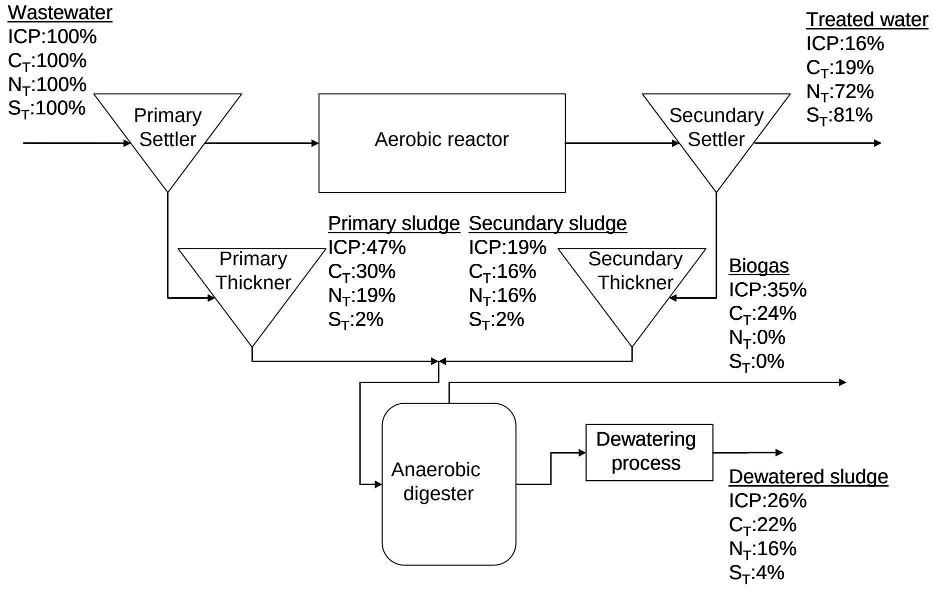

Figure 2. 3. *Energy flow (EF), total carbon (C_T), total nitrogen (N_T) and total sulphur (S_T) balance of the different streams of the $WWTP_2$.*

The distribution of C_T, as expected, showed a similar pattern than the energy flow (Figure 2.3). 30% and 16% of the C_T is transferred to the primary and secondary sludges respectively, 35% is oxidized in the active sludge unit, and 19% of the C_T remained in the treated water. The C_T transferred to the sludge line is then transformed into biogas (24% of the initial C) and 22% remains in the dewatered digested sludge.

On the other hand, total nitrogen (N_T) and total sulphur (S_T) distribution showed different behaviours. As expected, considering that the activated sludge unit was not designed neither operated to remove nitrogen, 72% of the N_T remained in the treated water. Only 19% and 16% of the initial nitrogen was found in the primary and secondary sludges respectively. As for S_T, 81% of the initial S_T remained in the treated water and only 4% was found in the primary and secondary sludges.

2.3.5 New approaches to optimize WWTP energy balance

Results show than the anaerobic digestion technology plays an important role in energy self-sufficiency of a WWTP. Different factors contribute to energy balance; among others, the low electric efficiency of the commonly used CHP engines seems to be of special relevance. The interest of new equipments with higher electric efficiency has aroused in recent years. The solid oxide fuel cell (SOFC)-based CHP could offer a net electric efficiency around 50-60% (Papurello *et al.*, 2014). This engine is very sensitive to several contaminants as sulphur, siloxane, aromatic and halogenated compounds, etc. Hence, a proper biogas cleaning unit is required that could increase significantly the operational cost (Papurello *et al.*, 2014). Nevertheless, the economic analysis showed that SOFCs could successfully compete with other cogeneration technologies shortly (Trendewicz and Braun, 2013, Siefert and Litster, 2014).

Another important factor is the energy consumed in the WWTP, mainly in the aerobic treatment. Big efforts to optimize the activated sludge system have been done since some time ago. WWTP reconfiguration, optimal control of the aeration systems, equipments maintenance and replacement, can lead to higher energy efficiencies and savings (Pittoors *et al.*, 2014). Liu *et al.* 2011 showed that the standard oxygen transfer rate of a 10-year-old aeration system is significantly reduced, and its replacement could lead to energy savings of over 10%, with a payback period less than 14 months. Nevertheless, maximising biogas production through pre-treatments and co-digestion, seem to be the best strategy (Alatriste-Mondragón *et al.*, 2006, Silvestre[1] *et al.*, 2011). The improvement of the primary settler efficiency could also lead to improve the biogas production. Notwithstanding, biological nutrients removal (BNR), nitrogen and/or phosphorous, rely on the availability of easy biodegradable organic carbon, and therefore primary settler is generally absent in WWTPs adopting BNR processes to have enough organic matter to remove the nutrients (Bolzonella *et al.*, 2006).

New developments for nutrient removal that does not require organic matter could be an alternative to maximize energy recovery without compromising nutrient removal efficiency. In this sense, partial nitritiation/anammox (PN/A) process could be an interesting option. Nowadays, exists more than 100 installation working in WWTP (Lackner *et al.*, 2014), but all of them process side streams (centrate from the sludge dewatering). The inclusion of PN/A in the main water stream of a WWTP presents limitations, due to the presence of organic matter, low N concentration and low temperature. Nevertheless, once those limitation will be overcome, the combination PN/A and anaerobic digestion could turn WWTP to neutral energy consumers, or even so in net energy producers.

2.4 CONCLUSIONS

Biogas can supply between 39% and 76% of the total energy consumed in wastewater treatment. Energy production strongly depends on organic matter concentrations in the initial wastewater, while energy consumption mainly depends on the operation of the active sludge system. The payback period of a CHP engine fuelled with biogas is dependent on the price of electric energy, but it will be less than 2 years should the price of energy be ≥ 216 €·kJ^{-1}.

It has been demonstrated that elemental composition is a good tool to estimate the energy content of the different streams of the WWTP as well as to perform an energy flow analysis of the whole plant. The energy flow analysis shows that 67% of the initial energy content of raw WW is transferred to the sewage sludge and that the anaerobic digestion process is able to recover 52% transforming it into biogas. These results suggest that anaerobic digestion is a good technology to recover energy from WW, although it is necessary to apply some strategies to increase biogas energy supply.

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[1] Chapter 4

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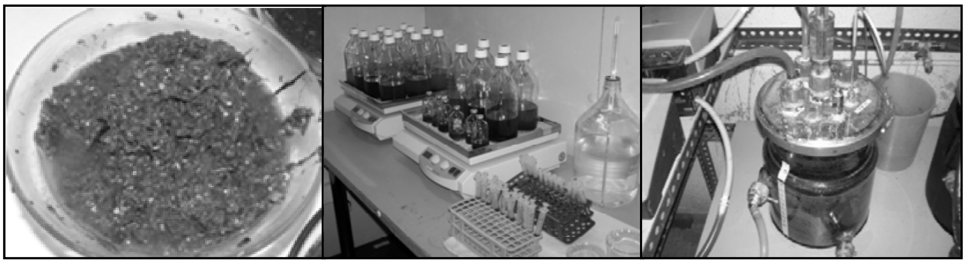

CHAPTER 3

CO-DIGESTION AS STRATEGY TO OPTIMISE BIOGAS PRODUCTION FROM SEWAGE SLUDGE: ADVANTAGES AND LIMITATIONS

The aim of this review is analyze the benefits and drawbacks of SS co-digestion practices, from lab scale tests to industrial experiences, since it can help to attain energy self-sufficiency in a wastewater treatment plant (WWTP). Household organic wastes or food wastes are the co-substrates traditionally receiving more attention, followed by wastes with high grease content. Other industrial wastes, such as recovered glycerol or crops, have been also analyzed as potential SS co-substrates. Many studies have been carried out in bench-scale continuous stirred digesters under mesophilic conditions, the organic loading rate (OLR) being the parameter that has more influence on the increase in methane production. Although few works reported inhibition episodes, some episodes related to organic overloading and fatty acid accumulation during SS co-digestion with high doses of grease wastes (>60% volatile solids in the feed) have been found. Besides this, pre-treatment appears to be a necessary step, when using food waste, in order to remove the inert fractions and reduce particulate size. This operation increases both investment and operational costs while limiting its implementation. Few research studies were focused on the global impact of SS co-digestion in a WWTP, the main problem being the increase of nitrogen and organic matter in the effluent returning to the water line. Finally, few works have ever paid attention to the implications of the ultimate end-use of the resulting digested sludge; to the evidence of change in its composition and/or to the increase in potentially toxic elements, or at least they have not been reported.

3.1 INTRODUCTION

Industrial and municipal wastewater treatment plants (WWTPs) are designed to remove contaminants, such as organic matter, nitrogen, phosphorous, etc., meeting the standards of regulation prior to the water being discharged. Although WWTPs have reached a high level of efficiency, a huge amount of sewage sludge (SS) is produced which should be properly managed. In parallel, the global energy crisis and a greater awareness of the consequences of climate change have awarded great importance to renewable energies as a source for WWTPs. In this context, anaerobic digestion (AD) has gained prominence as a technology capable to stabilize SS simultaneously producing renewable energy (biogas) which could supply between 40% and 70% of the total electrical energy demand of a WWTP (Shizas and Bagley, 2004, Silvestre *et al.*, 2015a[1])

Different strategies to increase biogas production have been studied in the last few years in order to finally attain energy self-sufficiency in a WWTP. Some approaches are based on the use of pre-treatments and/or SS co-digestion with others substrates. The right selection of SS pre-treatments can increase biogas production and volatile solid reduction, might improve the dewatering properties of the digested sludge and, in some cases, enhance pathogen removal (Carlsson *et al.*, 2012). These different procedures namely biological, mechanical, thermal and thermo-chemical pre-treatments and their effects on SS AD have been exhaustively analyzed in different literature surveys (Pérez-Elvira *et al.*, 2006, Carrère *et al.*, 2010). Thermal treatments are often the most effective, their main drawback being the cost of investment in new equipments, since the highest biogas increments are usually obtained at high temperatures and high pressures.

Anaerobic co-digestion is defined as the digestion of a mixture of different organic wastes with different origins and compositions in order to make the most of their composition's complementary characteristics. The right co-substrate selection can lead to an increase in biogas production. Moreover, the use of a co-substrate can also attenuate temporal variations in quantity and quality of the sludge and of each of the organic wastes used, unifying management methodologies and sharing treatment facilities to make a more efficient use of all shared equipment, thus reducing overall operational costs (Alatriste-Mondragón *et al.*, 2006). Co-digestion is not a new concept but, due to the enormous potential of co-substrate mixtures, different literature reviews have been

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[1] Chapter 2

published (Alatriste-Mondragón *et al.*, 2006; Mata-Alvarez *et al.*, 2011, 2014).

The selection of substrates –especially their composition–, and the proportion of each one in the feed mixture, is the key to success as it is necessary to obtain the best chemical composition to optimize the activity of the biomass involved in the anaerobic process (carbon-nitrogen ratio, pH, alkalinity, etc.); to avoid the inhibition of different components (ammonia, volatile fatty acids, intermediate products, etc.); to optimize methane production (Mata-Alvarez *et al.*, 2014); and to improve the dewaterability of the final effluent (Pérez-Elvira *et al.*, 2006).

The success of SS co-digestion strategies can be shown in some full-scale experiences, although scarce information is found in literature due to the industrial sector's lack of interest to publish their results in cientific publications (Mata-Alvarez *et al.*, 2014). Nonetheless, full-scale SS co-digestion operations face various technical and economic constraints which have reduced their interest as a suitable strategy to increase energy production from SS. The lacking and confusing legislation regulating SS co-digestion in WWTPs is one of the major non-technical limitations, coupled with waste availability and the lack of knowledge about adequate co-digestion strategies or their impact in the overall operation of a WWTP.

Several criteria used for co-digestion selection is discussed, and different lab-bench SS co-digestion studies and full-scale trails are also analyzed in this work. The aim of this literature survey is to identify and analyze the main constraints of full-scale SS co-digestion application, using results obtained in the different laboratory, bench and full scale research studies carried out in the last 20 years.

3.2. CO-SUBSTRATE SELECTION CRITERIA

The selection of co-substrates and mixture design usually is a quandary between economic and technical criteria. From a technical point of view, the most adequate substrates for co-digestion would be those that maximize methane production without producing instability or toxicity to the anaerobic biomass. From an economical point of view, different aspects must be taken into account, such as the increase of the anaerobic sludge produced and its dewaterability, the possible pre-treatment of the co-substrate, the recirculation impact of rejected water from others units of the WWTP, or the possible increase in maintenance costs, apart from logistics costs (transport and storage) related to waste availability within the geographical area of the WWTP. Bellow, the different co-substrate selection criteria are discussed, according to which objective wants or needs to be primarily attended.

3.2.1. Economic feasibility

Full scale co-digestion aims to impact the facility's energy balance. It tries to satisfied with the extra biogas generated during co-digestion the energy demand of the WWTP; hence, there might be an economic profit in saving electric energy, and these savings should be higher than any maintenance costs arising related to the new process.

In this regard, transport costs are the main criteria to follow for co-substrate selection, limiting the use of certain organic substrates. Within this idea, food waste (FW) and the organic fraction of municipal solid wastes (OFMSW)– have been used for lab, bench and full scale co-digestion operations, as FW availability is usually widespread in the same areas where urban WW is generated.

Besides this, some co-substrates need a specific pre-treatment which entails an increase in economic costs and further technical requirements for the process. The need of pre-treatments depends on the characteristics and composition of the co-substrate itself. Sometimes, co-substrates need a reduction in particulate size to improve homogenization with the SS (Rosenwinkel and Meyer, 1999, Krupp *et al.*, 2005, Bolzonella *et al.*, 2006) or, if they contain impurities (metal pieces, glass, sand, stones), they require their removal prior to be used as co-digestion substrate. Bolzonella *et al.* (2006) concluded that the treatment cost of the OFMSW to be used as co-substrate in SS anaerobic digestion was $50€\cdot T^{-1}_{OFMSW}$, amounting to 14% of the total costs of a WWTP; and investment costs for a OFMSW sorting line were estimated at 750,000€. Edelmann *et al.* (2000) reported that energy consumption for the pre-treatment of OFMSW (macerator, screw pump, pasteurization unit, and stirred storage tank) was of 35 kWh of electricity and 50 kWh of heat per tonne of waste. This study concludes the economic feasibility of the co-digestion process to be negative due to the price of the energy sold in this time period, despite the actual electricity and heat surplus obtained (over 70 $kWh\cdot T^{-1}$ and 210 $kWh\cdot T^{-1}$, respectively).

Recently, another waste generated inside WWTPs called grease waste (GW) or solids trapped in the dissolved air flotation (DAF) unit, has stirred up strong interest as it can optimize the entire plant operation, reducing disposal costs as well as avoiding co-substrate transport costs (Silvestre *et al*., 2011[1])

As for its maintenance costs, different issues have been reported such as the formation, floating and accumulation of inorganic material at the bottom of the reactor, etc. Foam formation has been reported by different authors when oil and grease waste is added to the digester (Pitk *et al*., 2013). Fibrous wastes, like grass silage, have a tendency to float, blocking pipes and pumps (Thamsiriroj and Murphy, 2010). Some substrates could contain inert material like sand and stones which, decanted in the digesters would reduce their active volume or block the system's piping and valves (Krupp *et al*., 2005; Edelmann *et al*., 2000). Thus, stirring should be an adequate approach when co-digestion is implemented in order to reduce the formation of floating layers and foams, and/or to avoid decanting episodes. In this regard, depending on the co-substrate some digester modifications should be carried out and, therefore, the cost of such modifications must be considered in an economic feasibility study.

3.2.2. Process stability

As some components contained in the wastes or intermediary components produced during the anaerobic digestion could be inhibitory or toxic for the microorganism's activity (this toxicity produces a total failure of microbiologic activity; while inhibition produces a partial loss of activity in the biomass), the selection and dosage of the co-substrate should be accurately selected.

In this sense, the main disadvantage of using wastes containing high amounts of grease as co-substrate is the accumulation of intermediary compounds as long chain fatty acids (LCFA) produced during its degradation which could inhibit microorganism activity. LCFAs are formed during lipid and fat degradation and later degraded to acetic acid and hydrogen. The inhibitory effect of LCFAs varies depending on the type of LCFA, the concentration of each one, the AD temperature operation and the specific AD population. This inhibitory mechanism is related with their adsorption in the cell membrane, hampering the functionalities of the bacterium. Acetogenic bacteria are more sensible to LCFA inhibition than hydrogenotrophic bacteria, and a thermophilic anaerobic biomass is also more sensible than its mesophilic counterpart (Alves *et. al*, 2001; Palatsi *et. al*, 2009).

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[1] Chapter 4

Several authors reported an inhibition of the process at high GW ratios in the feed mixture (60-70% in volatile solids (VS) basis), producing a decrease in methane production (Figure 3.1) and in VS removal (Figure 3.2), and probably due to high concentrations of LCFAs. Girault *et al.*, (2012) reported LCFA concentrations between 6,000-7,000 mg L^{-1} when GW reached up to 70% of the VS inlet. Silvestre *et al.* (2011)[1] suggested that the acclimatization of the biomass brought about by slowly increasing the grease waste dose, could be the right strategy to increase fat degradation and reduce the inhibitory effect of LCFAs.

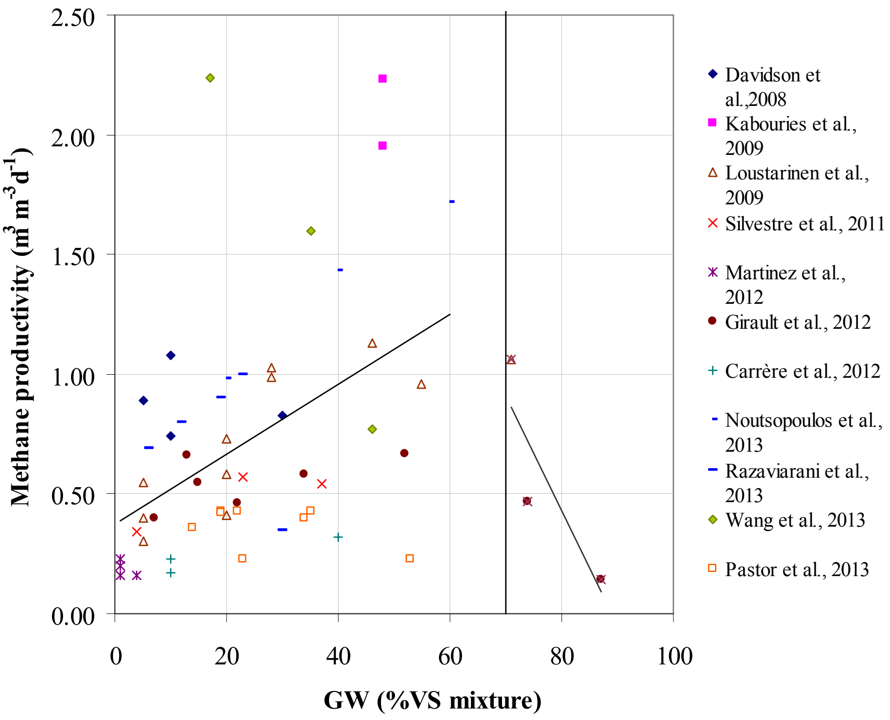

Figure 3. 1. *Methane production as a function of the percentage of grease waste (VS) present on the feeding mixture of different sewage-grease waste co-digestion studies carried out at lab and bench scale.*

Another non-desired issue in any co-digestion operation which has also been widely reported in literature is the inhibition of the AD process due to high VFA accumulation when operated at a high organic loading rate

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[1] Chapter 4

(OLR). Murto *et al.* (2004) reported that during SS and potato processing waste co-digestion, when increasing OLR to 5.9 $kg_{SV} \cdot m^{-3}d^{-1}$, high VFA accumulations resulted in the depletion of bicarbonate and the reduction of its buffering capacity, leading to a decrease in pH. Cechi *et al.* (1996) reported an imbalance process when sewage sludge was co-digested with macroalgae in thermophilic conditions: VFAs rose to levels higher than 5 $g \cdot L^{-1}$, amounting to more than 20% due to propionic acid. Methane percentages on the biogas were also substantially affected reaching mean values as low as 45%, and biogas yield values were extremely low showing a collapse of the digester. The reason for this failure was attributed to the inhibition of methanogenic activity due to the toxicity of hydrogen sulphide. Silvestre *et al.* (2015b)[1] reported high process instability when crude glycerine was co-digested with SS under thermophilic conditions, and with VFA concentrations above 1.5 $g \cdot L^{-1}$.

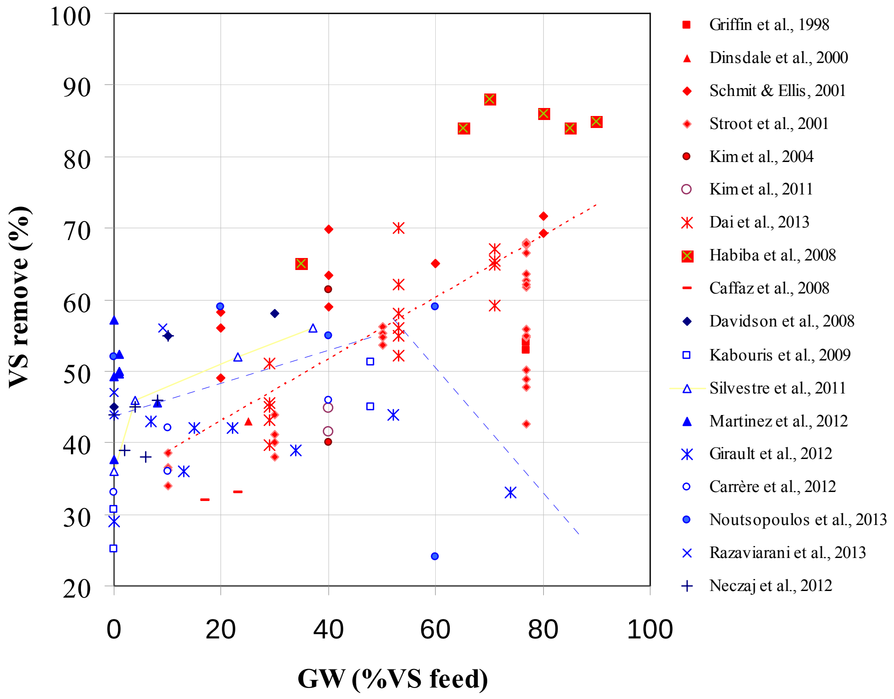

Figure 3. 2. *Volatile solids removal efficiency as an function of the percentage (on VS basis) of the food waste (red symbols) and grease waste (blue symbols) during lab and bench scale anaerobic co-digestion experiments*

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[1] Chapter 6

3.2.3. Sustain a stable biogas production rate

The total amount of SS produced and its composition depends on the treated wastewater flow, which varies over the different seasons (i.e. fluctuations due to seasonal industrial activities or the effect of tourism on WWTPs during summer). Consequently, substantial variations in the anaerobic digester's performance, mainly due to hydraulic retention time (HRT) and/or OLR variations occur and, therefore biogas production varies throughout the year. The addition of co-substrates can modulate these variations and allow for a performance closer to stationary conditions, with a positive impact on microorganisms activity.

In addition, to stabilize seasonal variations in biogas co-digestion could increase biogas production by increasing the OLR. SS AD processes are usually operated at low OLR values of 0.5-1.5 $kg_{VS}·m^{-3}·d^{-1}$ and at high HRT of 20 to 40 days (Silvestre *et al.*, 2015[1]) due to low solid concentrations in the SS and the high volume of the typical SS anaerobic digester. Hence, these anaerobic digesters offer an opportunity to increase the OLR, adding co-substrates, and a higher biogas production could be expected.

Within this idea, co-digestion would involve a higher control and knowledge of the AD process and an adequate management and storage of the co-substrates. Nonetheless, the main studies carried out at lab and bench scale, have been performed with constant SS concentration values or with a constant co-substrate addition, which is the case for some industrial operations. In this regard, SS co-digestion depends on co-substrate availability, which may change in composition and quantity over time. Few studies about the effect of the change in co-substrates over time are found in literature. Fonoll *et al.* (2015) reported that changes in the kind of fruit used as co-substrate did not affect the anaerobic digestion process, with a rapid recovery to basal level.

3.2.4. Balance the nutrients composition of the feed

The presence of nutrients is essential to the growth and activity of the microorganisms involved in an anaerobic digestion process. Carbon is essential as a source of energy to generate new cellular material and to produce methane and carbon dioxide; and nitrogen and phosphate are needed for cellular growth. The carbon/nitrogen (C/N) ratio is an important parameter for bacteria to adequately carry out their activity, as bacteria consume 20 to 30 times more carbon than nitrogen (Sosnowoski

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[1] Chapter 2

et al., 2003). A deficit in nitrogen implies a reduction in the production of enzymes and, as a whole, a reduction in anaerobic biomass activity (Procházka *et al*., 2012). An excessive nitrogen concentration, especially in the NH_3^+-N form could be inhibitory to microbiologic activity (Yenigün *et al*., 2013).

SS is characterized by low C/N ratio values (6-16), thus an increase in the C/N ratio results in higher biogas yields and better specific activities. Sosnowoski *et al.* (2003) reported better performances of the anaerobic process when increasing the C/N ratio from 9 to 14 with the addition of the OFMSW to the SS anaerobic digestion. On the other hand, Bouallagui *et al.* (2009) reported a 44% improvement in biogas yield when reducing the C/N ratio from 34.2 (fruit and vegetable alone) to 24.76 (SS co-digestion).

3.2.5. Improve digestate dewaterability

Anaerobic effluents contain high amounts of water, its dehydration being necessary to reduce sludge management costs. The dewatering process involves high costs in terms of energy consumption, reaching close to 25% of the total energetic costs of a WWTP (Pérez-Elvira *et al*., 2006). Therefore, it is important to know the impact on the rheological properties of co-substrate addition during SS anaerobic digestion, as said addition could improve or worsen the dewatering process.

Adding co-substrates leads to higher biogas production due to the presence of higher quantities of organic matter in the digester, but if this extra organic matter is not degraded, the effluent may contain higher concentrations of organic matter which could hinder the dewatering process (Liu *et al*., 2012, Silvestre *et al*., 2011[1], Girault *et al*., 2012). The dewatering process generates a solid fraction usually managed outside the WWTP, and a liquid fraction that is reintroduced to the water line. If said liquid fraction contains higher concentrations of organic matter, oxygen consumption in the WWTP will increase and, therefore, lead to an increase in operational costs which should be taken into account.

In the same way, NH_4^+-N effluent concentrations can increase when SS is co-digested with high nitrogen content wastes, leading to an expected higher consumption of oxygen for wastewater depuration. Pitk *et al.* (2013) reported an increase of NH_4^+-N concentrations in the effluent from 783 to 3,700 $mg{\cdot}L^{-1}$ in SS co-digestion with animal by-products, while Borowsaki and Weatherley, (2013) reported a 1,615 to 2,221 $mg{\cdot}L^-$

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[1] Chapter 4

^{1} increase in SS-poultry manure co-digestion. Heo *et al.* (2003) showed a 50% NH_4^+-N increase when high quantities of FW were added to SS anaerobic digestion. Similarly, Nowak *et al.* (2007) showed that NH_4^+-N concentrations were 2 folds higher in the water rejected when SS co-digested with organic waste in the WWTP of Loewen (Austria), where it was necessary to convert a primary settler to a nitrification/denitrification reactor to handle the extra ammonia nitrogen generated. An increase in NH_4^+-N and P concentrations in the digested effluent was also observed in a SS-OFMSW anaerobic co-digestion in the Treviso WWTP. In this case, the extra NH_4^+-N was treated in an aerobic digester with an increase in oxygen demand of 5-10% and an extra addition of organic matter. With this solution, the nitrogen feedbacks to the activated sludge process derived from bio-waste treatment were lower than the 4% of total N usually entering the Treviso WWTP (Cavinato *et al.*, 2013). With regards to P, more than 80% of P was recovered through struvite crystallization (Bolzonella *et al.*, 2006).

As for the characteristics of the solid fraction, the use of a co-substrate could increase the quantities of dewatered anaerobic sludge and, as a consequence, increase their external management costs. For this reason, knowing the optimal OLR to be added to the system is essential to limit the increase of organic matter in the effluent. However, some authors reported an increase in VS removal efficiencies with high co-substrate additions and high OLR values in comparison to a simple SS anaerobic digestion (Stroot *et al.*, 2001, Dai *et al.*, 2012; Loustarinen *et al.*, 2009), and some works reported an improvement in the dewatering proprieties of the digestate after co-digestion, while others state the contrary.

Habiba *et al.* (2009) showed that active sludge co-digestion with vegetables and fruit waste improved the filterability of the final effluent due to the presence of fibrous particles which formed a protection layer on the filter media reducing obstruction problems. Hidaka *et al.* (2013) reported similar results in SS anaerobic co-digestion with grass from public green areas. Silvestre *et al.* (2014)[1] showed opposed results for grease waste and sewage sludge anaerobic co-digestion under mesophilic and thermophilic temperature conditions. While the anaerobic digestate dewatering proprieties improved during mesophilic co-digestion, in comparison with SS anaerobic digestion, thermophilic co-digestion showed worse dewatering proprieties. Jensen *et al.* (2014) did not find any impact on the dewatering proprieties during SS co-digestion working with different glycerine dosage.

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[1] Chapter 5

3.3. CO-DIGESTION IMPLEMENTATION

In lab and pilot SS co-digestion experiments, FW has been the most studied co-substrate, followed by GW, slaughterhouse wastes and crude glycerine. Other organic wastes such as crop residues, industrial wastes (coffee waste, oils, chocolate, fruit juices, etc.) and lignocelluloses wastes have been also studied as potential co-substrates. These wastes are typically characterized by high organic matter concentrations which have a great effect on the OLR but maintain the HRT in similar values than in SS mono-digestion. Besides this, some organic liquid waste streams, usually generated in the food or pharmaceutical/chemical industry (i.e. alcohols such as glycerine recovered from biodiesel) are used. In this case, the impact on HRT in an anaerobic digestion process must be further analyzed both to avoid instability episodes in the digester and to reduce the impact on the activated sludge system (due to a water content increase in the liquid fraction of the dewatering unit).

The main lab and bench co-digestion studies are carried out simulating the most typical SS anaerobic digestion operating in a mesophilic continuous stirred tank reactor (CSTR) configuration with a HRT between 15 and 30 days. Even though, SS thermophilic anaerobic digestion has gained interest due to a lower HRT application and sludge hygienization.

3.3.1. Lab-scale studies

Tables 3.1, 3.2, 3.3 and 3.4 show the characterization of several food wastes, grease wastes, slaughterhouse wastes, and glycerine, respectively. Tables 3.5, 3.6, 3.7, 3.8, and 3.9 show the major operation and control parameters of SS anaerobic co-digestion with different organic wastes. The majority of studies used a primary sludge (PS) and waste activated sludge (WAS) mixture as main substrate (Griffin *et al*., 1997, Stroot *et al*., 2001, Sosnowoski *et al*., 2003, Kim *et al*., 2004, Kim *et al*., 2006, Kim *et al*., 2011, Dai *et al*., 2012), but some authors only used WAS as the main co-substrate for the co-digestion process (Laffite-Trouqué and Forster, 2000, Misi and Forster, 2002, Athanasoulia *et al*., 2012; Dinsdale *et al*., 2000, Heo *et al*., 2003, Habiba *et al*., 2009, Caffaz *et al*., 2008, Liu *et al*., 2012, Cavinato *et al*., 2013). Only two studies analyzed anaerobic co-digestion using just PS (Schmit and Ellis, 2001, Gomez *et al*., 2006).

3.3.1.1 Food Waste

FW comprises those wastes collected from households, restaurants, hotels, canteens and markets. FW is characterized by a high variability in composition depending on different factors, such as climate, collection system and frequency, seasonality, culture practices, etc. (Foster-Carneiro *et al.*, 2008). Important differences were observed in kinetics and methane yield depending on the nature and composition of the organic waste (Rao and Singh, 2004, Forster-Carneiro *et al.*, 2008). The main components of FW are fruit, vegetables, paper, cereal waste (bread, rice), meat and fish waste, garden waste, etc. In the majority of studies, the particulate size of FW was reduced (< 3mm) before co-digestion. Table 3.1 shows the characterization of several FWs coming from different origins. TS concentrations showed great differences, covering between 468 to 36 g·L^{-1}, where 71% to 95% corresponded to VS. C/N ratio values varied between 52 and 13, depending on the sample.

Table 3. 1. Main characteristics of food wastes coming from different origins

Parameters	**TS** g·L^{-1}	**VS** g·L^{-1}	**COD** g·L^{-1}	**C/N** g·g^{-1}	**TKN** mg·L^{-1}	**SO$_4^{-2}$-S** mg·L^{-1}	**pH** -
OFMSW[1]	468	394	542	52	-	-	-
FVW[2]	44	39	-	23	1026	143	4.57
FW (canteen)[3]	38	36	-	17	897	59	4.13
FW (supermarket)[4]	45	33	-	24	1159	316	3.86
FW (household)[5]	36	33	-	14	863	48	3.54
FW (canteen)[6]	212	197	-	13	544	-	4.70
FVW (market)[7]	68	56	65	36	1360	-	-
OFMSW[8]	259	247	385	17	6026	545	-
OFMSW[9]	283	220	393	14	7503	308	-
OFMSW[10]	422	300	641	21	7331	435	-

[1]Nielfa *et al.*, 2015; [2,3,4,5]Cabbai *et al.*, 2013; [6]Dai *et al.*., 2012; [7]Habiba *et al.*, 2009;[8,9,10]Silvestre *et al.*, 2015c[1]

FW samples were collected from different sources: markets (Dinsdale *et al.*, 2000, Habiba *et al.*, 2009, Caffaz *et al.*, 2008, Liu *et al.*, 2012, Cavinato *et al.*, 2013), restaurants and dining halls (Kim *et al.*, 2004, Kim *et al.*, 2006, Kim *et al.*, 2011, Dai *et al.*, 2012, Liu *et al.*, 2012, Cavinato *et al.*, 2013) and the OFMSW (Griffin *et al.*, 1997, Dinsdale *et al.*, 2000, Schimit and Ellis, 2001, Stroot *et al.*, 2001, Sosnowoski *et al.*, 2003,

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[1] Chapter 7

Gomez *et al*., 2006). Depending on the source, the presence of impurities like plastics, cans, glasses, etc., varies and usually requires a pre-treatment to remove them and to avoid hydrodynamic problems inside the reactor or pumping problems (Krupp *et al*., 2005).

Many studies about SS co-digestion with FW are found in literature at both laboratory and pilot scale, plus some industrial scale applications in different WWTPs. Table 3.5 shows a summary of different studies found in literature about SS-FW co-digestion at lab and bench scale. A wide range of SS-FW mixtures were analyzed in different studies, from a 90:10 SS:FW ratio on a VS basis (Heo *et al*., 2003) to a 10:90 SS:FW ratio on a TS basis (Habiba *et al*, 2009). The SS-FW co-digestion process was analyzed using different digester configurations: CSTR (Griffin *et al*., 1997, Stroot *et al*., 2001, Heo *et al*., 2003, Gómez *et al*., 2006, Caffaz *et al*., 2008, Dai *et al*., 2012, Liu *et al*., 2012, Cavinato *et al*., 2013); two anaerobic sequential batch reactors (ASBR) both operating in mesophilic temperature conditions (Habiba *et al*., 2009, Kim *et al*., 2011, Kim *et al*., 2006, Kim *et al*., 2004) or with a first thermophilic one, followed by a second mesophilic digester (Kim *et al*., 2004, Kim *et al*., 2006, Kim *et al*., 2011); a two-phased anaerobic digestion (TPAD), composed by two CSTRs, the first under thermophilic and the second under mesophilic temperature conditions (Dinsadale *et al*., 2000, Sosnowoski *et al*., 2003, Schmit and Ellis, 2001). Also, a wide range of working volumes has been used with digesters varying between 2L and 1600L (Habiba *et al*., 2009; Liu *et al*., 2012).

Figure 3.3 shows methane production as a function of the OLR applied in the different studies found in literature. As it can be seen, a strong correlation is present, where the higher the OLR the higher the methane production, showing a strong influence of the OLR in methane production. Figure 3.2 shows VS removal as a function of the FW percentage (on a VS basis) in the mixture, showing an increase in VS removal efficiencies with higher FW percentages in the mixture. The operational parameters of the digesters (HRT and OLR) widely varied with the different studies. HRT varied from 47 days (Gomez *et al*., 2006) to 4 days (Stroot *et al*., 2001), and OLR varied from 0.3 to 22 $kg_{VS}·m^{-3}·d^{-1}$ (Habiba *et al*., 2009; Dai *et al*., 2012). Methane yield and production (per m^3 digester) varied from 0.09 to 0.90 $m^3·kg_{VS}^{-1}$ (Stroot *et al*., 2001; Griffin *et al*., 1997) and 0.08 to 3.05 $m^3·m^{-3}·d^{-1}$ (Habiba *et al*., 2009; Stroot *et al*., 2001), respectively.

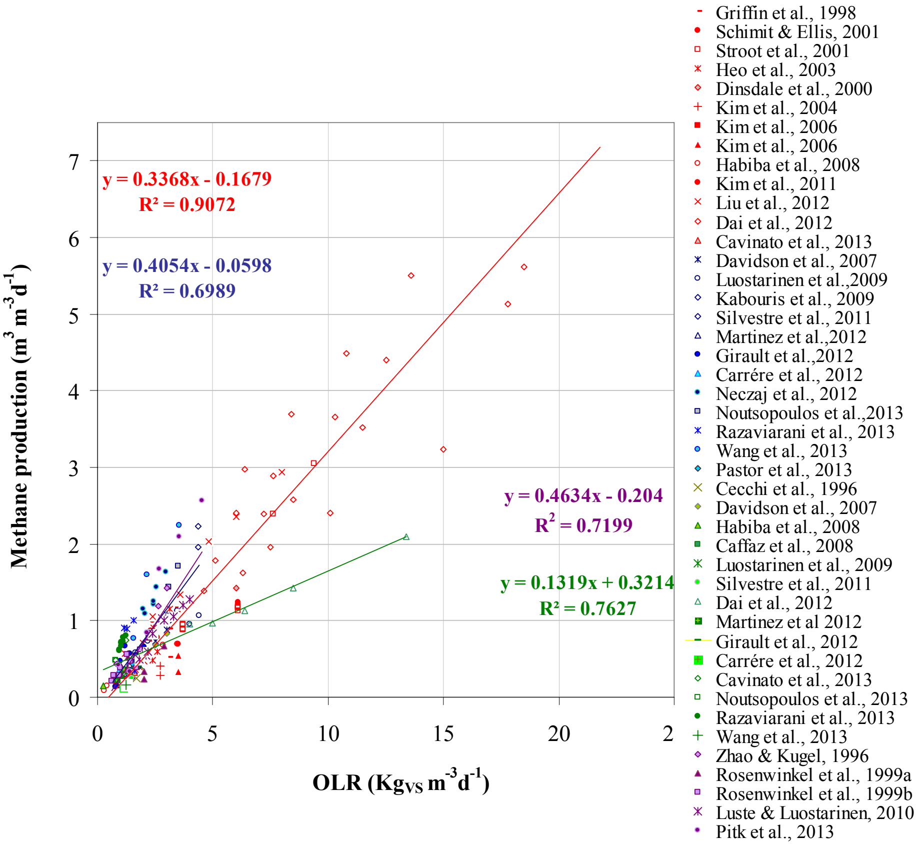

Figure 3.3. *Methane production as a function of the OLR applied in the different anaerobic digestion experiments: sewage sludge-food waste anaerobic co-digestion (red symbols), sewage sludge-grease waste anaerobic co-digestion (blue symbols), sewage sludge-slaughterhouse waste (purple symbols) and simple sewage sludge anaerobic digestion (green symbols).*

Thermophilic anaerobic co-digestion showed higher biogas production than mesophilic co-digestion. Kim *et al*. (2004, 2006 and 2011) found an increase in methane production of +41%, +59% and +80%, respectively, when operating the first digester under thermophilic temperature conditions followed by a mesophilic digester with different OLR and HRT values using a 60:40 SS:OFMSW mixture ratio (on a TS basis). Griffin *et al*. (1997) compared mesophilic and thermophilic anaerobic co-digestion at a HRT of 20 days and at 3.10 $kg_{SV}\cdot m^{-3}\cdot d^{-1}$ and a mixture with 77% of OFMSW (TS basis), showing a 71% increase in methane production under thermophilic compared to mesophilic conditions.

Cavinato *et al*. (2013) reported a 50% increase in methane yield during the thermophilic SS-OFMSW co-digestion (25% VS_OFMSW) in comparison with the mesophilic co-digestion working under the same operational conditions.

3.3.1.2. Grease waste

The main characteristics of different grease waste samples are shown in Table 3.2. Both total solid and fat concentrations considerably varied depending on the origin of the GW. Table 3.6 shows the major operational and control parameters at lab and bench scale SS anaerobic co-digestion experiments with GW. Most of the studies are centred in the grease waste produced in the dissolved air flotation (DAF) unit of the WWTP (Silvestre *et al*., 2011[1]; Martínez *et al*., 2012; Noutsopoulos *et al*., 2013; Girault *et. al*., 2012). Loustarinen *et al.* (2009) and Neczaj *et al.* (2012) studied SS anaerobic co-digestion with a grease trap coming from a meat processing plant, and Kabouries *et al*. (2009) and Wang *et al.* (2013) with fats, oils and grease (FOG) coming from the FOG-trap of several restaurants. Carrère *et al.* (2010) studied SS co-digestion with fatty wastewater coming from restaurants while Pastor *et al.* (2013) focused its research in the use of used oils from restaurant kitchens.

Different SS:GW mixtures ratios were analyzed: from a 99:1 ratio (Davidsson *et al*., 2008) to a 10:90 ratio on a VS basis (Noutsopoulos *et al*., 2013). All studies were carried out in a CSTR with a reactor volume between 2L and 1200L (Carrère *et al*., 2010 Razaviarani *et al*., 2013a). HRT and OLR were comprised between 10 to 30 days and 0.79 - 4.41 $kg_{VS}·m^{-3}·d^{-1}$, respectively.

As in the case of the SS-FW anaerobic co-digestion, OLR is the parameter having the highest influence in methane production (Figure 3.3), showing higher increases in methane production the higher the OLR applied. GW addition was limited due to LCFA inhibition and therefore, the maximum OLR applied to the digester without producing instability of the anaerobic process was lower than the OLR applied for SS co-digestion with FW.

Figure 3.1 shows methane production as a function of the percentage of GW on the mixture (on a VS basis) showing that when GW addition was higher than 60% of the total volatile solids in the mixture, methane production decreased. Most studies were carried out under mesophilic temperature conditions, except for the works of Kabouries *et al*. (2009)

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[1] Chapter 4

and Silvestre *et al.* (2014)[1]. Kabouries *et al.* (2009) compared mesophilic and thermophilic SS anaerobic co-digestion with a mixture composed of 48% of the VS found in polymer-dewatered fat, oils and grease. Under the same operational conditions (HRT and OLR), methane production increased in +355% and +298% in thermophilic and mesophilic conditions, respectively. Silvestre *et al.* (2014)[1] showed a lower increase in biogas production for thermophilic than mesophilic co-digestion, probably due to LCFA accumulation and the slow adaptation to fat of the thermophilic biomass present.

Table 3. 2. *Main characteristics of grease waste samples from different origins*

Parameters	**TS**	**VS**	**pH**	**Fat**	**SO_4^{-2}-S**	**COD**	**NKT**	**C/N**
	mg·L^{-1}	mg·L^{-1}	-	g·kg^{-1}	g·kg^{-1}	g·L^{-1}	mg·L^{-1}	g·g^{-1}
GW$^{1}_{\text{(FOG restaurants}}$	777	777	5			2698		
GW$^{2}_{\text{(FOG restaurants)}}$	258	257				1510		
GW$^{3}_{\text{(FOG restaurants)}}$	968	955				>1500		
GW$^{4}_{\text{(FOG)}}$	32	30	4.2					22
GW$^{5}_{\text{(DAF WWTP)}}$	146	123		47	61	298	4287	20
GW$^{6}_{\text{(DAF WWTP)}}$	160	143		100	37	321	3556	39
GW$^{7}_{\text{(DAF WWTP)}}$	126	101		38	42	258	3166	23
GW$^{8}_{\text{(DAF WWTP)}}$	75	63		15	127	177	3428	10
GW$^{9}_{\text{(DAF WWTP)}}$	96	86	5.5	-	-	196	2200	42
GW$^{10}_{\text{(meat processing plant)}}$	25	25	5.1	-	-	-	-	-
GW$^{11}_{\text{(DAF WWTP)}}$	173	170	4.4	-	-	-	-	-

[1]Razaviarani and Buchanan, 2014 ; [2]Razaviarani *et al.*, 2013a ; [3]Wang *et al.*, 2013 ; [4]Wan *et al.*, 2011 ; [5,6,7,8] Silvestre *et al.*, 2011[1];[9]Girault *et al.*, 2012 ; [10]Luostarinen *et al.*, 2009 ; [11]Davidsson *et al.*, 2008.

3.3.1.3. Slaughterhouse wastes

Different slaughterhouse wastes have been also studied, such as the blood of slaughtered pigs and sausage processing floats (Zhao and Kugel, 1996); stomach contents and slaughter flotation tailings (Rosenwinkel and Meyer, 1999); a mixture of the content of the digestive tract, drum sieve wastes, DAF sludge and grease sludge (Luste and Loustarinen, 2010); or Category 2 and Category 3 animal by-products (ABP) (Pitk et al., 2013). Table 3.3 shows a characterization of the different slaughterhouse waste

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[1] Chapter 5

samples. Both TS concentration and fat content vary significantly depending on the origin of the slaughterhouse waste.

Table 3. 3. *Characterization of grease waste samples from different origins*

Parameters	**ST** $g{\cdot}L^{-1}$	**VS** $g{\cdot}L^{-1}$	**COD** $g{\cdot}L^{-1}$	**C/N** $g{\cdot}g^{-1}$	**TKN** $g{\cdot}L^{-1}$	**FAT** %	**pH** -
Sterilized mass[1]	960	870	-	9.3	60	-	-
Digestive tract content[2]	130	120	-	-	-	-	6.6
Drumsieve waste[3]	140	140	-	-	-	-	6.8
DAF sludge[4]	78	68	-	-	-	-	6.8
Grease Trap Sludge[5]	159	158	-	-	-	-	5.6
Cow meat and fat[6]	886	854	2295	-	3	76	-
Pig meat and fat[7]	57	56	1475	-	14	47	-
Confiscates[8]	25	22	385	-	26	5	-
Pig Stomach[9]	183	180	377	-	12	9	-
Rumen content[10]	117	109	152	-	1	0.2	-
Waste blood[11]	197	184	263	-	32	-	-
Meat tissue[12]	383	362	870	7.9	3	18	-
Intestines[13]	294	281	726	6.0	3	7	-
Post flotation sludges[14]	219	185	1207	8.8	2	6	-
Bristles[15]	236	224	1211	7.0	2	2	-

[1]Pitk *et al.*, 2013; [2,3,4,5]Luste and Luosterinen, 2010; [6,7,8,9,10,11]Palatsi *et al.*, 2011; [12,13,14,15]Borowski *et al.*, 2015

Table 3.7 shows the major operational and control parameters for sewage sludge co-digestion with slaughterhouse wastes. All authors used primary and secondary sludges as main co-substrates. The anaerobic configuration applied in the majority of these studies was carried out in a CSTR, with the exception of Zhao and Kugel, (1996) who used a two stage configuration, with first a hyper-thermophilic (75ºC)-CSTR followed by a mesophilic-CSTR. The volume of the digester varied between 4L and 2,000L (Luste and Luostarinen, 2010; Rosenwinkel and Mayer, 1999). HRT varied from 12.5 to 44 days (Zhao and Kugel, 1996; Rosenwinkel and Meyer, 1999). The OLR applied to the digester was between 0.84 and 4.54 $kg_{VS}{\cdot}m^{-3}{\cdot}d^{-1}$ (Zhao and Kugel, 1996; Pitk *et al.*, 2013). All studies were carried out under mesophilic temperature conditions (35-37ºC) although Zhao and Kugel, (1996) first applied a hyper-thermophilic digestion prior to a secondary mesophilic one. Figure 3.3 shows methane production as a function of the OLR applied in the different sludge co-digestion studies with slaughterhouse wastes. Just as with other wastes, the higher the OLR the higher the methane production.

In this case, and according to the high lipid content characterizing of slaughterhouse wastes, the co-digestion process behaviour is very similar to the behaviour of the same process using grease waste.

3.3.1.4. Glycerine

Crude glycerine (CGY) is the main by-product generated during biodiesel production. CGY comprises variable amounts of components such as alcohols, water, inorganic salts coming from the biodiesel catalysts, free fatty acids, un-reacted triglycerides, and methyl esters (Siles *et al.*, 2009). Table 3.4 shows the characterization of different crude glycerine samples. The main difference between these samples lays in their pH values. Several CGY samples showed an alkali pH (8.0-12.6) and others acid pH values (5.0-3.3). This difference is related with the biodiesel process applied. Therefore, depending on this process, CGY could contain different inhibitor components like methanol, Na or soaps.

Table 3. 4. *Crude glycerine sample characterization*

Parameters	**Units**	**G[1]**	**G[2]**	**G[3]**	**G[4]**	**G[5]**	**G[6]**	**G[7]**	**G[8]**	**G[9]**	**G[10]**
TS	$mg\cdot L^{-1}$	782	829	933	-	972	277	-	926	961	781
VS	$mg\cdot L^{-1}$	743	746	844	-	-	240	-	924	917	744
pH	-	10.4	-	-	10.7	5.0	9.0	8-9	5.5	12.6	3.3
Methanol	%	5.1	11	12	7.1	-	-	-	-	-	-
Glycerine	%	46.5	72.3	66.0	50.6	-	-	80	-	-	-
Soaps	%	-	-	-	26.5	-	-	-	-	-	-
Na	$g\cdot L^{-1}$	-	-	-	-	-	-	17	-	-	-
K	$mg\cdot L^{-1}$	-	-	-	-	-	-	454	-	-	-
SO_4^{-2}-S	$g\cdot L^{-1}$	-	-	-	-	-	-		1.7	2.7	0.5
PO_4^{-3}-P	$mg\cdot L^{-1}$	-	-	-	-	-	-	-	228	101	168
COD	$g\cdot L^{-1}$	-	1056	912	1000		1631	1140	1517	1070	1397
TN	$mg\cdot L^{-1}$	-	-	-	-	372	-	-	-	-	-
TP	$mg\cdot L^{-1}$	-	-	-	-	9.6	-	-	-	-	-
C/N	$g\cdot g^{-1}$	-	-	-	-	-	-	-	588	3338	78

[1]Nartker *et al.*, 2014 ; [2,3]Jensen *et al.*, 2014 ; [4]Athanasoulia *et al.*, 2014; [5]Fountoulakis *et al.*, 2010; [6]Razaviarani and Buchanan, 2015 ; [7]Nghiem *et al.*, 2014 ; [8,9,10] Silvestre *et al.*, 2015b[1]

CGY has stirred up strong interest as co-substrate for the co-digestion process, since it is easily digestible, has a high biogas potential and can be

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[1] Chapter 6

easily stored for long periods of time (Castrillón *et al*., 2011; Fountoulakis *et al*., 2010, Silvestre *et al*., 2015b).

The main co-digestion research studies were carried out with a mixture of primary and secondary sludges (Fountoulakis *et al*., 2010, Razaviarani *et al*., 2013b, Jensen *et al*., 2014). Nartker *et al*. (2014) and Athnasaulia *et al*. (2014) studied CGY co-digestion with a primary sludge and a waste activated sludge respectively. A CSTR was the reactor configuration chosen in all of these studies. Athnasoulia *et al*. (2014) selected a two-stage configuration, working in two serial CSTRs. The volume of the digester ranged between 0.85L (Jensen *et al*., 2014) and 1,200L (Razaviarani *et al*., 2013b). The glycerine-sludge mixtures analyzed varied from 0.5% vv (Jensen *et al*., 2014) and 8% vv (Nartker *et al*., 2014). HRTs applied were from 12.5 to 32 days and the OLR was between 1.03 and 2.88 $kg_{VS}{\cdot}m^{-3}{\cdot}d^{-1}$. Volumetric methane production (VMP) (per m^3 of digester) increased from +7% to +259% when glycerine was 2% vv to 7% vv of the co-digestion mixture (Nartker *et al*., 2014). Athanasoulia *et al*. (2012) showed biogas production increases between +216% (2% vv glycerine at 12.3 days) and +519% (3% vv glycerine at 14 days) (Table 3.8).

Several studies showed instability during the process due to VFA accumulation, –mainly propionic acid–, when glycerine was added in a determinate ratio. Fountoulakis *et al*. (2010) found an unstable process with high VFA concentrations in the effluent when the glycerine content was increased by 3%. Razaviarani *et al*. (2013b) also showed a considerable decline in methane production and VS removal efficiencies when the proportion of VS due to glycerine increased to 31% (2% v/v). However, Nartker *et al*. (2014) did not show instability during the process until the proportion of glycerine in the co-digestion mixture rose to 8% vv. Since the main mechanism of inhibition during CGY co-digestion processes appears to be the accumulation of VFAs produced by a fast CGY fermentation rather than the presence of toxic compounds (Jansen *et al*., 2014), a higher HRT together with the use of just a primary sludge (higher alkalinity than the activated sludge) applied by Nartker *et al*. (2014) when compared with others studies, could explain the higher doses of glycerine the system was capable to accept before the process finally fell.

3.3.1.5. Other wastes

Many other wastes have been also tested to be used as co-substrate in SS anaerobic digestion such as: potato wastes from industrial food processing (Zhao and Kugel, 1996, Murto *et al.*, 2004), landfill leachates (Carriere *et al.*, 1993, Montusiewicz and Lebiocka, 2011), shredded grass from public green spaces (Hidaka *et al.*, 2013), confectionary wastes (Laffite-Trouqué and Forster, 2000), macroalgae (Cecchi *et al.*, 1996), olive mill effluents (Carriere et al., 1993, Athanasoulia *et al.*, 2012), cheese whey (Carriere *et al.*, 1993), wood chips and corn starch (Converti *et al.*, 1997), a mixture of OFMSWs, enzyme industry waste, paper mill sludge (Einola *et al.*, 2001), carbohydrate-rich food processing waste (Björnsson *et al.*, 2000), a mixture of poultry wastes, fruit and vegetable wastes, cattle slurries (Misi and Forster, 2002), a mixture of cattle manure and food wastes (Marañon *et al.*, 2012), poultry manure (Borowoski and Weatherley, 2013), and primary clarifier skimmings (Alanya *et al.*, 2013) (Table 3.9).

Most of these studies were carried out in CSTR reactors, although some alternative systems were used. For example, Carriere *et al.* (1993) studied SS co-digestion with olive mill effluents and cheese whey in anaerobic contact digesters (ACD) by thickening and recycling part of the digested sludge in the feed. This operational strategy allowed for an increment in the solids retention time while also maintaining the hydraulic retention time. Another system was studied by Zhao and Kugel, (1996) who analyzed SS anaerobic co-digestion with a potato processing concentrate using a two staged system: first a thermophilic CSRT operated with low HRT (2.5 days), followed by a second mesophilic CSRT with a higher HRT (10 days). Laffite-Trouqué and Forster, (2000) used a system composed of two ASBR, first operating at thermophilic temperature conditions with a low (4h) HRT and a second digester operating at mesophilic conditions with an 8-15 days HRT.

The HRT applied in the different co-digestion experiments was between 4.5 and 27 days (Carriere *et al.*, 1993). The OLR applied to the digester varied considerably and in most cases OLR values were higher than 7 $kg_{VS}{\cdot}m^{-3}{\cdot}d^{-1}$ (Einola *et al.*, 2001; Hidaka *et al.*, 2013; Alanya *et al.*, 2013). Methane yields varied between 0.12 and 0.77 $m^3{\cdot}kg_{VS}^{-1}$ (Lafitte-Trounqué and Forster, 2000; Alanya *et al.*, 2013), and methane production was found to be between 0.03 and 2.50 $m^3_{CH4}{\cdot}m^{-3}{\cdot}d^{-1}$ (Cecchi *et al.*, 1996; Carriere *et al.*, 1993).

Some authors reported operating conditions (HRT, OLR and mixture) which caused instability of the anaerobic digestion process. Carriere *et al.*

(1993) reported instability in SS anaerobic co-digestion with olive mill effluents (25:75 ratio on a COD basis) at an OLR of 12 $kg_{COD}·m^{-3}·d^{-1}$ and 4.5 days of HRT. Murto *et al.* (2004) reported instability of the anaerobic process in SS anaerobic co-digestion with potato processing industrial wastes with OLRs of 5.9, 5.3 and 4.4 $kg_{VS}·m^{-3}·d^{-1}$when the amount of co-substrate was 72%, 80% and 84% of the volatile solids found in the feed mixture.

Most studies were carried out under mesophilic temperature conditions (35º) but some authors compared the co-digestion process under mesophilic and thermophilic conditions. Cecchi *et al.* (1996) showed a lower increase in methane production during SS co-digestion with macroalgae under thermophilic than mesophilic temperature conditions. Marañón *et al.* (2012) reported a lower increase in methane yield during co-digestion with a mixture of cattle manure, sewage sludge and food waste when operating under thermophilic instead of mesophilic temperature conditions.

3.3.2. Full scale trials

Despite the huge amount of lab and bench scale experiments, and the great scientific knowledge attained, full scale SS co-digestion trials are scarce and most of them have been carried out using food waste as co-substrate. Several works have analyzed and described industrial-scale SS-FW anaerobic co-digestion trials in different WWTPs (Pahl *et al.*, 2008, Park *et al.*, 2011, Pavan *et al.*, 2007, Rintala and Järvinen, 1996). In a WWTP in Frutigen (Sweden) a two-digester serial operating SS co-digestion with OFMSW under mesophilic conditions was studied. The OFMSW was pre-treated pasteurizing it in a thermophilic aerobic digester. The co-digestion mixture was composed of 80% of SS and 20% of the OFMSW, resulting in a 27% increase in biogas production in comparison with simple SS anaerobic digestion (Edelmann *et al.*, 2000).

Krupp *et al.* (2005) carried out a comparative study to find the most environmentally friendly option to treat 12,000 $t·year^{-1}$ of organic waste using a life cycle assessment approach. Composting, anaerobic mono-digestion and co-digestion with sewage sludges were the treatments considered. Anaerobic co-digestion was the option with the lower impact on climate change. The process was implemented in the WWTP of Wiesbaden (Germany) with 350,000 inhabitant equivalent.

Bolzonella *et al.* (2006) showed results obtained from a SS-FW co-digestion trial performed in the WWTP of Treviso (Italy). The addition of

10 t·d^{-1} of OFMSW with a 60:40 SS FW ratio on a VS basis, increased biogas production from 3,500 to 17,500 m^3·month^{-1}.

In the WWTP of Velenje (Slovenia), FW was added to the SS anaerobic digesters, with a total volume of 2,000 m^3 at an HRT of 20 days. OLR was 0.8 kg$_{VSS}$·m^{-3}·d^{-1} but during the co-digestion process OLR was increased to 1 kg$_{VSS}$·m^{-3}·d^{-1}, resulting in an 80% increase in biogas production (Zupancic *et al.*, 2008).

Rosenwinkel and Meyer (1999) studied mesophilic co-digestion with a mixture of stomach content (5.6% TS) and flotation tailings at a WWTP in Rheda (Germany). The mixture was composed of 77% of the totals solids, the OLR increased (+17%) from 0.78 to 1.26 and the HRT was reduced from 21 to 18 days. The gas production rate suffered a 60% increase as compared with simple SS digestion.

3.4. CONCLUSIONS

In general, SS anaerobic co-digestion with different wastes has been widely studied. Nevertheless, few factors have been thoroughly analyzed, OLR and the percentage of co-substrate in the mixture are the two factors more deeply studied. Most of the studies have been carried out under mesophilic conditions, there being very few studies performed in the thermophilic range. The study of the temporal variations in the substrate during co-digestion is also an aspect scarcely analyzed; even if it is an important issue to be considered in full scale trials. The influence of co-digestion on the overall performance of a WWTP is another issue in need of more attention, as well as the economic and energetic impact of the addition of other co-substrates to WWTPs. It is also worth mentioning that despite the huge amount of scientific knowledge attained, full scale SS co-digestion operations are scarce; administration issues come as the main reason that hamper its application. Nevertheless, developing SS co-digestion operational strategies with organic wastes of different origins and composition could be an interesting approach to promote the application of co-digestion at full scale WWTP.

Table 3. 5. *Operational and control parameters of the different lab and bench studies of the semi-continuous sewage sludge anaerobic digestion with the food waste*

Sludges	**Substrates**	**^{1}Mixture**	**Digester (volume)**	**^{2}HRT (d)**	**^{3}T (ºC)**	**^{4}OLR ($kg_{VS}\cdot m^{-3}d^{-1}$)**	**CH_4 ($L\cdot kg_{VS}^{-1}$)**	**CH_4 ($m^3\cdot m^{-3}d^{-1}$)**	**CH_4 (% biogas)**	**^{5}VFA ($mg\cdot L^{-1}$)**	**^{6}VS removal (%)**	**Reference**
^{7}PS:^{8}WAS (64:36)	^{9}OFMSW	23:77(^{10}TS)	^{11}CSTR (3L)	20	55	3.10	0.23	0.90	59	700	54	Griffin *et al.*, 1997
					35		0.90	0.52	54	2572	53	
WAS	^{12}FVW (market)	75:25 (VS)	Two-stage (CSTR /^{13}ITD)	13	30	26/ 6	0.25	1.14	68	1300	43	Dinsdale *et al.*, 2000
				17	30	4/ 18	-	-	-	530	43	
PS	OFMSW: paper (50%); newspaper (10%); grass (26%); dog food (14%)	80:20 (TS)	^{14}TPAD (^{15}Te+^{16}M)	15(5+10)	55-35	2.3	0.38	0.87	-	167	58	Schmit & Ellis, 2001
		60:40 (TS)				2.4	0.42	1.01	-	170	70	
		40:60 (TS)				2.4	0.34	0.82	-	934	65	
		20:80 (TS)				2.6	0.30	0.78	-	166	72	
		80:20 (TS)	CSTR	15	35	2.3-2.6	0.36	-	-	-	56	
			TPAD (Te+M)	15(3+12)	55-35	2.3-2.6	0.33	-	-	-	49	
		60:40 (TS)	CSTR	15	35	2.3-2.6	0.36	-	-	-	63	
			TPAD (Te+M)	15(3+12)	55-35	2.3-2.6	0.33	-	-	-	59	
PS:WAS (64:36)	OFMSW: paper and food waste	23:77 (TS)	CSTR (1L)	20	37	3.7	0.24	0.88	55	28	67	Stroot *et al.*,2001
				13		6.1	0.21	1.16	55	23	63	
				4		18.5	0.11	1.77	52	873	50	
PS:WAS (50:50)	OFMSW: potato (55%); fruit (28%); bread (5%); paper (2%); rice /spaghetti (10%)	100:0 (TS)	^{17}UASB (9L)	35	56	0.4	-	-	-	-	-	Sosnowoski *et al.*, 2003
		25:75 (TS)		38	56	1.5	-	-	-	-	-	
		0:100 (TS)	TPAD (Te+M) (23L)	30	56	2.8	-	-	-	-	-	
		100:0 (TS)	UASB (9L)	61	36	0.7	-	-	-	-	-	
		25:75 (TS)		28	36	3.1	-	-	-	-	-	

***Table 3.5.** Cont.*

Sludges	Substrates	[1]Mixture	Digester (volume)	[2]HRT (d)	[3]T (ºC)	[4]OLR ($kg_{VS}·m^{-3}d^{-1}$)	CH_4 ($L·kg_{VS}^{-1}$)	CH_4 ($m^3·m^{-3}d^{-1}$)	CH_4 (% biogas)	[5]VFA ($mg·L^{-1}$)	[6]VS removal (%)	Reference
[8]WAS	[18]FW: boiled rice (10-15%); vegetables (65-70%); meat and eggs (15-20%)	90:10 (VS)	[11]CSTR (3.5L)	20	35	1.2	0.19	0.23	85	0	39	Heo *et al*., 2003
				13		1.8	0.22	0.40	78	-	36	
				10		2.4	0.22	0.48	76	-	34	
		70:30 (VS)		20		1.3	0.23	0.30	81	-	44	
				13		2.0	0.24	0.47	72	-	40	
				10		2.6	0.23	0.60	71	-	38	
		50:50 (VS)		20		1.6	0.34	0.53	69	-	56	
				13		2.4	0.38	0.91	65	-	55	
				10		3.2	0.37	1.15	63	-	54	
[7]PS:WAS	FW (dining hall)	60:40 ([10]TS)	[19]ASBR ([15]Te+[16]M) (4L+4L)	10	55-35	2.7	0.28	0.41	-	-	61	Kim *et al*., 2004
				10	35-35	2.7	0.19	0.29	-	-	40	
PS	[9]OFMSW: fruit, potato, bread, paper	100:0 (TS)	CSTR (3L)	47	35	2.5	-	-	-	-	-	Gomez *et al*., 2006
				40		2.5	-	-	-	-	-	
				37		3.3	-	-	-	-	-	
				37		3.6	-	-	-	-	-	
		22:78 (TS)		47		2.5	-	-	-	-	-	
				40		2.4	-	-	-	-	-	
				40		3.0	-	-	-	-	-	
				37		3.8	-	-	-	-	-	
				37		9.0	-	-	-	-	-	
				37		4.1	-	-	-	-	-	
				37		4.3	-	-	-	-	-	

Table 3.5. *Cont.*

Sludges	Substrates	[1]Mixture	Digester (volume)	[2]HRT (d)	[3]T (ºC)	[4]OLR ($kg_{VS}\cdot m^{-3}d^{-1}$)	CH_4 ($L\cdot kg_{VS}^{-1}$)	CH_4 ($m^3\cdot m^{-3}d^{-1}$)	CH_4 (% biogas)	[5]VFA ($mg\cdot L^{-1}$)	[6]VS removal (%)	Reference
[7]PS:[8]WAS	[18]FW (dining hall)	-	[19]ASBR ([15]Te+[16]M) (4L+4L)	-	55-35	3.5	0.10	0.34	-	-	-	Kim *et al*., 2006
		-	ASBR (M+M) (4L+4L)	-	35-35		0.15	0.54	-	-	-	
WAS	[12]FVW (market)	100:0([10]TS)	ASBR(2L)	20	35	0.3	0.13	0.04	57	480	55	Habiba *et al*., 2009
		65:35 (TS)				0.3	0.28	0.08	57	560	65	
		35:65 (TS)				0.4	0.34	0.14	58	640	84	
		30:70 (TS)		10		1.0	0.34	0.33	59	690	88	
		20:80 (TS)				1.6	0.26	0.40	60	800	86	
		15:85 (TS)				1.9	0.29	0.52	59	850	84	
		10:90 (TS)				2.9	0.25	0.68	60	928	85	
		0:100 (TS)				3.5	0.20	0.70	58	2400	81	
WAS	FVW: wholesale market; kitchen wastes	100:0 (VS)	CSTR(200L)	28	35-37	0.8	0.04	0.09	60	-	24	Caffaz *et al*., 2008
		83:17 (VS)	CSTR(200L)	44		0.9	0.06	0.16	62	-	32	
		77:23(VS)	CSTR(200L)	34		1.1	0.22	0.20	57	-	33	
PS:WAS	FW (dining hall)	60:40 (VS)	ASBR(M+Te) (4L+4L)	7	55-35	6.1	0.20	1.24	-	-	45	Kim *et al*., 2011
			ASBR(M+M) (4L+4L)	8	35-35	3.5	0.18	0.69	-	-	42	

Table 3.5. *Cont.*

Sludges	Substrates	[1]Mixture	Digester (volume)	[2]HRT (d)	[3]T (°C)	[4]OLR ($kg_{VS}·m^{-3}d^{-1}$)	CH_4 ($L·kg_{VS}^{-1}$)	CH_4 ($m^3·m^{-3}d^{-1}$)	CH_4 (% biogas)	[5]VFA ($mg·L^{-1}$)	[6]VS removal (%)	Reference
[8]WAS	[18]FW: markets; canteen, restaurant	100:0 (VS)	[11]CSTR (380L)	22	37	1.22	0.09	0.11	61	-	-	Cavinato *et al.*, 2013
		75:25 (VS)				1.60	0.21	0.32	60	-	-	
		100:0 (VS)			55	1.66	0.30	0.48	62	-	-	
		75:25 (VS)				2.21	0.35	0.77	62	-	-	
[7]PS:WAS	FW canteen: rice, meat, veg., oil	100:0 (VS)	CSTR(6L)	30	35	4.0	0.24	0.95	65	1268	38	Dai *et al.*, 2012
				16		6.4	0.18	1.13	65	784	29	
		71:29 (VS)		30		4.6	0.30	1.39	61	666	51	
				16		7.5	0.26	1.96	60	462	45	
		47:53 (VS)		30		5.1	0.35	1.79	57	525	62	
				16		8.5	0.30	2.58	56	352	56	
		29:71 (VS)		30		6.0	0.40	2.40	56	380	70	
				16		10.3	0.36	3.66	55	288	65	
		0:100 (VS)		16		10.8	0.42	4.49	51	446	79	
WAS	FW *canteen; [12]FVW **market	50:25*:25**	CSTR(1m^3)	100	35	1.20	0.47	0.56	63	203	70	Liu *et al.*, 2012
				50		2.40	0.44	1.05	61	195	66	
				33		3.60	0.40	1.34	60	230	63	
				25		4.80	0.42	2.04	58	250	65	
				20		6.00	0.40	2.35	55	500	65	
				15		8.00	0.34	2.94	56	570	62	

Notes of Table 3.5: [1]Mixture: Sludge to substrate ratio; [2]HRT: Hydraulic retention time; [3]T : Temperature; [4]OLR : Organic loading rate; [5]VFA : Volatile fatty acids; [6]VS : on volatile solids basis; [7]PS: Primary sludge; [8]WAS: Waste activated sludge; [9]OFMSW: Organic fraction of municipal solid waste; [10]TS: on total solids basis; [11]CSTR: Continuous stirred tank reactor; [12]FVW: Fruit and vegetable waste; [13]ITQ: Inclined tubular digester; [14]TPAD: Temperature phased anaerobic digestion; [15]Te: Thermophilic; [16]M: Mesophilic; [17]UASB: Upflow anaerobic sludge blanket; [18]FW: Food waste; [19]ASBR: Anaerobic sequencing batch reactor

Table 3. 6. *Operational and control parameters of the SS anaerobic co-digestion with grease waste*

Sludges	Substrates	[1]Mixture	Digester (volume)	[2]HRT (d)	[3]T (ºC)	[4]OLR ($kg_{VS}·m^{-3}d^{-1}$)	CH_4 ($L·kg_{VS}^{-1}$)	CH_4 ($m^3·m^{-3}d^{-1}$)	CH_4 (% biogas)	[5]VFA ($mg·L^{-1}$)	[6]VS removal (%)	Reference
[7]PS:[8]WAS (50:50 [9]v)	Grease trap sludge Sludge	100:0(VS)	[10]CSTR (35L)	10	35	3.00	0.38	0.83	71	-	-	Davidsson *et al.*, 2008
		95:5(VS)				3.00	0.30	0.89	70	-	-	
		90:10(VS)				3.00	0.36	1.08	69	-	-	
		100:0(VS)		13		2.50	0.7	0.68	65	-	45	
		90:10(VS)				2.50	0.30	0.74	66	-	55	
		70:30(VS)				2.40	0.34	0.83	69	-	58	
		70:30(VS)			52	4.45	0.51	2.23	69	644	51	
PS:WAS	Grease trap meat processing plant meat processing plant	100:00 (VS)	CSTR(4L)	20	35	1.08-1.16	0.32	0.27	61	<1000	44-48	Luostarinen *et al.*, 2009
				18		1.29-1.42	0.32	0.35	60		50-54	
				16		1.56-2.09	0.28	0.42	59		55-67	
		95:5 (VS)		20		1.14-1.22	0.35	0.30	63		22-27	
				18		1.38-1.51	0.35	0.40	63		48-52	
				16		1.67-2.23	0.37	0.55	63		50-63	
		80:20 (VS)		20		1.37-1.45	0.42	0.41	62		36-40	
				18		1.58-1.73	0.45	0.58	66		45-49	
				16		1.93-2.45	0.44	0.73	63		55-64	
		72:28 (VS)				2.80	0.44	1.03	60		51	
						3.13	0.45	0.99	65		62	
		54:46 (VS)				3.46	0.46	1.13	60		67	
		45:55 (VS)				3.99	0.32	0.96	63		72	
		29:71 (VS)				4.41	0.31	1.06	63		70	

Table 3.6. *Cont.*

Sludges	Substrates	[1]Mixture	Digester (volume)	[2]HRT (d)	[3]T (ºC)	[4]OLR (kg$_{VS}$·m^{-3}d^{-1})	CH_4 (L·kg$_{VS}$$^{-1}$)	CH_4 (m^3·m^{-3}d^{-1})	CH_4 (% biogas)	[5]VFA (mg·L^{-1})	[6]VS removal (%)	Reference
[7]PS:[8]WAS (40:60 VS)	Polymer-dewatered [11]FOG	100:0 (VS)	[10]CSTR (2L)	12	35	2.45	0.16	0.39	-	495	25	Kabouris *et al*., 2009
		100:0 (VS			52	2.45	0.20	0.49	-	719	31	
		52:48 (VS)			35	4.35	0.45	1.95	66	513	45	
PS:WAS (70:30 [9]v)	Grease trap [12]DAF unit [13]WWTP	100:00 (VS)	CSTR (5.5L)	20	35	0.90-2.10	0.25	0.25	71	-	36	Silvestre *et al*., 2011[1]
		96:4 (VS)				1.10-1.30	0.28	0.34	69	-	46	
		77:23 (VS)				1.20-2.00	0.37	0.57	71	-	52	
		63:37 (VS)				1.50-1.90	0.33	0.54	68	-	56	
WAS	Fatty wastewater restaurants	100:0 (v)	CSTR (2L)	21	35	1.12	0.12	0.13	65	-	33	Carrere *et al*., 2012
		90:10 (v)				1.01	0.17	0.17	77	-	36	
		60:40 (v)				0.91	0.36	0.32	70	-	46	
		90:10 (v)				0.84	0.27	0.23	74	-	42	
WAS	Grease trap DAF unit WWTP	100:0 (VS)	CSTR (200L)	24	36	1.90	0.26	0.49	66	-	29	Girault *et al*., 2012
		93:7 (VS)		30		1.80	0.18	0.40	66	-	43	
		87:13 (VS)		24		2.00	0.33	0.66	69	-	36	
		85:15 (VS)	CSTR (3.4L)	25		1.70	0.32	0.55	67	-	42	
		78:22 (VS)		26		1.60	0.29	0.46	67	-	42	
		66:34 (VS)		25		1.40	0.40	0.58	67	-	39	
		48:52 (VS)		24		1.20	0.55	0.67	69	-	44	
		26:74 (VS)		24		1.00	0.45	0.47	68	-	33	
		13:87 (VS)		25		0.80	0.16	0.14	67	-	17	

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[1] Chapter 4

Table 3.6. *Cont.*

Sludges	Substrates	[1]Mixture	Digester (volume)	[2]HRT (d)	[3]T (ºC)	[4]OLR ($kg_{VS}\cdot m^{-3}d^{-1}$)	CH_4 ($L\cdot kg_{VS}^{-1}$)	CH_4 ($m^3\cdot m^{-3}d^{-1}$)	CH4 (% biogas)	[5]VFA ($mg\cdot L^{-1}$)	[6]VS removal (%)	Reference
[7]PS:[8]WAS (30:70 [9]v)	Grease trap [12]DAF unit [13]WWTP	100:0 (VS)	[10]CSTR (3L)	30	35	0.77-0.84	0.20	0.23	64	881	38	Martínez *et al.*, 2012
		100:0 (VS)		26		0.88-0.95	0.17	0.28	65	757	49	
		100:0 (VS)		21		1.10-1.19	0.26	0.36	59	800	57	
		99:1 (VS)		30		0.77-0.84	0.19	0.23	66	730	50	
		99:1 (VS)		26		0.90-0.98	0.13	0.20	59	757	50	
		99:1 (VS)		21		1.11-1.19	0.09	0.16	59	815	52	
		92:8 (VS)		30		0.79-0.87	0.14	0.16	70	824	46	
PS:WAS (74:26 VS)	Grease trap DAF unit WWTP	100:0 (VS)	CSTR (3L)	15	35	1.90	0.29	0.56	65	580	52	Noutsopoulos *et al.*, 2013
		80:20 (VS)				2.90	0.34	0.98	66	600	59	
		60:40 (VS)				3.10	0.46	1.43	70	610	55	
		40:60 (VS)				3.50	0.49	1.72	70	750	59	
		40:60 (VS)				4.10	-	-	-	-	24	
		10:90 (VS)				8.30	-	-	-	-	-	
		81:19 (VS)				1.16	0.78	0.90	66	24	56	
		77:23 (VS)				1.58	0.63	1.00	64	28	-	
		70:30 (VS)				1.60	0.22	0.35	66	38	-	
WAS	Grease interceptor waste from a food service establishment	100:0(VS)	CSTR (6L)	20	37	1.24	0.13	0.16	59	-	-	Wang *et al.*, 2013
		54:46 (VS)				1.58	0.50	0.77	68	-	-	
		65:35 (VS)				2.16	0.75	1.60	70	-	-	
		83:17 (VS)				3.54	0.63	2.24	69	-	-	

Table 3.6. *Cont.*

Sludges	Substrates	[1]Mixture	Digester (volume)	[2]HRT (d)	[3]T (ºC)	[4]OLR ($kg_{VS}·m^{-3}d^{-1}$)	CH_4 ($L·kg_{VS}^{-1}$)	CH_4 ($m^3·m^{-3}d^{-1}$)	CH_4 (% biogas)	[5]VFA ($mg·L^{-1}$)	[6]VS removal (%)	Reference
[7]PS:[8]WAS (75:25 [9]v)	Oil used from restaurants	81:19 (VS)	[10]CSTR (30L)	30	38	0.91	0.47	0.43	65	<100	-	Pastor *et al.*, 2013
		81:19 (VS				0.91	0.46	0.42	66			
		77:23 (VS)				0.74	0.31	0.23	66			
		86:14 (VS)				1.21	0.30	0.36	60			
		47:53 (VS)				0.64	0.35	0.23	64			
		65:35 (VS)				0.98	0.27	0.27	63			
		78:22 (VS)				1.51	0.29	0.43	62			
		66:34 (VS)				0.97	0.41	0.40	62			
PS:WAS	Grease trap waste fro, meat processing plant	100:0 (VS)	CSTR(5.5L)	10	37	2.66-3.37	0.18	0.54	68	2320-880	44	Nieczaj *et al.*, 2012
		98:2 (VS)				2.35	0.20	0.47	71	897-840	39	
		96:4 (VS)				2.36	0.21	0.50	70	800-874	45	
		94:6 (VS)				1.93	0.23	0.44	71	600-606	38	
		92:8 (VS)				1.98	0.21	0.42	74	640-577	46	
		90:10 (VS)				2.44-2.87	0.23	0.61	72	611-704	55	

Table 3.6. *Cont.*

Sludges	Substrates	[1]Mixture	Digester (volume)	[2]HRT (d)	[3]T (ºC)	[4]OLR ($kg_{VS}\cdot m^{-3}d^{-1}$)	CH_4 ($L\cdot kg_{VS}^{-1}$)	CH_4 ($m^3\cdot m^{-3}d^{-1}$)	CH_4 (% biogas)	[5]VFA ($mg\cdot L^{-1}$)	[6]VS removal (%)	Reference
[7]PS:[8]WAS (75:25 [9]v)	Grease trap [12]DAF unit [13]WWTP	100:0 (VS)	[10]CSTR(1200L)	20	36	1.03	0.61	0.63	68	4	47	Razaviarani *et al.*, 2013a
		100:0 (VS)				0.95	0.56	0.53	65	6	-	
		100:0 (VS)				1.12	0.51	0.57	66	8	-	
		100:0 (VS)				0.94	0.58	0.54	65	16	44	
		100:0 (VS)				1.22	0.50	0.61	66	17	-	
		100:0 (VS)				1.12	0.62	0.70	70	13	-	
		100:0 (VS)				1.03	0.57	0.59	71	4	-	
		94:6 (VS)				1.01	0.68	0.69	67	7	-	
		88:12 (VS)				1.26	0.63	0.80	68	7	-	

Notes of Table 3.6:[1]Mixture: Sludge to substrate ratio; [2]HRT: Hydraulic retention time; [3]T : Temperature; [4]OLR : Organic loading rate; [5]VFA : Volatile fatty acids; [6]VS : on volatile solids basis; [7]PS: Primary sludge; [8]WAS: Waste activated sludge; [9]V: on volume basis; [10]CSTR: Continuous stirred tank reactor; [11]FOG: fat, oil and grease; [12]DAF: Dissolved air flotation;[13]WWTP: Waste Water Treatment Plant

***Table 3. 7.** Operational and control parameters of the SS anaerobic co-digestion with slaughterhouse wastes*

Sludges	Substrates	[1]Mixture	Digester (volume)	[2]HRT (d)	[3]T (ºC)	[4]OLR (kg$_{VS}$·m^{-3}d^{-1})	CH$_4$ (L·kg$_{VS}$$^{-1}$)	CH$_4$ (m^3·m^{-3}d^{-1})	CH$_4$ (% biogas)	[5]VFA (mg·L^{-1})	[6]VS removal (%)	Reference
[7]PS:[8]WAS	Slaughtered pig blood	90:10 ([9]v)	Two stage	12.5 (total)	-	2.6	0.45	1.19	72	6076	52	Zhao and Kugel, 1996
		90:10 (v)	[10]CSTR_[11]Te (0.65 m^3)	2.5 (1º st)	75							
		90:10 (v)		10.0 (2º st)	37							
		90:10 (v)	CSRT_[12]M (1.95 m^3) "	16.5 (total)	-	0.8	0.51	0.43	74	3752	58	
		90:10 (v)		2.5 (1º st)	75							
		90:10 (v)		14.0 (2º st)	37							
PS:WAS	Sausage processing floats	90:10 (v)	Two stage	12.5 (total)	-	3	0.47	1.42	69	4896		Zhao and Kugel, 1996
		90:10 (v)	CSTR_Te (0.65 m^3)	2.5 (1º st)	75	-		-	-			
		90:10 (v)	CSTR_M (1.95 m^3)	10.0 (2º st)	37	3.0	0.47	1.42	69	167	45	
PS:WAS	Stomach content	0:100 (v)	CSTR (2m^3)	44	37	3.2([13]TS)	0.06(TS)	0.19	-	-	-	Rosenwinkel and Meyer, 1999
		0:100 (v)		25		5.8(TS)	0.00(TS)	-	-	-	-	
		70:30 (v)		20		3.1(TS)	0.11(TS)	0.34	-	5314	-	
		65:25 (v)		25		2.0(TS)	0.17(TS)	0.34	-	4619	-	
		65: 25 (v)		17		2.9(TS)	0.23(TS)	0.67	-	-	-	
		100:0 (v)		20		1.3(TS)	0.28(TS)	0.36	-	1863	-	
		100:0 (v)		25		1.2(TS)	-	-	-	1544	-	
		87.5:12.5 (v)		25		1.3(TS)	0.40(TS)	0.52	-	1730	-	
		87.5:12.5 (v)		17		2.0(TS)	0.12(TS)	0.24	-	-	-	

Table 3.7. *Cont*

Sludges	Substrates	[1]Mixture	Digester (volume)	[2]HRT (d)	[3]T (ºC)	[4]OLR ($kg_{VS}\cdot m^{-3}d^{-1}$)	CH_4 ($L\cdot kg_{VS}^{-1}$)	CH_4 ($m^3\cdot m^{-3}d^{-1}$)	CH_4 (% biogas)	[5]VFA ($mg\cdot L^{-1}$)	[6]VS removal (%)	Reference
[7]PS:[8]WAS	Slaughter flotation tailings	0:100 ([9]v)	[10]CSTR(2 m^3)	44	37	1.27([13]TS)	0.37	0.56	-	0	-	Rosenwinkel and Meyer, 1999
				30		1.87(TS)	0.33	0.62	-	678	-	
				25		2.24(TS)	0.35	0.78	-	5314	-	
		75:25 (v)		25		0.68(TS)	0.43	0.29	-	4619	-	
				20		0.99(TS)	0.38	0.38	-	-	-	
				15		1.67(TS)	0.41	0.68	-	-	-	
		87.5:12.5 (v)		25		0.63(TS)	0.34	0.21	-	-	-	
				20		0.94(TS)	0.31	0.29	-	210	-	
				15		1.46(TS)	0.32	0.47	-	90	-	
PS:WAS	[14]ABP materials *digestive tract*	66:34 (VS)	CSTR (4L)	25	35	1.8	0.34	0.50	56	150	63	Luste and Luostarinen, 2010
				20		2.4	0.40	0.83	64	200	34	
				14		3.3	0.38	1.05	66	90	41	
				25		2.1	0.37	0.55	61	900	56	
				20		2.8	0.43	1.03	65	100	37	
				14		3.7	0.40	1.20	65	80	37	
		46:54 (VS)		25		2.2	0.34	0.60	63	500	66	
				20		2.9	0.41	1.00	65	100	34	
				14		4.0	0.39	1.28	67	80	38	

Table 3.7. *Cont.*

Sludges	Substrates	[1]Mixture	Digester (volume)	[2]HRT (d)	[3]T (ºC)	[4]OLR ($kg_{VS}\cdot m^{-3}d^{-1}$)	CH_4 ($L\cdot kg_{VS}^{-1}$)	CH_4 ($m^3\cdot m^{-3}d^{-1}$)	CH_4 (% biogas)	[5]VFA ($mg\cdot L^{-1}$)	[6]VS removal (%)	Reference
[7]PS:[8]WAS	[14]ABP Cathegory 2-3 Stirilized 133ºC 3bar 20 min	100:0([15]w)	[10]CSTR (4.5L)	20.0	37	1.40	0.23	0.33	-	-	47	Pitk *et al.*, 2013
		100:0([15]w)		22.5		0.90	0.09	0.42	-	-	42	
		97.5:2.4 (w)				2.13	0.40	0.84	-	-	57	
		95.0:5.0 (w)				2.68	0.62	1.67	-	-	64	
		92.5:7.5 (w)				3.55	0.59	2.10	-	-	61	
		80.0:10. (w)				4.54	0.55	2.56	-	-	-	
SS	SHW 50% intestines 21% meat tissue 21% post flotation sludge 8% bristle (w)	100:0w	CSTR (5 dm^3)	20	35	2.15	0.33	0.71	77	1879	51	Borowoski *et al.*, 2015
		100 :0		15		3.03	0.36	1.1	72	1406	51	
		70 :30		20		2.98	0.61	1.82	77	1823	61	
		70 :30		15		3.97	0.75	3	74	2227	57	
		50 :50		20		3.1	0.89	2.8	68	2350	62	
		50 :50		15		4.1	0.76	3.12	67	3774	62	

Notes of Table 3.7:[1]Mixture: Sludge to substrate ratio;[2]HRT : Hydraulic retention time; [3]T : Temperature; [4]OLR : Organic loading rate;[5]VFA : Volatile fatty acids; [6]VS : on volatile solids basis;[7]PS: Primary sludge;[8]WAS: Waste activated sludge;[9]V:on volume basis;[10]CSTR: Continuous stirred tank reactor;[11]Te: termophilic; [12]M: mesophilic; [13]TS: on total solids basis; [14]ABP: Animal by-products; [15]w: on basis weight

Table 3. 8. *Operational and control parameters of the SS anaerobic co-digestion with glycerine*

Sludges	Substrates	[1]Mixture	Digester (volume)	[2]HRT (d)	[3]T (ºC)	[4]OLR ($kg_{VS}\cdot m^{-3}d^{-1}$)	CH_4 ($L\cdot kg_{VS}^{-1}$)	CH_4 ($m^3\cdot m^{-3}d^{-1}$)	CH_4 (% biogas)	[5]VFA ($mg\cdot L^{-1}$)	[6]VS removal (%)	Reference
[7]PS:[8]WAS	crude glycerine biodiesel manufacturing	100:0 ([9]v)	[10]CSTR (1L)	24	35	-	-	-			-	Fountoulakis *et al.*, 2010
		99:1(v)				-	-	1.25			-	
		97:3 (v)				-	-				-	
		100:0 (v)	CSTR (3L)	23-25		-	-	0.37			-	
		99:1(v)		23-25		-	-	0.78			-	
PS:WAS (3:1)	Glicerina Conola oil biodiesel production	100:0(VS)	CSTR (1200L)	20	36	0.90	0.50	0.45		7.3	45	Razaviarani *et al.*, 2013b
		100:0(VS)				0.81	0.58	0.47		3.48	43	
		100:0(VS)				0.82	0.61	0.50		5.78	45	
		87:13 (VS)				1.03	0.90	0.93		4085	51	
		78:22(VS)				1.04	0.83	0.86		42.1	53	
		70:30(VS)				1.18	0.38	0.45		91.1	31	
PS	crude glycerine	100:0(VS)	CSTR (4L)	32	37	1.11	-	0.35			-	Nartker *et al.*, 2014
		81:19(VS)				0.98	0.22	0.38			60	
		70:30(VS)				1.26	0.36	0.56			66	
		67:33 (VS)				1.70	0.40	0.72			66	
		55:45(VS)				1.67	0.43	0.65			59	
		51:49(VS)				1.90	0.48	0.78			64	
		41:59(VS)				1.88	0.43	0.68			56	
		36:64(VS)				2.09	0.40	0.75			56	
		39:61(VS)				2.54	0.45	1.02			63	
		31:69(VS)				2.42	0.60	1.02			65	
		35:65(VS)				2.88	0.50	1.26			67	

Table 3.8. *Cont.*

Sludges	Substrates	[1]Mixture	Digester (volume)	[2]HRT (d)	[3]T (ºC)	[4]OLR ($kg_{VS}\cdot m^{-3}d^{-1}$)	CH_4 ($L\cdot kg_{VS}^{-1}$)	CH_4 ($m^3\cdot m^{-3}d^{-1}$)	CH_4 (% biogas)	[5]VFA ($mg\cdot L^{-1}$)	[6]VS removal (%)	Reference
[8]WAS	crude glycerine	100:0([9]v)	Two_series [10]CSTR (40L+60L)	12.3	37	-	-	0.3 (B)				Athanasoulia *et al.*, 2012
		100:0(v)		14				0.21				
		100:0(v)		16.4				0.23				
		100:0(v)		19.7				0.20				
		98:2 (v)		12.3				1.10				
		98:2 (v)		14				1.00				
		98:2 (v)		16.4				0.90				
		98:2 (v)		19.7				0.80				
		97:3(v)		12.3				1.40				
		97:3(v)		14				1.30				
		97:3(v)		16.4				1.00				
		97:3(v)		19.7				0.90				
[7]PS:WAS (58:32)	crude glycerine	100:0 (v)	CSTR (0.85L)	17	37	1.81	0.40	0.72			53	Jensen *et al.*, 2014
						2.14	0.51	1.08			51	
						2.16	0.38	0.82			55	
						1.92	0.36	0.70			52	
		99.5:0.5(v) (1 pulse day)				2.22	0.36	0.80			62	
		99.5:0.5(v) (4 pulse day)				2.43	0.39	0.94			58	
		98:2(v) (4 pulse day)				3.68	0.34	1.25			75	
		99.5:0.5(v) (1 pulse day)				2.22	0.35	0.78			62	

Notes of Table 3.8:[1]Mixture: Sludge to substrate ratio; [2]HRT: Hydraulic retention time; [3]T : Temperature; [4]OLR : Organic loading rate;[5]VFA : Volatile fatty cids; [6]VS : on volatile solids basis; [7]PS: Primary sludge;[8]WAS: Waste activated sludge; [9]V: on volume basis; [10]CSTR: Continuous stirred tank reactor.

Table 3. 9. *Operational and control parameters of the SS anaerobic co-digestion with other organic wastes*

Sludges	Substrates	^{1}Mixture	Digester (volume)	^{2}HRT (d)	^{3}T (ºC)	^{4}OLR ($kg_{VS}\cdot m^{-3}d^{-1}$)	CH_4 ($L\cdot kg_{VS}^{-1}$)	CH_4 ($m^3\cdot m^{-3}d^{-1}$)	CH_4 (% biogas)	^{5}VFA ($mg\cdot L^{-1}$)	^{6}VS removal (%)	Reference
^{7}PS:^{8}WAS	Olive mill effuents	67:33 $_{(^{9}COD)}$	^{10}ACD (130 dm^3) (^{11}SRT:30 d)	13.0	37	4.2$_{(COD)}$	-	1.40	64	-	66$_{(COD)}$	Carrieri *et al*., 1993
		60:40 $_{(COD)}$		9.0		4.8$_{(COD)}$		1.50	64		64$_{(COD)}$	
		40:60 $_{(COD)}$		8.0		7.7$_{(COD)}$		2.44	63		65$_{(COD)}$	
		55:45 $_{(COD)}$		8.5		6.9$_{(COD)}$		2.48	63		63$_{(COD)}$	
		44:56 $_{(COD)}$		8.1		7.0$_{(COD)}$		2.50	64		71$_{(COD)}$)	
		25:75 $_{(COD)}$		4.5		12($_{COD)}$		0.50	44		-	
PS:WAS	Chesse whey	58:42 $_{(COD)}$	ACD (130 dm^3) (SRT:21.5 d)	18.9	37	2.7$_{(COD)}$	-	0.82	73	-	65$_{(COD)}$	Carrieri *et al*., 1993
		44:56 $_{(COD)}$		14.6		3.5$_{(COD)}$		1.27	73		70$_{(COD)}$	
		100:00 $_{(COD)}$		27.0		1.6$_{(COD)}$		0.42	76		57$_{(COD)}$	
		42:58 $_{(COD)}$		12.3		4.3 $_{(COD)}$		1.73	63		65$_{(COD)}$	
		42:58 $_{(COD)}$		12.3				1.72	69		74$_{(COD)}$	
		42:58 $_{(COD)}$		12.3				1.73	66		79$_{(COD)}$	
PS:WAS	Landfill lechate (young)	58:42 $_{(COD)}$	^{12}CSTR (130 dm^3)	15	37	2.07$_{(COD)}$	-	0.81	65	-	65$_{(COD)}$	Carrieri *et al*., 1993
		54:46 $_{(COD)}$		25		1.14$_{(COD)}$		0.27	44		44$_{(COD)}$	
		70:30 $_{(COD)}$		15		2.85$_{(COD)}$		1.07	67		67$_{(COD)}$	
		100:00$_{(COD)}$		25		2.05$_{(COD)}$		0.62	63		63$_{(COD)}$	
				2.5	75	-		-	-	3877		
				10.0	37	1.67	0.39	0.65	65	101	34	
		80:20 (^{13}v)		12.5	-	-	-	-	-	-		
				2.5	75	0.37	0.46	0.17	71	4829	46	
				10.0	37	-	-	-	-	205		

Table 3.9. *Cont.*

Sludges	Substrates	[1]Mixture	Digester (volume)	[2]HRT (d)	[3]T (ºC)	[4]OLR ($\mathrm{kg_{VS}\cdot m^{-3}d^{-1}}$)	CH_4 ($\mathrm{L\cdot kg_{VS}^{-1}}$)	CH_4 ($\mathrm{m^3\cdot m^{-3}d^{-1}}$)	CH_4 (% biogas)	[5]VFA ($\mathrm{mg\cdot L^{-1}}$)	[6]VS removal (%)	Reference
[7]PS:[8]WAS	Macroalgae (Venice lagoon)	100:0 ([14]TS)	[12]CSTR	14.5	37	1.70	0.15	0.25	63	460	29	Cecchi *et al.*, 1996
		81:19 (TS)		14.7	37	2.60	0.22	0.57	71	320	31	
		83:17 (TS)		11.2	37	4.40	0.18	0.79	72	33	27	
		62:38 (TS)		11.7	37	4.20	0.17	0.69	69	610	26	
		60:40 (TS)		11.2	55	5.30	0.00	0.03	23	6080	4	
		40:60 (TS)		12.3	55	5.50	0.13	0.79	71	1390	21	
PS:WAS	Wood chips[a] & corn starch[b]	50:34[a]:15[b] ([13]v)	CSTR (2L)	20	37	0.8$_{([9]\mathrm{COD})}$	0.48$_{([15]\mathrm{VSr})}$	-	-	-	11	Converti *et al.*, 1997
		67:33 (TS)				1.4$_{(\mathrm{COD})}$	0.45$_{(\mathrm{VSr})}$				15	
						2.2$_{(\mathrm{COD})}$	0.48$_{(\mathrm{VSr})}$				15	
						3.4$_{(\mathrm{COD})}$	0.43$_{(\mathrm{VSr})}$				12	
						4.6$_{(\mathrm{COD})}$	0.34$_{(\mathrm{VSr})}$				13	
						6.1$_{(\mathrm{COD})}$	0.30$_{(\mathrm{VSr})}$				8	
PS:WAS	Potato processing concentrate	50:50 (v)	Two stage CSRT_[16]Te (0.65 m^3)	12.5	75/37	-						Zhao and Kugel, 1996
				2.5		-				3877		
				10.0		1.67	0.39	0.65	65	101	34	
		80:20 (v)	CSRT_[17]M (1.95 m^3)	12.5		-						
				2.5		0.37	0.46	0.17	71	4829	46	
				10.0		-				205		

Table 3.9. *Cont.*

Sludges	**Substrates**	**^{1}Mixture**	**Digester (volume)**	**^{2}HRT (d)**	**^{3}T (ºC)**	**^{4}OLR ($kg_{VS}\cdot m^{-3}d^{-1}$)**	**CH_4 ($L\cdot kg_{VS}^{-1}$)**	**CH_4 ($m^3\cdot m^{-3}d^{-1}$)**	**CH_4 (% biogas)**	**^{5}VFA ($mg\cdot L^{-1}$)**	**^{6}VS removal (%)**	**Reference**
^{8}WAS	Confectionary Wastes sugar Syrups	*mixture 1 **mixture 2 (lower co-substrate)	^{18}SBR (^{16}Te+^{17}M) (5+5L)	4h-8d*	55/35	0.63	0.12	-		-	-	Lafitte-Trouqué and Forster, 2000
				4h-12d*		0.42	0.34	-		-	-	
				4h-12d**		0.42	0.30	-		-	-	
				4h-15d**		0.41	0.31	-		-	-	
			^{12}CSTR (10L)	20d*	35	0.33	0.36	-		-	-	
				20d**		0.33	0.28	-		-	-	
^{7}PS:WAS	^{19}OFMS^{a} Enzyme industry waste^{b} Paper mill sludge^{c}	30:20^{a}:20^{b}:30^{c13}v	CSTR (5L)	14	35	7.00	0.30	-	-	-	-	Einola *et al*., 2001
		50:30^{a}:20^{b}:0^{c}v					0.38					
		50:20^{a}:20^{b}:10^{c}v					0.28					
		70:0^{a}:30^{b}:0^{c}v				4.00	0.18					
		60:40^{a}:0^{b}:0^{c}v				7.00	0.33					
PS:WAS (68:32) (^{6}VS)	Carbohydrate-rich food processing waste	28:72(VS)	CSRT (0.5L)	-	35	-	0.48$_{(15VSr)}$	-	-	-	10.8	Björnsson *et al*., 2000
		28:72(VS)					0.45$_{(VSr)}$				14.5	
		28:72(VS)					0.48$_{(VSr)}$				14.7	
		28:72(VS)					0.43$_{(VSr)}$				12.4	
		28:72(VS)					0.33$_{(VSr)}$				12.5	
		28:72(VS)				5.9 $_{(^{9}COD)}$	0.30$_{(VSr)}$				7.5	

Table 3.9. *Cont.*

Sludges	Substrates	[1]Mixture	Digester (volume)	[2]HRT (d)	[3]T (ºC)	[4]OLR ($kg_{VS}·m^{-3}d^{-1}$)	CH_4 ($L·kg_{VS}^{-1}$)	CH_4 ($m^3·m^{-3}d^{-1}$)	CH_4 (% biogas)	[5]VFA ($mg·L^{-1}$)	[6]VS removal (%)	Reference
[8]WAS thickened	Poultry waste^1 Fruit and vegetable waste^2 Cattle slurry^3	52.5:17.5^1:15^2:15^3 ([14]TS)	CSTR (8.8L)	20	35	1.85	0.192	-	-	1572	-	Misi and Forster, 2002
		35:35^1:15^2:15^3 (TS)				1.80	0.190			1779		
		17.5:52.5^1:15^2:15^3 (TS)				1.57	0.250			440		
		0:70^1:15^2:15 (TS)				1.51	0.250			486		
		70:0^1:15^2:15^3 (TS)				1.86	0.162			816		
		52.5:17.5^1:15^2:15^3 (TS)				1.89	0.163			2271		
[7]PS:WAS (32:68) ([13]v)	Potato processingIndustrial waste	28:72 (VS)	[12]CSTR (0.5L)	19.7	35	1.6	0.42	0.67	70	0	-	Murto *et al.*, 2004
				19.0		1.5	0.42	0.63	70	0	-	
				13.4		1.9	0.42	0.80	70	0	-	
				9.3		3.1	0.42	1.30	70	50	-	
				7.1		4.2	0.42	1.76	70	250	-	
				5.3		5.9	0.00	0.00	-	0	-	
PS:WAS (30:70) (v)		20:80 (VS)		18.0		1.6	0.42	0.67	70	0	-	
				12.6		2.7	0.42	1.13	70	-	-	
				9.1		4.0	0.42	1.68	70	0	-	
				7.0		5.3	0.00	0.00	-	180	-	
		99.6:0.4(VS)		19		1.44	0.76	-		-	-	
		99.1:0.9(VS)		18		1.45	0.69	-		-	-	

Table 3.9. *Cont.*

Sludges	**Substrates**	**[1]Mixture**	**Digester (volume)**	**[2]HRT (d)**	**[3]T (ºC)**	**[4]OLR ($kg_{VS}·m^{-3}d^{-1}$)**	**CH_4 ($L·kg_{VS}^{-1}$)**	**CH_4 ($m^3·m^{-3}d^{-1}$)**	**CH_4 (% biogas)**	**[5]VFA ($mg·L^{-1}$)**	**[6]VS removal (%)**	**Reference**
[7]PS:[8]WAS (60:40) ([13]V)												
PS:WAS (31:69) (v)	Landifill lechate (5-10 years)	100:0 ([6]VS)	[12]CSTR (40L)	20	35	1.44	0.65	-		-	-	Montusiewicz and Lebiocka, 2011
		16:80 (VS)		20.2		1.5	0.42	0.63	70	0.00	-	
		16:80 (VS)		10.4		3.9	0.42	1.64	70	0.22	-	
		16:80 (VS)		9.7		4.4	0.00	0.00	-	-	-	
PS:WAS (60:40) (v)		100:0 (VS)		20		1.44	0.65	-		-	-	
		99.6:0.4(VS)		19		1.44	0.76	-		-	-	
		99.1:0.9(VS)		18		1.45	0.69	-		-	-	
WAS	Olive mill wastewater	100:0 ([13]v)	[12]CSTR1/ CSTR2 (40L+60L)	12.3	37	2.34 ([9]COD)	-	-	-	<100	37 ([20]VSS)	Athanasoulia *et al.*, 2012
				14.0		2.06 (COD)					41 (VSS)	
				16.4		1.76 (COD)					37 (VSS)	
				19.7		1.47 (COD)					36 (VSS)	
		70:30 (v)		12.3		5.57 (COD)					28 (VSS)	
				14.0		4.89 (COD)					27 (VSS)	
				16.4		4.18 (COD)					30 (VSS)	

Table 3.9. *Cont.*

Sludges	Substrates	[1]Mixture	Digester (volume)	[2]HRT (d)	[3]T (ºC)	[4]OLR ($kg_{VS}\cdot m^{-3}d^{-1}$)	CH_4 ($L\cdot kg_{VS}^{-1}$)	CH_4 ($m^3\cdot m^{-3}d^{-1}$)	CH_4 (% biogas)	[5]VFA ($mg\cdot L^{-1}$)	[6]VS removal (%)	Reference
[7]PS:[8]WAS	Cattle manure[a] Food waste[b]		[12]CRST (3.75L)	22		0.58	0.75	0.62	70	-	53	Marañon *et al.*, 2012
				22	35	0.53	0.74	0.60	69	-	57	
		10:70[a]:20[b]([21]w)		20		0.57	0.74	0.55	68	-	54	
				18		0.56	0.65	0.43	67	-	57	
				22		0.62	0.44	0.55	71	-	59	
				22		0.64	0.42	0.53	66	-	56	
				20		0.63	0.42	0.58	67	-	56	
				18	55	0.48	0.33	0.49	68	-	56	
				22		0.51	0.59	0.49	72	-	56	
		20:70:10 (w)		"		0.56	0.57	0.47	68	-	54	
				20		0.59	0.60	0.45	68	-	57	
				18		0.70	0.55	0.38	68	-	53	
[7]PS:[8]WAS (60:40) ([13]v)	Shredded grass from public green spaces	100:0([14]TS)	CSTR (4L)	20	35	1.70 (TS)	0.30	-	-	-	-	Hidaka *et al.*, 2013
		91:9 (TS)				2.62 (TS)	0.39					
		66:34 (TS)				6.24 (TS)	0.36					
		50:50 (TS)				8.00 (TS)	0.38					
		100:0 (VS)				0.81	0.58	0.47	59	3	43	
		100:0 (VS)				0.82	0.61	0.50	59	6	45	
		87:13 (VS)				1.03	0.90	0.93	64	4085	51	
		78:22 (VS)				1.04	0.83	0.86	64	42	53	
		70:30 (VS)				1.18	0.38	0.45	53	91	32	

Table 3.9. *Cont.*

Sludges	Substrates	[1]Mixture	Digester (volume)	[2]HRT (d)	[3]T (ºC)	[4]OLR ($kg_{VS}·m^{-3}d^{-1}$)	CH_4 ($L·kg_{VS}^{-1}$)	CH_4 ($m^3·m^{-3}d^{-1}$)	CH_4 (% biogas)	[5]VFA ($mg·L^{-1}$)	[6]VS removal (%)	Reference
[7]PS:[8]WAS	Lixiviation of sugar beet pulp	-	[12]CSTR (5L)	30	35	1.1	-	0.23	69	-	-	Montañes *et al.*, 2013
				20		1.2	-	0.13	70	-	-	
				15		1.8	-	0.29	68	-	-	
				10		2.2	-	0.68	69	-	-	
				6		5.0	-	0.31	67	-	-	
PS:WAS (50:50) ([13]v)	Poultry mannure	100:0 (VS)	CSTR ($3dm^3$)	20	35	1.03	0.24	0.27	68	1901	36	Borowski and Weatherley, 2013
		100:0 (VS)				1.55	0.23	0.37	67	1089	34	
		70:30 (VS)		30		1.57	0.25	0.40	68	2084	49	
		70:30 (VS)				2.36	0.20	0.48	67	1585	43	
PS:WAS (50:50) ([21]w)	Primary clarifier skimming	100:0 ([9]COD)	CSTR (13L)	13	35	4.1 (COD)	-	0.77	-	-	51	Alanya *et al.*, 2013
		73:27 (COD)				5.6 (COD)	0.24 (COD)	1.34			59-66	
		61:39 (COD)				6.7 (COD)	0.26 (COD)	1.74			59-66	
		54:46 (COD)				7.6 (COD)	0.26 (COD)	1.99			59-66	
		36:64 (COD)				11.0 (COD)	0.31 (COD)	3.39			59-66	

Notes of Table 3.9:[1]Mixture: Sludge to substrate ratio;[2]HRT : Hydraulic retention time;[3]T : Temperature;[4]OLR : Organic loading rate;[5]VFA : Volatile fatty acids;[6]VS : on volatile solids basis;[7]PS: Primary sludge;[8]WAS: Waste activated sludge;[9]COD: Chemical oxygen demand;[10]ACD:Anaerobic contact digester; [11]SRT: solid retention time; [12]CSTR: Continuous stirred tank reactor[13]V: on basis volume;[14]TS: on total solids basis; [15]VSr; [16]Te:Thermophilic;[17]M: Mesophilic; [18]ASBR: Anaerobic sequencing batch reactor; [19]OFMSW: Organic fraction of municipal solid waste;[20]VSS: on basis volatile suspended solids; [21]w:on basis weight

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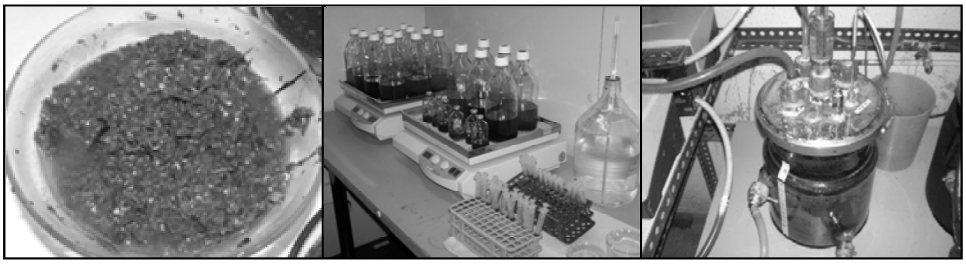

CHAPTER 4

BIOMASS ADAPTATION OVER ANAEROBIC CO-DIGESTION OF SEWAGE SLUDGE AND TRAPPED GREASE WASTE

The feasibility of sewage sludge co-digestion using intermediate waste generated inside a wastewater treatment plant, i.e. trapped grease waste from the dissolved air flotation unit, has been assessed in a continuous stirred lab reactor operating at 35ºC with a hydraulic retention time of 20 days. Three different periods of co-digestion were carried out as the grease waste dose was increased. When the grease waste addition was 27% of the COD fed (23% volatile solids fed (organic loading rate 3.0 $kg_{COD}\cdot m^{-3}\cdot d^{-1}$), an increase in volumetric methane production of 128% was reported. Specific activity tests suggested that anaerobic biomass had adapted to the co-substrate. The adapted inoculum showed higher acetoclastic methanogenic and β-oxidation syntrophic acetogenic activities but lower hydrogenotrophic methanogenic activity. The results indicate that a slow increase in the grease waste dose could be a strategy that favours biomass acclimation to fat-rich co-substrate, increases long chain fatty acid degradation and reduces the latter's inhibitory effect.

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4.1 INTRODUCTION

Sludge production in the European Union has been increasing for several years. More than 10 million tons dry matter of sewage sludge (SS) were produced in 2006 (Laturnus *et al.*, 2007), representing about 58 kg dry matter per inhabitant-equivalent and year (Mogoarou, 2000). As society demands cleaner water, and because of the strict regulations contained in the 91/271/EEC Council Directive (CEC, 1991), new wastewater treatment plants (WWTPs) will be built and the existing ones will be optimized; this will probably cause a further increase in sludge production.

SS contains a high percentage of organic matter (60–70% of the dry matter) and nutrients such as phosphorous and nitrogen, which can be recycled for agricultural use if the SS is free of heavy metals and other pollutants. The anaerobic digestion process is a well-known technology that improves SS quality for agricultural use, while at the same time producing biogas that can supply between 40–60% of the energy required to run a WWTP (Shizas and Bagley, 2004). Different strategies have been proposed to increase biogas production and optimize SS anaerobic digestion (Appels *et al.*, 2008; Kalogo and Monteith, 2008). One of these is the co-digestion of SS with other organic wastes, while increasing the load of biodegradable organic matter and improving the biochemical conditions of the different microorganism populations that develop. Since the optimum carbon-to-nitrogen ratio (C/N) is between 20–30 (Parkin and Owen, 1986), and SS has a C/N ratio of between 6 and 16, co-digestion with other organic waste with a high C/N ratio could improve the nutrient balance and increase the amount of degradable carbon and, consequently, the biogas yield (Sosnowski *et al.*, 2007).

Two factors that limit co-digestion are the associated transport cost of co-substrates and the addition of new, external waste to the WWTP. One possible option is to use intermediate waste generated inside the WWTP, such as the grease waste (GW) trapped in the dissolved air flotation (DAF) unit. This would lead to an optimization of the entire plant, since the costs of managing the GW to landfill will decrease, and its high fat content could increase biogas yield.

Various authors have reported increased methane yields during the co-digestion of SS with different types of fats. Davidsson *et al.* 2008 reported an increase of 9–27% when 10–30% grease, on a volatile solid (VS) basis, was added to an SS anaerobic reactor. Loustarinen *et al.* (2009) reported an increase of 60% when SS was co-digested with the grease trapped from a meat-processing plant (46% VS added), and

Kabouris *et al*. (2009) found that methane yields were 2.6 times higher when they added oil and grease from restaurants and food outlets (48% total VS load).

Fats are degraded following a specific anaerobic chain reaction and a metabolic route, which is different from that of proteins and carbohydrates. In the first step, the neutral fats are hydrolyzed (lipolyzed) into free long-chain fatty acids (LCFAs) and glycerol, catalyzed by extracellular lipases. The free LCFAs are converted into acetate and H_2 by acetogenic bacteria through a β-oxidation process, and finally methane is produced by methanogenic bacteria (Masse *et al*., 2002).

As the fat concentration in SS is generally low, the introduction of fatty wastes into a SS anaerobic reactor can change the biochemical activities of the different groups of anaerobic microorganisms. The acclimation of anaerobic sludge to a specific substrate leads to a new bacterial population that can be different from the mother culture (Gavala and Lyberatos, 2001) or at least result in a new bacterial population distribution (Palatsi *et al*., 2010).

The aim of this paper is: i) to characterize and to determine the methane potential of trapped GW from the dissolved air flotation unit of a WWTP, ii) to assess the feasibility of the co-digestion of SS and GW, and iii) to analyse biomass adaptation during co-digestion with GW.

4.2 MATERIAL AND METHODS

4.2.1 Substrates

In order to characterize the trapped GW from the DAF unit of a WWTP, four samples (GW_1, GW_2, GW_3 and GW_4) were taken from four different WWTPs (Barcelona, Spain). The SS used in the anaerobic experiments was a mixture of 70% primary sludge and 30% activated sludge. It was sampled every second week and kept refrigerated at 4ºC. GW_2, the co-substrate used in the continuous experiment, was sampled twice (GW_2 and GW_2') and kept frozen. Figure 4.1 shows an illustration of the different GW analyzed.

The inoculum (In1) used for batch and for the start-up of the continuous reactor was the effluent from a full scale anaerobic mesophilic digester. Biomass adaptation was assessed by comparing the activity of In1 with the adapted inoculum (In2), sampled at the end of the continuous experiment. Inocula were stored at 35ºC before using, in order to avoid the decrease of their activity. Storage time was less than 3 days in all the cases.

Figure 4. 1. *Illustration of the grease wastes analyzed*

4.2.2 Analytical methods

Total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), total chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), ammonia nitrogen (NH_4^+-N), total and partial alkalinity (TA, PA), sulfate (SO_4^{2-}-S) and fat concentrations were measured according to Standard Methods (APHA, AWWA, WEF, 1995). The elemental composition (carbon (C), nitrogen (N) and hydrogen (H)) was determined by catalytic oxidation combined with gas chromatography (LECO Instruments).

The biogas composition (CH_4, H_2, and CO_2) and the volatile fatty acids (VFA) (acetate, propionate, i-butyrate, n-butyrate, i-valerate and n-valerate acids) were determined with a gas chromatograph (CO-300 Varian, USA) using, respectively, a packed column with a thermal conductivity detector (TCD), and a capillary column with a flame ionization detector (FID). LCFA concentration was determined in accordance with Palatsi *et al*. (2009). Samples were lyophilized and digested at 90ºC using chlorotrimethylsilane (CTMS) as a catalyst to form fatty acid methyl ester (FAME), which was then identified and quantified by gas chromatograph (GC 3800 Varian, USA) with a capillary column and a FID detector.

4.3.3 Anaerobic biodegradability test

The methane potential of the GW and SS were determined by means of anaerobic biodegradability tests based on Campos *et al*. (2008). Glass vials with a capacity of 1.2 L were filled with 0.5 L of a mixture of inoculum In1 (5 $g_{VSS}\cdot L^{-1}$), substrate (5 $g_{COD}\cdot L^{-1}$) and deionized water. The mixture was supplemented with macro/micronutrients (NH_4Cl, K_2HPO_4,

$MgSO_4$, $CaCl_2$, $FeCl_2$, H_3BO_3, $ZnCl_2$, $CuCl_2$, $MnCl_2$, $(NH_4)_6Mo_7O_2$, $CoCl_2$, $NiCl_2$, EDTA, HCl, $NaSeO_3$, resazurine) and bicarbonate ($1g_{NaHCO3}\cdot g_{COD}^{-1}$), following Ferrer *et at.* (2010). The vials were bubbled with N_2 and placed in an orbital shaker inside a cabin at 35ºC. A control vial without substrate was included to assess the residual methane potential of the inoculum, thereby enabling the net methane potential to be calculated. The methane potential was determined as the final accumulated methane production per initial organic content of the substrate on COD basis (MP_{COD}; $NL_{CH4}\cdot kg_{COD}^{-1}$) or VS basis ($MP_{VS}$; $NL_{CH4}\cdot kg_{VS}^{-1}$).

4.3.4 Continuous experiment

A continuous experiment was carried out in a 7 L continuous stirred tank reactor (CSTR) with a working volume of 5.5 L. Figure 4.2 shows an illustration of the lab experimental set-up. The CSTR was operated at mesophilic range (35ºC), with a hydraulic retention time (HRT) of 20 days. The reactor was fed twice a day with a temporized peristaltic pump. Biogas production was measured with a volumetric milligas counter (Ritter Apparatebau GMBH & Co KG, model MGC- 10). The influent and effluent characteristics were measured once a week and biogas composition twice a week.

Figure 4.2. *Lab experimental set-up used to carry out the continuous experiment*

The continuous experiment was conducted over 302 days divided into five different periods. During periods I and II, SS alone was digested, while co-digestion with GW was performed during periods III, IV and V. The addition of GW was evaluated in terms of volumetric methane production ($Nm^3 \cdot m^{-3} \cdot d^{-1}$), methane yield ($Nm^3 \cdot t_{VSsinlet}^{-1}$) and organic matter removal (on COD and VS basis).

4.3.5 Biomass adaptation assessment

In order to assess the adaptation of the bacterial community to GW, a new biodegradability test of GW_2' was performed with the initial inoculum (In1) sampled in the WWTP, and the adapted inoculum (In2), sampled during period V of the continuous experiment. Specific activity tests of both inocula were also performed.

The specific activity tests, based on Soto *et al.* (1993), were carried out in 120 mL glass vials, filled with 50 mL of anaerobic biomass (5 $g_{VSS} \cdot L^{-1}$), macro and micro nutrients and bicarbonate ($1g_{NaHCO3} \cdot g_{COD}^{-1}$), and bubbled with N_2 to remove the air in the headspace. The specific substrates and their initial concentrations were: 10 mM acetate, 6.2 mM iso-butyrate and iso-valerate (1:1), 0.3 mM oleate, and 0.7–4.6 mM hydrogen (Table 4.4). The vials were placed in an orbital shaker inside a cabin at a temperature of 35ºC. Every experiment was conducted in triplicate. As in the anaerobic biodegradability test, a control vial without substrate was included to assess the residual methane production of the inoculum, and thereby to calculate the net methane production. The initial and maximum slopes of the accumulated net methane curve were calculated to determine the specific substrate activities.

4.4. RESULTS AND DISCUSSION

4.4.1 Substrate characterization

The main characteristics of the GW and the SS are shown in Table 4.1. The GW samples had different compositions, but all of them had a higher organic matter content (COD and VS) and C/N ratio than the SS. The total COD and VS content of the GW varied between 177–321 $g_{COD} \cdot kg^{-1}$ and 63–143 $g_{VS} \cdot kg^{-1}$ respectively, while the SS samples presented average values of 44±8 $g_{COD} \cdot kg^{-1}$ and 23±4 $g_{VS} \cdot kg^{-1}$ respectively. There was a wide range of fat content in the GW (15–100 $g_{fat} \cdot kg^{-1}$) and it was higher than in the SS. These differences can be attributed to raw wastewater characteristics and the difference in efficiency between the flotation units of each WWTP as well as the presence of little pieces of plastic bags that could be accounted as VS, but are not biodegradable. GW_2 presented the highest fat concentration, at 70% of the VS.

Table 4. 1. *Characterization of trapped grease waste (GW) and sewage sludge (SS)*

Parameters	Units	GW_1	GW_2	GW_3	GW_4	SS
TS	$g{\cdot}kg^{-1}$	146 ± 1	160 ± 4	126 ± 1	75 ± 3	32 ± 5
VS	$g{\cdot}kg^{-1}$	123 ± 1	143 ± 3	101 ± 1	63 ± 2	23 ± 4
VS	*%TS*	*84*	*89*	*80*	*84*	*70*
COD	$g{\cdot}kg^{-1}$	298 ± 20	321 ± 30	258 ± 37	177 ± 5	44 ± 8
Fat	$g{\cdot}kg^{-1}$	47 ± 1	100 ± 4	38 ± 2	15 ± 2	0.20 ± 0.05
Fat	*% VS*	*38*	*70*	*38*	*24*	*1*
SO_4^{2-} -S	$mg{\cdot}kg^{-1}$	61 ± 4	37± 1	42 ± 3	127 ± 1	19 ± 0
TKN	$mg{\cdot}kg^{-1}$	4,287 ± 47	3,556 ± 51	3,166 ± 53	3,428 ± 53	2,000 ± 294
NH_4^+ -N	$mg{\cdot}kg^{-1}$	659 ± 12	348 ± 4	377 ± 8	353 ± 24	841 ± 109
NH_4^+ -N	*%TKN*	*15*	*10*	*12*	*12*	*42*
C/N	$g{\cdot}g^{-1}$	20	39	23	10	10

The sulfate (SO_4^2 -S) and TKN concentration in the GW was also high compared to the SS. Sulfate is an important parameter, since it can promote the growth of sulfate reducing bacteria (SRB) that can compete with methanogenic archaea. However, there is only an active competence when the COD/SO_4^{2-} ratio is below 1.7–2.7 (Choi and Rim, 1991). The GW samples had a COD/SO_4^{2-} ratio higher than 1,000, and so competition between SRB and methanogenic archaea can be neglected. Nitrogen is also an important parameter to take into account, not only because NH_4^+-N is an important inhibitor with regard to acetoclastic methanogenic archaea (Angelidaki *et al.*, 1993), but also because the rejected wastewater from SS dewatering, which is usually returned to the water line, may have a higher nitrogen concentration and can therefore affect the performance of activated sludge reactor.

4.4.2 Anaerobic biodegradability test

The methane potential (MP) and the ratio of biodegradable COD to the VS ratio (COD_{AB}/VS) of the four samples of GW and the SS are shown in Table 4.2. The methane potential of the SS on a COD basis (MP_{COD}) reached the highest value, being 68% of the maximum theoretical value

(237±4 $NL_{CH4}·kg_{COD}^{-1}$). In the case of GW, MP_{COD} was in the range of 44-61% of the maximum value. However, methane potential on VS basis (MP_{VS}) of the GW was around 50% higher than MP_{VS} of the SS, showing that the percentage of biodegradable VS is higher in GW than in SS. Thus, COD_{AB}/VS ratio of GW is higher than COD_{AB}/VS ratio of SS in all the cases (2.8-2.0 and 1.4 respectively).

Table 4. 2. *Methane potential (MP) estimated during the biodegradability test and biodegradable COD to VS ratio (COD_{AB}/VS) of the substrate tested*

Waste	**MP_{VS}**	**MP_{COD}**	**MP**	**COD_{AB}/VS**
	$NL_{CH4}·kg_{VS}^{-1}$	$NL_{CH4}·kg_{COD}^{-1}$	$NL_{CH4}·kg^{-1}$	$g·g^{-1}$
GW_1	483 ± 37	215 ± 17	69 ± 5	2.2 ± 0.1
GW_2	473 ± 53	232 ± 17	58 ± 7	2.0 ± 0.2
GW_3	529 ± 11	207 ± 4	53 ± 1	2.5 ± 0.0
GW_4	432 ± 27	154 ± 10	27 ± 2	2.8 ± 0.1
SS	322 ± 6	237 ± 4	9 ± 0	1.4 ± 0.0

The evolution of the MP_{VS}, VFA and H_2 partial pressure (PH_2) of two GW samples (GW_2 and GW_4) and the SS are shown in Figure 4.3a. The MP_{VS} curve of GW_1 and GW_3 presented a similar trend, to that of GW_2 (data not shown), but with shorter lag phase in accordance with its lower fat content. As can be seen in Figure 4.3a, gas production in GW_2 started on the fifth day of the experiment, while in GW_4 and the SS, gas production was detected on the first day. The long lag phase in GW_2 could be attributable to an adaptation or inhibition period for the microorganisms due to the high fat concentration (699 $g_{FAT}·kg_{VS}^{-1}$). GW_4 had a lower fat content (238 $g_{FAT}·kg_{VS}^{-1}$) and behaved similarly to the SS. However, the final methane potential was higher for GW_2, in line with its higher fat content.

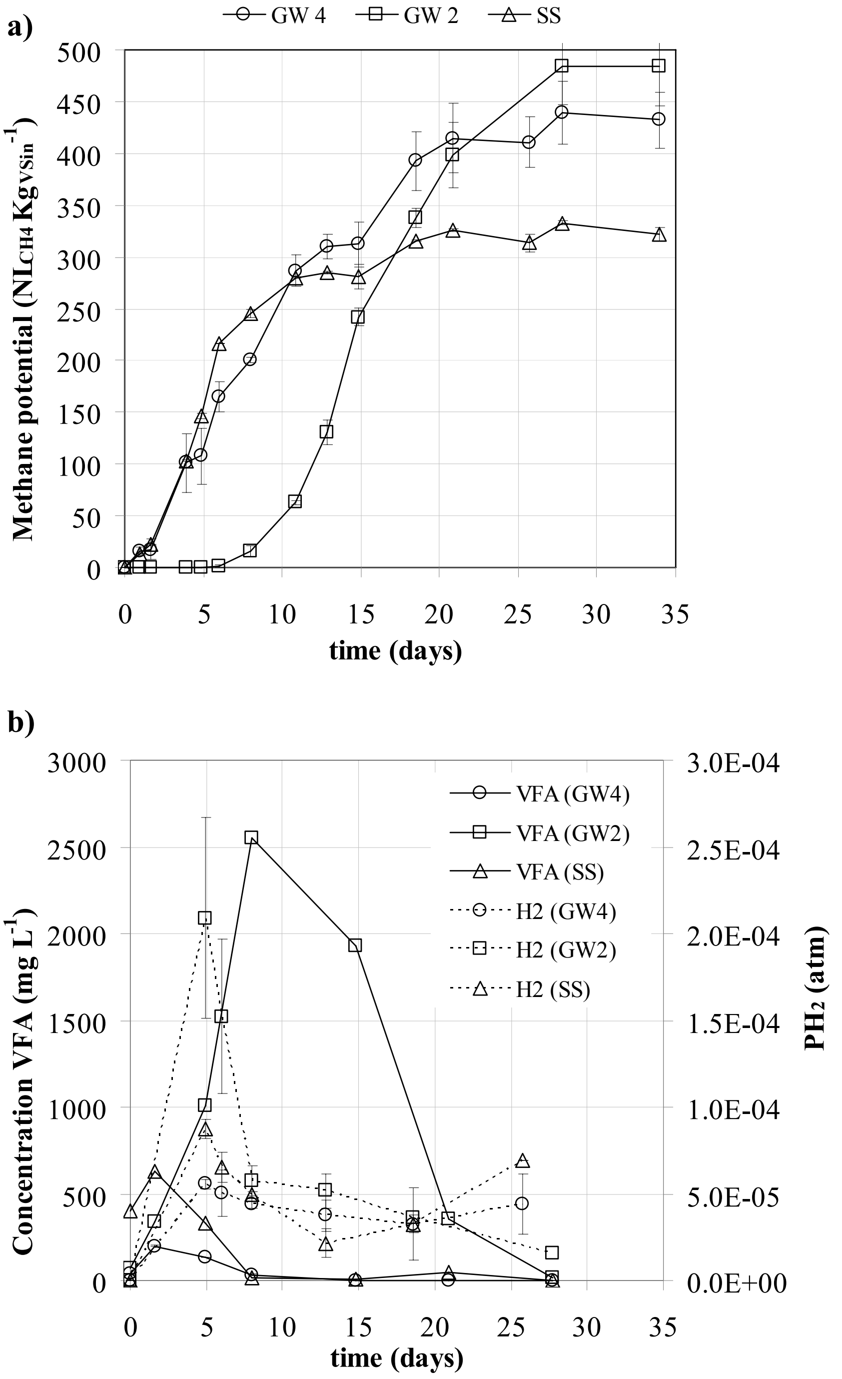

Figure 4. 3. *Evolution of accumulated methane (a), VFA concentration and* H_2 *partial pressure (*PH_2*), (b) of* GW_2*,* GW_4 *and SS during the biodegradability test.*

The evolution of VFAs and PH_2 (Figure 4.3b) were similar in the cases of GW_4 and the SS, reaching maximum values on day one. In the case of GW_2, the VFA concentration increased until day eight, reaching a value much higher than that observed for the SS and GW_4 vials. VFA accumulation was in accordance with H_2 accumulation and the long lag phase observed was probably due to the inhibitory effect of LCFAs (Palatsi *et al.*, 2009). The adaptation to GW_2 by the microbial consortia was reflected first in the consumption of H_2 and thereafter in a rapid decrease of VFA concentration.

In order to study the adaptation of microorganisms, GW_2 was selected as the co-substrate for the continuous experiment. The higher fat concentration, and consequently the longer lag phase but also the higher CH_4 production, allows the differences in CH_4 production and the adaptation of biomass to be clearly seen.

4.4.3 Continuous experiment

The continuous experiment, as previously mentioned, was divided into five different periods: periods I and II corresponded to anaerobic digestion with SS, while periods III, IV and V corresponded to co-digestion with GW_2. Figure 4.4 shows the evolution of methane production over the 302 days of the experiment and the average organic loading rate (OLR) of each period. Table 4.3 synthesizes the main parameters of each period.

The start-up (period I) was performed with the SS at a low OLR (1.11 $kg_{COD}·m^{-3}·d^{-1}$). After 25 days of digestion, the OLR was increased to 2.2 $kg_{COD}·m^{-3}·d^{-1}$ (period II). A steady state was reached in this period and the average volumetric methane production was 0.25±0.1 $Nm^3·m^{-3}d^{-1}$. SS co-digestion with GW_2 started on day 138. During the co-digestion periods, the OLR was increased in three stages by adding different amounts of GW2: 2.4 $kg_{COD}·m^{-3}·d^{-1}$ (period III), 3.0 $kg_{COD}·m^{-3}·d^{-1}$ (period IV) and 3.6 $kg_{COD}·m^{-3}·d^{-1}$ (period V). The percentage of GW_2 in each period corresponded to 8%, 27% and 39% of the inlet COD respectively.

The addition of GW (periods III and IV) resulted in an increase in biogas production and methane yield (on a VS basis), and higher organic matter removal efficiencies. Methane production increased by 36% and 128% in periods III and IV respectively, as compared with period II, and methane yield increased by 12% and 48% (Table 4.3). The average organic matter removal efficiency (on a COD and VS basis) in period II was 35%COD and 36%VS, while in period III and period IV it was

40%COD and 46%VS, and 55%COD and 52%VS respectively (Table 4.3). Biogas composition was around 70% v/v methane in all periods.

However, a further OLR increase (period V) did not result in greater methane production and was in accordance with the lower COD removal efficiency compared with period IV, and the higher residual LCFAs in the effluent during this period (1,349 ± 211 mg·L^{-1}). This can be explained by the inhibition of LCFAs, or by the fact that the biomass could not degrade the extra organic loading.

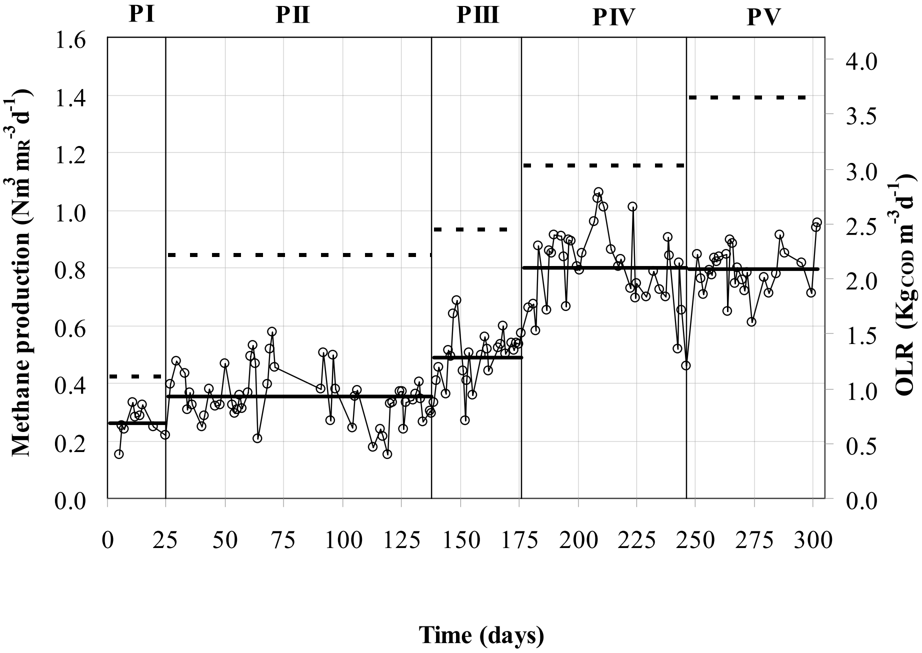

Figure 4. 4. *Methane production and organic loading rate (OLR) during continuous operation (Nomenclature: circles represent the weekly methane production; continuous line represents the average methane production of each period; dotted line represents the average OLR).*

Table 4. 3. *Operation and process parameters obtained during continuous experiment*

Parameters	**Units**	**Period I (25 days)**	**Period II (113 days)**	**Period III (41 days)**	**Period IV (159 days)**	**Period V (59 days)**
$GW_{influent}$	$\%VS_{influent}$	0	0	4±2	23±4	37±3
$GW_{influent}$	$\%COD_{influent}$	0	0	8±1	27±4	39±2
HRT^1	days	20 ± 2	20 ± 3	20 ± 2	20 ± 1	20 ± 2
$OLR_{COD}{}^1$	$kg_{COD}\cdot m^{-3}\ day^{-1}$	1.1 ± 0.1	2.2 ± 0.6	2.4 ± 0.3	3.0 ± 0.2	3.6 ± 0.5
$OLR_{VS}{}^1$	$kg_{VS}\cdot m^{-3}\ day^{-1}$	nm	1.5 ± 0.6	1.2 ± 0.1	1.6 ± 0.4	1.7 ± 0.2
$COD_{Removed}{}^2$	%	46 ± 9	35 ± 13	40 ± 8	55 ± 8	44 ± 5
$VS_{Removed}{}^2$	%	-	36 ± 7	46 ± 3	52 ± 11	56 ± 1
$Biogas^1$	$Nm^3\cdot m^{-3}d^{-1}$	0.26 ± 0.06	0.35 ± 0.10	0.49 ± 0.10	0.80 ± 0.14	0.80 ± 0.08
$CH_4{}^2$	$Nm^3\cdot m^{-3}d^{-1}$	0.18 ± 0.04	0.25 ± 0.07	0.34 ± 0.07	0.57 ± 0.10	0.54 ± 0.10
$CH_4{}^2$	$Nm^3\cdot T_{VSinlet}{}^{-1}$	-	249 ± 80	278 ± 58	369 ± 85	331 ± 32
$\%\ CH_4{}^3$	% v/v biogas	71 ± 1	72 ± 3	70 ± 1	70 ± 2	68 ± 1
VFA^2	$mg\cdot L^{-1}$	< 100	< 100	< 100	< 100	< 100
LCFA	$mg\cdot L^{-1}$	nm	514 ± 98	323 ± 82	516 ± 62	1,349 ± 211
TA^3	$g\ CaCO_3\cdot L^{-1}$	3.1 ± 0.1	5.7 ± 1.1	5.9 ± 0.7	5.9 ± 0.4	5.4 ± 0.5
PA^3	$g\ CaCO_3\cdot L^{-1}$	2.0 ± 0.1	3.6 ± 0.9	4.3 ± 0.1	4.1 ± 0.1	3.7 ± 0.3
pH^3	-	8.1 ± 0.2	7.8 ± 0.4	7.5 ± 0.2	7.4 ± 0.2	7.4 ± 0.3
T^1	ºC	35 ± 0	34 ± 1	35 ± 0	36 ± 0	36 ± 0

Note: Average and S.D of the daily (^{1}), weekly (^{2}) or twice week (^{3}) samples of each period

The LCFA concentration that produces inhibition in anaerobic biomass depends on the LCFA type. Shin *et al.* (2003) reported that the half maximal inhibitory concentration (IC_{50}) in the mesophilic range was 1,500 mg·L^{-1} for oleate (C18:0) and 1,100 mg·L^{-1} for palmitate (C16:0). The acetogenic and methanogenic populations have been described as the populations most sensitive to LCFA inhibition. With regard to methanogenic archaea, acetoclastic populations show a higher inhibition degree than hydrogenotrophic (Lalman and Bagley, 2001; Pereira *et al.*, 2004; Templer *et al.*, 2006; Palatsi *et al.*, 2010). In this study, the total LCFA in period V was slightly over 1,300 mg·L^{-1}, mainly palmitate (80%) and oleate (20%). These figures are close to the inhibitory concentration reported in the literature, but the low and stable VFA content (below 100 mg·L^{-1}), as well as a stable pH and alkalinity values (Table 4.3), showed that was performed without inhibition nor organic overloading. On the other hand, the palmitate removal efficiency decreased from 63% in period IV to 28% in period V. The lower solubility of palmitate in comparison with oleate (Novak and Kraus, 1973) may explain this lower removal efficiency and it is in line with the fact there was no increase in biogas resulting from the increase in organic loading.

Luostarinen *et al.* (2009) reported similar results during SS co-digestion with grease trap sludge from a meat processing plant. When grease trap sludge was increased from 46% to 58% of the feed VS, the methane yield did not increase, but no signs of inhibition were observed. Similarly, Luste and Luostarinen (2010) reported a stable anaerobic co-digestion of SS with a mixture of animal by-products when the HRT was decreased from 25 days to 14 days, but the highest methane yield was reported with an HRT of 20 days. This reinforces the hypothesis that under these conditions, the degradation kinetics of LCFAs is the rate-limiting stage, and inhibition phenomena are not relevant.

Methane yield depends on substrate origin and composition, as well as operational conditions (mainly temperature and HRT). Davidsson *et al.* (2007) reported a methane yield increase similar to the one in this study when a small percentage of kitchen grease waste was added, but when the co-substrate was 28% of VS-influent, the increase of the methane yield was only 28%, a figure much lower than that reported in this study. This shows that, besides composition and operational conditions, biomass adaptation to fats (and LCFAs) has an important role.

4.4.4 Biomass adaptation assessment

In order to assess the adaptation of the bacterial community to GW, two different inocula were tested: the initial inoculum (In1) sampled in the WWTP, and the adapted inoculum (In2), sampled during period V of the continuous experiment. GW biodegradability tests as well as specific activity tests were performed with both inocula.

GW biodegradability tests were carried out with a new sample from the same WWTP (GW_2'). This new sample presented lower water content and consequently higher COD, TS, VS and fat concentration (1,059 $g_{COD}\cdot kg^{-1}$, 180 $g_{TS}\cdot kg^{-1}$, 160$g_{VS}\cdot kg^{-1}$ and 398 $g_{FAT}\cdot kg^{-1}$) than the previous sample (GW_2). Nevertheless, the fat/VS and COD_{AB}/VS ratios (0.8 $g\cdot g^{-1}$ and 2.2 $g\cdot g^{-1}$ respectively) were similar, to those of the GW_2 sample.

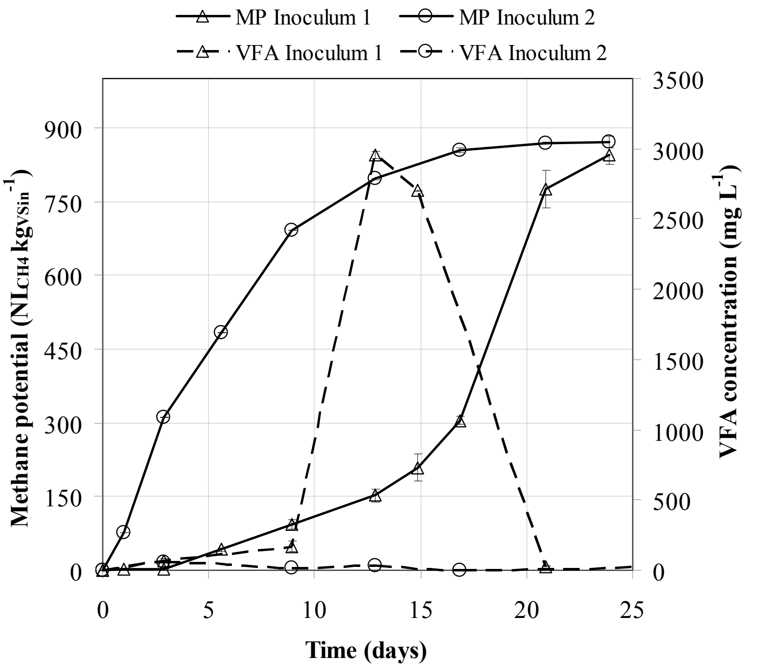

Figure 4. 5. ***Evolution of accumulated methane and VFA concentration during the biodegradability test using the initial (In1) and the adapted (In2)*** *inocula.*

The final MP_{VS} of GW_2' was similar with the two different inocula; 842 $Nm^3_{CH4}·kg_{VS}^{-1}$ when using the initial inoculum (In1), and 872 $Nm^3_{CH4}·kg_{VS}^{-1}$ with the adapted inoculum (In2) (Figure 4.5). However, the methane accumulation pattern was very different: 1) a significant reduction in the initial lag phase with In2 (close to zero), and 2) a sigmoid type curve for accumulated methane in the case of In1, while In2 followed a logistic type curve. This could be related to the adaptation of inoculum, with an increase in the concentration of microorganisms involved in the lipid degradation (Vavilin *et al.*, 2008).

In addition, specific substrate activity tests were performed to analyse possible changes in the biochemical activity of the different anaerobic populations. Both inocula were submitted to acetate, hydrogen, butyrate, valerate and oleate activities. The initial slope and the maximum slope, as well as the lag phase, were used to compare the different inocula (Table 4.4).

The lag phase was 0.4 days in the acetate activity test of In1, whereas the lag phase of In2 was zero (Figure 4.6a). The initial and maximum slopes of In2 were equal, while the maximum slope of In1 was double the initial slope. However, the maximum In2 slope was 6.3 times higher than that of In1. The high OLR during periods IV and V (Table 4.3) could explain the higher or more active acetoclastic methanogenic population of In2 compared to In1. On the other hand, the methanogenic hydrogenotrophic activity (H_2 activity) of In1 was higher than that of In2. The H_2 activity of In1 had a maximum slope almost twice as high as the maximum slope of acetate activity, whereas there was no In2 gas production with H_2 initial concentrations of 3.3 and 4.6 mM (Table 4.4). This can be explained by the low concentration or/and the inhibition of the hydrogenotrophic methanogenic population in In2. However, when the initial H_2 concentration was decreased to 1.3 and 0.7 mM, there was H_2 activity in In2, but in all cases lower values than in In1 were reported.

There was a lag phase of one day in methane production in the acetogenic activity test (butyrate and valerate substrates) and similar initial and maximum slopes for both inocula, but differences were found in the butyrate and valerate degradation rates. While the initial In1 degradation rate for valerate was higher than butyrate, there was a lag phase of 10 days for valerate degradation in the In2 test, and the butyrate degradation rate was higher than in the In1 test (Figure 4.6 b).

The increase in butyrate activity obtained in this study is in accordance with the increase in butyrate activity reported by Alves *et al.* (2001) after the long term digestion of lipids. The decrease in valerate activity can be attributed to the fact that valerate, together with propionate, are minor intermediates of oleate degradation (Weng and Jerris, 1976; Batstone *et al.*, 2002), and therefore biomass does not adapt to their presence.

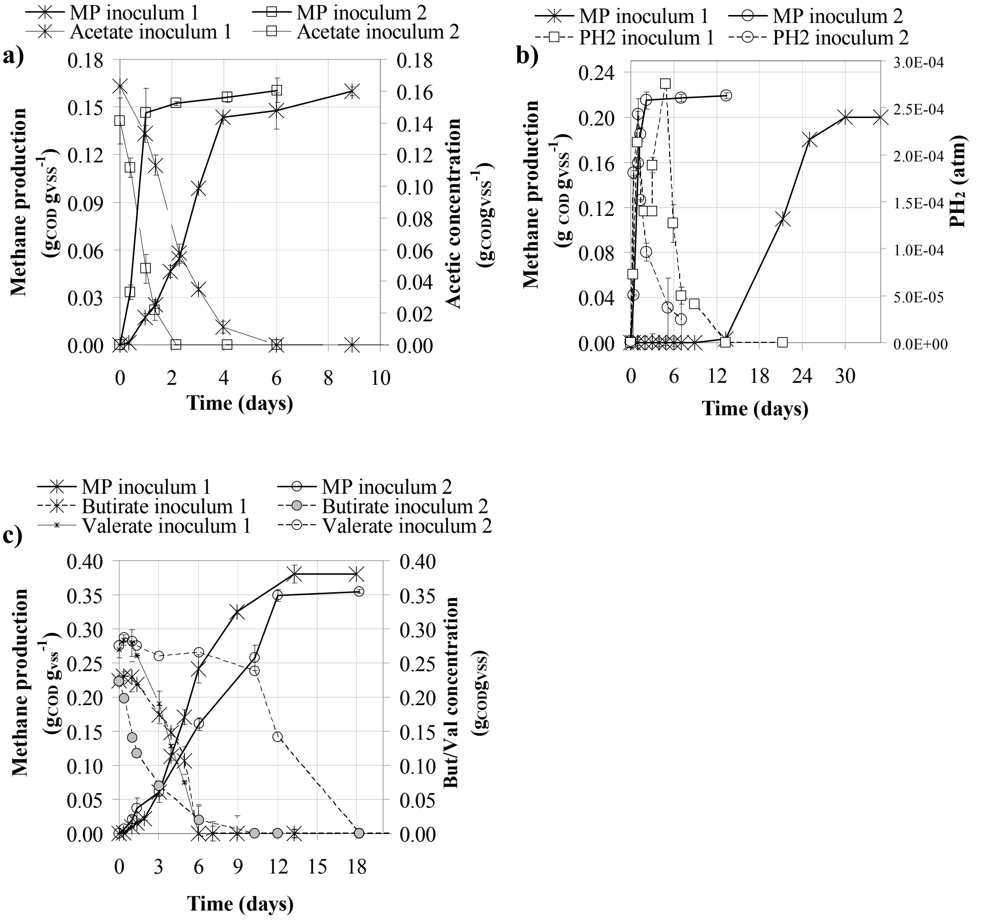

Figure 4. 6*. Evolution of accumulated methane, VFA concentration and* P_{H2} *during specific activity tests over acetate (a), oleate (b), and butirate & valerate (c) with the initial (In1) and the adapted (In2) inocula*

Table 4. 4. *Lag phase duration, initial and maximum slope of specific activity essays for initial (In1) and adapted (In2) inocula.*

		Inoculum 1			***Inoculum 2***		
		Lag Phase	Initial Slope	Maximum slope	Lag Phase	Initial Slope	Maximum slope
Substrate		days	$mg_{COD\text{-}CH4}\cdot g_{VSS}^{-1}d^{-1}$	$mg_{COD\text{-}CH4}\cdot g_{VSS}^{-1}d^{-1}$	days	$mg_{COD\text{-}CH4}\cdot g_{VSS}^{-1}d^{-1}$	$mg_{COD\text{-}CH4}\cdot g_{VSS}^{-1}d^{-1}$
Acetate	(10mM)	0.4	16	21	0	132	132
Butyrate & Valerate	(6.2 mM)	1	13	60	0	12	63
Oleate	(0.3 mM)	1	13	14	0	128	157
Hydrogen	(4.6 mM)	1	10	30	-	inhibited	-
Hydrogen	(3.3 mM)	1	10	100	-	inhibited	-
Hydrogen	(1.3 mM)	-	not tested	-	1	20	22
Hydrogen	(0.7 mM)	-	not tested	-	1	20	22

The activity test with oleate as substrate, named oleate activity test, was performed with a low concentration (0.3mM) to avoid inhibition. However, In1 presented a lag phase of 15 days (Figure 4.c), while no lag phase was reported in In2. Furthermore, the In2 maximum slope was 11 times higher than the In1 maximum slope (Table 4.4), which is in line with the biodegradability essays. In2 was able to adapt to the GW, and consequently the LCFA degradation rate increased. This suggested an increase in β-oxidation synthrophic acetogens that degraded LCFA, and probably limited LCFA inhibition.

Changes in the activities of anaerobic methanogenic and acetogenic populations after they have been in contact with fats have been studied, but results are somewhat contradictory. Palatsi *et al.* (2009) and Nielsen and Ahring (2006) studied changes in methanogenic activity after an inhibitory oleate pulse in the thermophilic range. Both authors reported an increase in acetoclastic and hydrogenotrophic activity, but Palatsi *et al.* (2009) found a higher increase in hydrogenotrophic activity, and Nielsen and Ahring (2006) reported a higher increase in acetoclastic activity. Calveiro *et al.* (2001) observed an increase in acetoclastic methanogenic activity after an oleate organic shock, but with an eight-fold decrease in hydrogenotrophic activity. However, after a hydraulic shock, hydrogenotrophic activity was doubled and the acetoclastic activity was half the initial value. In this case biomass had been continuously fed in with a non-inhibitory concentration of oleate in the mesophilic range. Alves *et al.* (2001) studied the effect of increasing fat concentration on the influent of a mesophilic fixed-bed reactor. The results showed that methanogenic activity did not change significantly.

As can be seen, different behaviours of methanogenic populations have been reported after exposure to fats, and no general conclusion can be drawn. The differences might be attributable to the adaptation procedure of the biomass. In some cases pulses of oleate were used, while in others a continuous oleate feeding, below inhibitory concentrations, was applied. In this case, changes in the activity of the anaerobic populations, could explain the adaptation process: 1) a slow increase in the grease waste fed would allow a gradual increase in the acetogenic population able to degrade LCFAs; 2) as butyrate degraders are the main population involved in the β-oxidation of fat (Rinzema *et al*., 1994), an increase in butyrate activity would be expected; and 3) as fat degradation results in around 70% of acetate (Batstone *et al*., 2002), acetoclastic methanogenic archaea should be the population that adapts most.

4.5 CONCLUSIONS

GW is a suitable co-substrate for the anaerobic digestion of sewage sludge. When GW addition was 27% of COD fed (23% of VS fed) (HRT=20 days, OLR= 3.0 $kg_{COD}·m^{-3}·d^{-1}$), an increase of 128% in the methane production was reported. The specific activity tests of the inocula confirmed that the anaerobic biomass had adapted to GW. Acetate and β-oxidation synthrophic acetogenic activity in the adapted inoculum were 6.3 and 11.2 times higher than for the initial inoculum. These results suggested that acclimatization of biomass brought about by slowly increasing the grease waste dose, could be a good strategy for increasing fat degradation and reducing the inhibitory effect of LCFAs.

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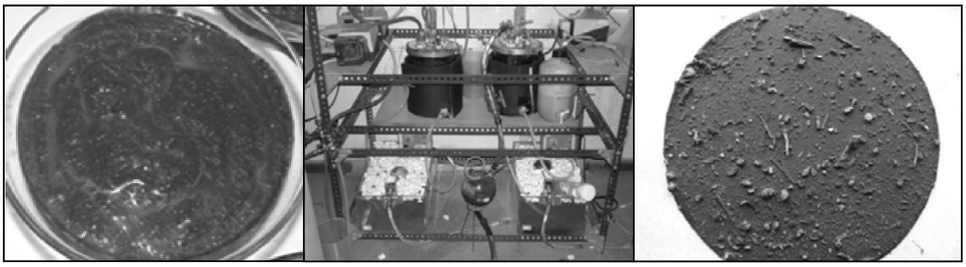

CHAPTER 5

THERMOPHILIC ANAEROBIC CO-DIGESTION OF SEWAGE SLUDGE WITH GREASE WASTE: EFFECT OF LONG CHAIN FATTY ACIDS IN THE METHANE YIELD AND ITS DEWATERING PROPERTIES

Thermophilic co-digestion of sewage sludge with three different doses of trapped grease waste (GW) from the pre-treatment of a WWTP has been assessed in a CSTR bench-scale reactor. After adding 12% and 27% of grease waste (on COD basis), the organic loading rate increased from 2.2 to 2.4 and 2.8 $kg_{COD}\cdot m^{-3}\cdot d^{-1}$ respectively, and the methane yield increased 1.7 and 2.2 times. Further GW increase (37% on COD basis) resulted in an unstable methane yield and in long chain fatty acids (LCFA) accumulation. Although this inestability, the presence of the volatile fatty acids in the effluent was negligible, showing good adaptation to fats of the thermophilic biomass. Nevertheless, the presence of LCFA in the effluent worsens its dewatering properties. Specific methanogenic activity tests showed that the addition of grease waste ameliorates the acetoclastic activity, and suggests that the tolerance to LCFA can be further enhanced by slowly increasing the addition of lipid-rich materials.

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5.1. INTRODUCTION

Wastewater Treatment Plants (WWTPs) play an important role in the protection of the environment, but they also work with energy intensive processes that use up high amounts of non-renewable energy. In the current context of scarcity of resources any likely savings on energy have become an important issue for the sustainable management of WWTPs.

Disposal of sewage sludge (SS) generated as a by-product in WWTPs, is a problem of growing significance, representing up to 50% of the entire operating costs of all WWTPs (Appels *et al*., 2008). Anaerobic digestion, for its ability to transform organic matter into biogas, is the usual treatment method employed to stabilize organic matter and to reduce solids, destroying at the same time most of the pathogens. However, in most cases biogas production from SS can be further optimized.

Thermophilic conditions can optimize the process accelerating the biochemical reactions and increasing the efficiency of organic matter degradation to methane. The growth rates of thermophilic bacteria are 2-3 times higher compared to those of its mesophilic homologues. Hence, this can lead to an increase of the biogas yield and of the organic loading rate (Zábranská *et al*. 2002). However, said increase of the biogas yield does not always occur, and thermophilic AD could also bring non-desirable consequences such as lower stability of the whole process, higher sensitiviy to inhibitors, higher energy requeriments, higher VFA concentrations in the effluent, and poor dewaterability (Zábranská *et al*. 2002, Han and Dague, 2005). Moreover, if a thermophilic inoculum is not available, biomass must be acclimated to temperature and so it will take more time to reach a stable biogas production (De la Rubia *et al*., 2005).

In order to optimize biogas production, co-digestion with other organic wastes is another attractive strategy. Adding highly biodegradable substrates increases the organic load and enhances the biochemical conditions that favour the growth of the different groups of bacteria involved in the process (Molinuevo-Salces *et al*., 2012). Grease waste (GW) from the dissolved air flotation unit of the pre-treatment step in WWTPs, as previously described by Silvestre *et al*. (2011)[1], is a suitable co-substrate for mesophilic SS anaerobic digestion The use of this intermediate waste generated inside the WWTP, leads to an optimization of the entire plant, since the costs of managing the GW to landfill decreases, and its high fat content increases biogas yield. Nevertheless, GW, as many other lipid rich wastes, has a high methane potential but its

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[1] Chapter 4

intermediate products (long chain fatty acids (LCFA)) could lead to an inhibition phenomena (Palatsi *et al*., 2009). LCFA inhibition depends on the type of LCFA; on microbial population and on temperature regime. Oleic acid, followed by palmitic and stearic acid, has been described as the LCFA with the highest inhibitor effect on the thermophilic biomass (Pereira *et al*., 2005, Hwu and Lettinga, 1997).

Many authors have studied anaerobic co-digestion of sewage sludge with different kinds of grease waste in mesophilic conditions, obtaining good performances (Luostarinen *et al*., 2009; Silvestre *et al*., 2011[1]; Pastor *et al*., 2013), but few studies have been conducted in a thermophilic regime. Kabouris *et al*. (2009) found that the thermophilic anaerobic co-digestion of grease coming from restaurants and kitchen waste produced less increase in methane yield, compared with the mesophilic digestion. Likewise, Dinsdale *et al*. (1996) studied the mesophilic and thermophilic anaerobic digestion of coffee wastes rich in fats, reporting a poor performance in the thermophilic regime, with a methane yield 5 times below the mesophilic yield.

Aside from biogas production, the dewatering properties of the SS are essential for the energetic and economic optimization of the WWTPs. It still remains unclear if AD —particularly thermophilic AD—, does or does not favour the SS dewatering properties as contradictory results have been reported in many studies (Borowski and Szapa, 2007; Novak and Kraus, 1973). Moreover, adding a new substrate could affect the dewatering properties of the digested sludge (Habiba *et al*., 2009). Hence, further studies are necessary in order to understand the behaviour of a thermophilic biomass in presence of grease waste, the characteristics of the digestate produced, and its implications regarding the global energetic balance of WWTPs.

This study aims, as a whole, to assess the anaerobic co-digestion of SS and trapped grease waste (GW) from the pre-treatment of a WWTP in thermophilic regime. The biodegradability of the substrates, the methane yield, the stability of the process —in relation to the presence or absence of intermediate-LCFAs—, the changes in the methanogenic activities of the biomass, and the dewatering properties of the effluent, were all used to assess the process performance. Moreover, results have been compared

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[1] Chapter 4

with the results obtained for mesophilic SS co-digestion with the same grease waste, as described and analyzed in Silvestre *et al.* (2011)[1].

5.2. MATERIAL AND METHODS

5.2.1 Characteristics of the substrates and inoculums

The SS used was a mixture of 70% primary sludge and 30% activated sludge (v/v) coming from a WWTP located in the La Llagosta (Barcelona). The SS was sampled every second week and kept refrigerated at 4ºC. The grease waste (GW) was sampled only once, from the same WWTP, and kept frozen before use.

The effluent of the full scale anaerobic mesophilic reactor from the WWTP was the inoculum used in the continuous thermophilic reactor. The biomass acclimatisation to the thermophilic conditions lasted 302 days.

Three samples from the anaerobic effluent, namely inoculum In1, In2 and In3, were taken at the end of three periods (PI, III, and IV) in order to analyse the changes in the biomass activity over time. In order to remove the residual organic matter, these inocula were stored for 3 days at a temperature of 55ºC before carrying out the specific activity tests.

5.2.2. Analytical methods

Total solids (ST), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), total chemical oxygen demand (COD), total Kjeldhal nitrogen (TKN), ammonia nitrogen (NH_4^+-N), total and partial alkalinity (TA, PA), as well as sulphate (SO_4^{2-}-S), phosphate (PO_4^{3-}-P) and fat concentration, were all determined according to Standard Methods (APHA, AWWA, WEF, 1995).

The biogas composition was determined using a gas chromatograph (VARIAN CO-300). CH_4 and CO_2 were determined with a packed column (Varian Haysep-Q 80-100 MESH) and a thermal conductivity detector (TCD), and H_2 with a capillary column (Varian Molecular Sieve 5A 80-100 MESH) and a flame ionization detector (FID), as described elsewhere Silvestre *et al.* 2011[1]. Volatile fatty acids (acetate, propionate, i-butyrate, n-butyrate, i-valerate and n-valerate acids) were determined by gas chromatography (VARIAN CO-300) with a flame ionisation detector (FID) and a capillary column (TRB-FFAP). LCFAs were determined, in

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[1] Chapter 4

accordance with Palatsi *et al.* (2009), using a gas chromatograph (GC 3800 Varian, USA) equipped with a capillary column and a FID detector.

5.2.3 Continuous experimental set-up

The continuous co-digestion experiment was performed in a 5.0 L anaerobic continuous stirred tank reactor (CSTR) (Figure 5.1). The reactor was built of glass with a water jacket connected to a thermostatic bath. The temperature was controlled at 55 ºC with a temperature probe connected to a data acquisition system (DAS, by STEP S.L.). The reactor was continuously stirred with a mechanical stirrer at 25 rpm. The reactor was fed twice a day (each 12 h) using a temporized peristaltic pump, allowing for 20 days of hydraulic retention time (HRT). Biogas production was measured with a volumetric gas counter (Ritter Apparatebau GMBH & CO. KG). The characteristics of the influent and effluent were measured once a week, and the biogas composition twice a week.

The experiment was held for 566 days divided into four different periods. Period I corresponds to the biomass acclimatisation, which lasted 302 days and where SS was the sole substrate, while periods II, III, and IV, correspond to the co-digestion of SS with different amounts of GW.

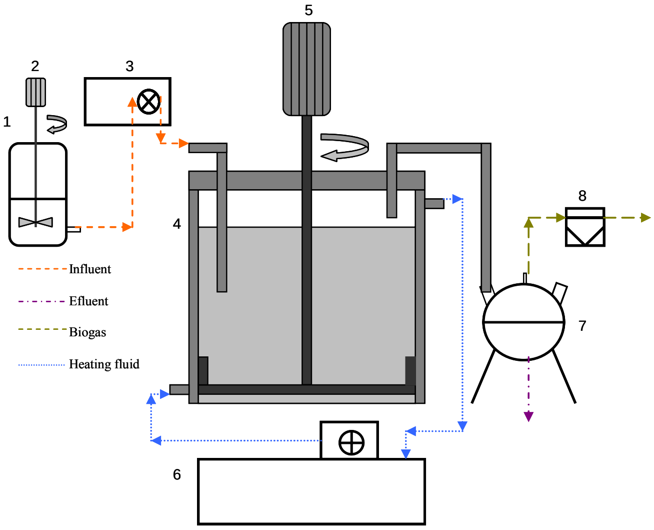

Figure 5. 1. *Schematic diagram of the experimental set-up: 1-feed bottle, 2-feed mixer, 3-feed pump, 4-reactor, 5- reactor stirrer, 6-water bath, 7- effluent bottle, 8-gas meter.*

5.2.4 Anaerobic biodegradability and specific activity tests

The methane potential (MP) of SS and GW was determined by means of an anaerobic biodegradability test (ABT). As explained elsewhere, 1.2 L capacity glass vials were filled with a mixture of 0.5 L of inoculum (5 $g_{VSS} \cdot L^{-1}$), substrate (5 $g_{COD} \cdot L^{-1}$) and deionised water. The thermophilic inoculum used was a sample taken from a thermophilic lab-scale reactor.

Changes in the activity of the methanogenic biomass through time were assessed by means of specific activity tests (Soto *et al.*, 1993). These tests were carried out in duplicate, in 120 mL glass vials filled with a mixture of 50 mL of the anaerobic biomass (5 $g_{VSS} \cdot L^{-1}$), macro and micro nutrients, bicarbonate (1 $g_{NaHCO3} \cdot g_{COD}^{-1}$) and specific substrates (acetic acid (10 mM) and hydrogen (4.6 mM)). Vials were bubbled with N_2, in order to ensure the complete removal of O_2, and airtight sealed with rubber stoppers and metallic clamps.

The time course of the methane production was monitored by gas chromatography, periodically sampling the head space of the vial. Methane production profiles were fitted to the modified Gompertz equation (eq. 1) (Lay *et al.*, 1998, Gadhamshetty *et al.*, 2010).

$$P = P_m \times \exp\left(-\exp\left[\frac{R_m \times e}{P_m} (\lambda - t) + 1 \right] \right) \qquad \text{(Eq 5.1)}$$

where P is the accumulated methane production ($mL_{CH4}\ g_{COD}^{-1}$), expressed as a time function (t, days), Pm is the methane production potential ($mL_{CH4} \cdot g_{COD}^{-1}$), Rm is the maximum methane production rate ($mL_{CH4} \cdot g_{COD}^{-1} \cdot day^{-1}$) and λ is the lag phase period of biogas production (day). The least-square sum of errors criterion was used in the fitting process and the Monte Carlo method was used to establish the 95% confidence interval on the estimated parameter values (Hauser, 2009). Both the parameter estimation and Monte Carlo analysis were run in MatLab (The Mathworks, USA).

5.2.5 Dewatering characteristics

The impact of the co-substrate on the dewatering characteristics of the effluents obtained in the continuous experiment was assessed by filterability and centrifugability tests (APHA, AWWA, WEF, 1995). The filterability test consisted in filtrating 20 mL of effluent applying a vacuum, and recording the volume filtered over time. Likewise, the centrifugability test consisted in centrifuging 40 mL of effluent for 5 minutes, at different spin speeds, and measuring the TS of the solid phase obtained.

5.3 RESULTS

5.3.1 Substrate characterization

The characteristics of the GW and SS are shown in Table 5.1. The GW showed high concentration of fat and organic matter —as COD and VS concentrations—, compared to the SS: the COD and VS were respectively 7 and 6 times higher in the GW than in the SS, while the GW fat content was nearly 97 times higher than in the SS. As for the presence of LCFAs in the GW, oleic acid represented almost 50% of the total amount of fat, followed by linoleic and palmitic acid which both respectivaly represented 17% and 18% of the overall amount of fat. As to the SS, oleic acid was also the most abundant LCFA, followed by mystirc and linoleic acids. The methane potential (MP) of both samples was also determined. As expected, the MP of the GW was much higher than the MP of the SS: 512 against 354 $L·kg_{VS}^{-1}$ respectivaly. The higher lipid concentration in the GW can explain this big difference.

Table 5. 1. *Characterisation of the grease waste (GW) and sewage sludge (SS)*

Parameters	Units	GW	SS
TS	$g·kg^{-1}$	160 ± 4	32 ± 5
VS	$g·kg^{-1}$	143 ± 3	23 ± 5
VS	%TS	89	70
COD	$g·kg^{-1}$	321 ± 30	44 ± 8
C/N	$g·g^{-1}$	39	10
Fat	$g·kg^{-1}$	107 ± 4	1.1 ± 0.0
Fat	% VS	75	4.8
Lauric Acid (12:0)	$g·kg^{-1}$	2.5 ± 0.1	0.1 ± 0.0
Myristic Acid (14:0)	$g·kg^{-1}$	2.1 ± 0.1	2.1 ± 0.1
Palmitic Acid (16:0)	$g·kg^{-1}$	19.3 ± 1	1.0 ± 0.0
Stearic Acid (18:0)	$g·kg^{-1}$	8.1 ± 0.8	0.8 ± 0.0
Oleic Acid (18:1)	$g·kg^{-1}$	52.6 ± 5.6	5.6 ± 0.0
Linoleic Acid (18:2)	$g·kg^{-1}$	21.3 ± 2.1	2.1 ± 0.0
Methane potencial (MP)	$L·kg_{VS}^{-1}$	512 ± 50	354 ± 12

5.3.2 Continuous experiments

Results from the continuous experiments are shown in Figure 5.2. During the start-up period, the reactor was fed with SS as the sole substrate (PI), and during each different co-digestion period with GW (PII, PIII and PIV). Period PI was in turn divided into three sub-periods (PIa, PIb, PIc), and period PIII was divided into two sub-periods (PIIIa and PIIIb), according to the changes in operational conditions. The methane production throughout all the periods is shown in Figure 5.2a, while Figure 5.2b shows the evolution of VFA concentrations over time (acetic, propionic and *n*-butyric acids). Average operational and process parameters are summarized in Table 5.2.

5.3.2.1. Start-up period (period PI)

The CSTR reactor was inoculated with a mesophilic anaerobic inoculum —a thermophilic inoculum was not available in the area— and the temperature was raised up to 55 ºC in one step. During this period (PI) the digester was fed with SS at an organic loading rate of 2.25 $\mathrm{kg_{COD}·m^{-3}}$ $\mathrm{d^{-1}}$ with a hydraulic retention time (HRT) of 20 days. As shown in Figure 5.2b, VFA concentrations rapidly increased, particularly for propionic acid (1.4 $\mathrm{g·L^{-1}}$) but also for acetic (0.4 $\mathrm{g·L^{-1}}$) and butyric (0.3 $\mathrm{g·L^{-1}}$) acids. The methane production rose during the first 220 days of operation (PIa in Figure 5.2a), notwithstanding the accumulation of VFA (Figure 5.2b). In order to favour the consumption of the VFAs accumulated, the stirring speed was reduced from 25 rpm to 5 rpm (period PIb). During this period the methane production decreased and the concentration of propionic acid remained at similar levels (PIb in Figure 5.1b). After 25 days, the stirring speed was set again to its initial value (25 rpm) and a quick consumption of propionic acid was observed, reaching values below 0.4 g $\mathrm{L^{-1}}$. (PIc, in Figure 5.2b).

This phenomenon, was previously described by Stroot *et al.* (2001). They reported that the accumulated propionate, produced during the co-digestion of municipal solid wastes (MSW) and SS, is consumed when the stirring speed is reduced. Some authors have attributed propionate accumulation to high H_2 partial pressures (Harper and Pohland, 1986; Mosey *et al*, 1989) since H_2 is a product of its degradation, but other researchers have stated that said accumulation is independent from H_2 partial pressures (Ren *et al.*, 1997; Inanc *et al.*, 1999). In this study, H_2 concentrations on the biogas were monitored weekly, resulting in negligible values during all the reactor operation. Likewise, intense stirring appears to inhibit the syntrophic oxidation of VFA, probably due to the disruption of the spatial juxtaposition of syntrophic bacteria and their hydrogenotrophic methanogenic partners (McMahon *et al*., 2001). During the slow stirring period (PIb) propionic acid was not degraded, but when the stirring speed was again increased, the accumulated propionate disappeared in just a few days. The spatial juxtaposition between syntrophic and methanogenic bacteria seems to be enhanced with slow stirring conditions, allowing for a quick propionate consumption when the stirring speed was reset to its initial value.

5.3.2.2 Co-digestion periods

The co-digestion periods with GW commenced when the digester was stabilized at the end of PI, shown by a stable concentration of VFAs below 0.3 g L^{-1}. Three mixtures of SS/GW were tested, dividing the run into three periods (PII, PIII, and PIV), where a fixed OLR of 2.4 ± 0.3 $kg_{COD}\cdot m^{-3}\cdot d^{-1}$ for period II and of 2.8 ± 0.4 $kg_{COD}\cdot m^{-3}\cdot d^{-1}$ for periods PIII and PIV were sustained. In period PII, a 12% COD of the influent was due to GW supplied, while for periods PIII and PIV, the GW respectively represented 27% and 37% COD. This gradual increase of GW resulted in an increase in the total lipid concentration of the influent, from 1.1 (period PI) to 9.3 $g\cdot L^{-1}$(period IV) (Table 5.2).

The increase of the OLR in period II, from 2.2 ± 0.2 (PI) to 2.4 ± 0.3 $kg_{COD}\cdot m^{-3}\cdot d^{-1}$, together with the addition of GW (12% of the COD influent) caused a 77% increment of the methane production (Figure 5.2a), while the next GW addition in period III, with a resulting OLR of 2.8 ± 0.4 $kg_{COD}\cdot m^{-3}\cdot d^{-1}$, led to a decrease in the methane production with a concomitant increase of propionic and acetic acids, with a values above 0.4 $g\cdot L^{-1}$ (period IIIa, in Figure 5.2a).

This suggested that the system suffered from an organic overload and/or a partial inhibition due to the fat increase in the feed, requiring more than 90 days to recover, hence showing a slow adaptation of the biomass. At the end of period PIII, the daily methane production was close to 0.40 $m^3\cdot m^{-3}$ d^{-1} and both propionic and acetic acids decreased down to 0.20 $g\cdot L^{-1}$ (period IIIb, in Figure 5.2a and 5.2b). In period PIV, a third mixture of GW-SS was introduced, and in less than a week the methane production decreased from 0.40 $m^3\cdot m^{-3}d^{-1}$ down to 0.23 $m^3\cdot m^{-3}d^{-1}$, clearly showing that the biomass was inhibited by the addition of the extra GW and the corresponding increase in fats. The reactor quickly recovered its methane production but remained unstable throughout the entire period with profound daily variations. Nevertheless, VFA concentrations remained below 0.2 $g\cdot L^{-1}$ for all period PIV.

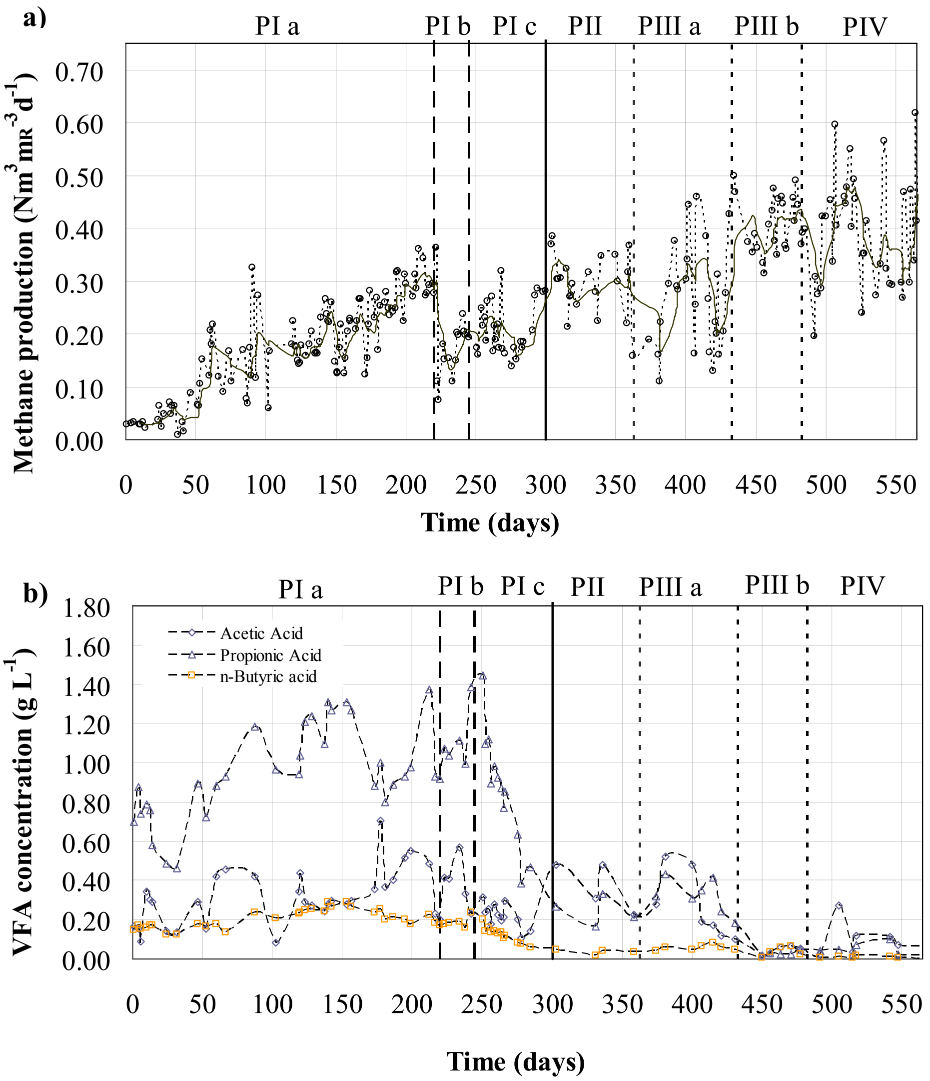

Figure 5. 2. *a) Methane production; and b) volatile fatty acids (VFA) evolution throughout the thermophilic reactor operation. Periods PI_a, PI_b and PI_c correspond to SS as the only substrate and PII, $PIII_a$, $PIII_b$ and PIV correspond to the co-digestion periods.*

Table 5. 2. *Operation and process parameters obtained during continuous experiment*

Parameters	**Units**	**Period I_c (53 days)**	**Period II (62 days)**	**Period III_b (48 days)**	**Period IV (82 days)**
$\mathbf{GW_{influent}}$	$\%VS_{nfluent}$	0	9 ± 2%	22 ± 3%	27 ± 4%
$\mathbf{GW_{influent}}$	$\%COD_{influent}$	0	12 ± 3%	27 ± 4%	37 ± 4%
$\mathbf{Fat_{influent}}$	$g\ L^{-1}$	1.1	3.0	6.6	9.3
$\mathbf{HRT^1}$	days	20 ± 2	20 ± 2	20 ± 2	21 ± 2
$\mathbf{OLR_{COD}{}^1}$	$kg_{COD}\ m^{-3}\ d^{-1}$	2.2 ± 0.2	2.4 ± 0.3	2.8 ± 0.3	2.8 ± 0.4
$\mathbf{OLR_{VS}{}^1}$	$kg_{VS}\ m^{-3}\ d^{-1}$	1.2 ± 0.1	1.2 ± 0.1	2.1 ± 0.3	1.9 ± 0.3
$\mathbf{COD_{Removed}{}^2}$	%	56 ± 3	40 ± 8	52 ± 15	44 ± 9
$\mathbf{VS_{Removed}{}^2}$	%	50 ± 11	46 ± 3	45 ± 9	44 ± 9
$\mathbf{Biogas^1}$	$Nm^3\ m^{-3}\ d^{-1}$	0.28 ± 0.10	0.46 ± 0.10	0.57 ± 0.10	0.55 ± 0.14
$\mathbf{CH_4{}^2}$	$Nm^3\ m^{-3}\ d^{-1}$	0.18 ± 0.09	0.32 ± 0.10	0.40 ± 0.07	0.38 ± 0.12
$\mathbf{CH_4{}^2}$	$Nm^3\ T^{-1}{}_{VSinlet}$	260 ± 45	300 ± 83	277 ± 69	243 ± 96
$\mathbf{\%\ CH_4{}^3}$	% v/v biogas	66 ± 4	70 ± 1	70 ± 1	69 ± 2
$\mathbf{VFA^2}$	$mg\ L^{-1}$	1200	< 100	< 100	< 100
$\mathbf{TA^3}$	$g_{CaCO3}\ L^{-1}$	5.1 ± 0.4	5.9 ± 0.7	4.0 ± 1.1	3.0 ± 0.7
$\mathbf{PA^3}$	$g_{CaCO3}\ L^{-1}$	2.7 ± 0.2	4.3 ± 0.1	2.7 ± 0.4	2.4 ± 0.3
$\mathbf{pH^3}$	-	7.4 ± 0.2	7.5 ± 0.2	7.5 ± 0.1	7.3 ± 0.2
$\mathbf{T^1}$	ºC	56 ± 1	55 ± 0	56 ± 2	55 ± 1

Note: Average and S.D of the daily (^{1}), weekly (^{2}) or twice weekly (^{3}) samples for each period

5.3.2.3 LCFA degradation

In order to further study the instability of the process due to fats, and its behaviour depending on the temperature regime (mesophilic and thermophilic), LCFAs of the influent and effluent of each period were analysed and compared with LCFA concentrations of the mesophilic experiment performed simultaneously in a reactor using the same GW-SS mixtures. The mesophilic digester performance was previously described in Silvestre *et al*. 2011[1].

Figure 5.3a shows influent and effluent LCFA concentrations for each period of the thermophilic reactor operation. As it can be seen, the overall amount of LCFAs in the influent increased from 1.0 $g·L^{-1}$, in period PI, to 3.0 $g·L^{-1}$, 6.6 $g·L^{-1}$and 9.3 $g·L^{-1}$, in periods PII, PIII and PIV respectively, and the most abundant LCFAs, in all cases, were oleic acid followed by palmitic acid. LCFA concentrations in the effluents were in all periods lower than in the influent, though different removal efficiencies were reported. Period $PIII_b$ showed a total LCFA removal efficiency of 89%. This is supported by the stable methane production, and a low VFA concentration (Figure 5.2). On the other hand, PII and PIV presented lower removal efficiencies of 56% and 68% respectively, being palmitic and stearic acids those showing the lowest removal efficiency values (results not shown). The accumulation of LCFAs agrees with the reported instability for some of the periods and the presence of VFAs in the effluent, suggesting that LCFA concentrations in the digester is a good indicator of the state of the system when SS is co-digested with a co-substrate rich in fats.

Figure 5.3b shows LCFA concentrations in the influent and effluent of the different co-digestion periods of the mesophilic reactor working with the same SS and GW, and operated simultaneously (results from its operation have been presented in Silvestre *et al*. 2011[1]). Comparing mesophilic and thermophilic periods with similar OLR and influent fat content, LCFA removal efficiencies were higher in the mesophilic reactor than in the thermophilic one. The mesophilic-PII LCFA removal efficiency was of 89% versus 56% for the thermophilic-PII, whereas during period PIII both efficiencies showed similar values (93% and 89% for mesophilic-PIII and thermophilic-PIII respectively), coinciding with the good and stable performance of the thermophilic period PIII (Figure 5.2, and Table 5.2). Nevertheless, the LCFA removal efficiency for period PIV was higher in the mesophilic regime (88%) compared to the thermophilic (68%), even if the influent fat content in the mesophilic-PIV

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[1] Chapter 4

was higher (11 $g{\cdot}L^{-1}$) than in the thermophilic-PIV (9 $g{\cdot}L^{-1}$). Likewise, Figure 5.4 compares the methane production ($m^3\ m^{-3}{}_R\ d^{-1}$) of the different periods of both temperature regimes, mesophilic and thermophilic. As it can be seen, the methane production was higher in the mesophilic reactor reached a maximum point, from which an additional increase in OLR triggers a decrease of the methane production. The thermophilic reactor shows a similar pattern, and in all cases its methane production values are lower than those of the mesophilic reactor, agreeing with the differences in LCFA concentrations in the reactor and the removal efficiencies observed.

The thermophilic anaerobic biomass has been described as being more sensitive to LCFAs than the mesophilic biomass. A LCFA concentration that produces inhibition depends, among other things, on the type of LCFAs present. Usually, both palmitic and stearic acids are the principal LCFAs to be accumulated and, as saturated acids, are known to degrade 5 times slower than un-saturated acids (Novak and Kraus, 1973). Since a concentration of 1 $g{\cdot}L^{-1}$ of a mixture of LCFAs was reported to produce inhibition of the thermophilic biomass in batch assays (Palatsi *et al.*, 2009), the LCFA concentration during period PIII (6.6 $g_{LCFA}{\cdot}L^{-1}$) seem to be the reason behind the instability of the system and accumulation of VFAs, although the VFA accumulation disappeared and the LCFA removal was of 89% after 90 days of operation. A further increase, up to 9.2 $g{\cdot}L^{-1}$ of LCFAs (period PIV on Figure 5.2) produced instability in the daily biogas production and high LCFA concentrations in the effluent (3.0 $g{\cdot}L^{-1}$) (Figure 5.3a), though values for VFAs remained below 0.2 $g{\cdot}L^{-1}$. This phenomenon was previously described by Girault *et al.* (2012): high LCFA accumulations during the mesophilic co-digestion of SS and wastes rich in fats did not produce VFA accumulation, suggesting that the inhibition could be related to a mass transfer limitation due to the deposition of LCFA on the surface of the biomass aggregates, as highlighted by Pereira *et al.* (2005).

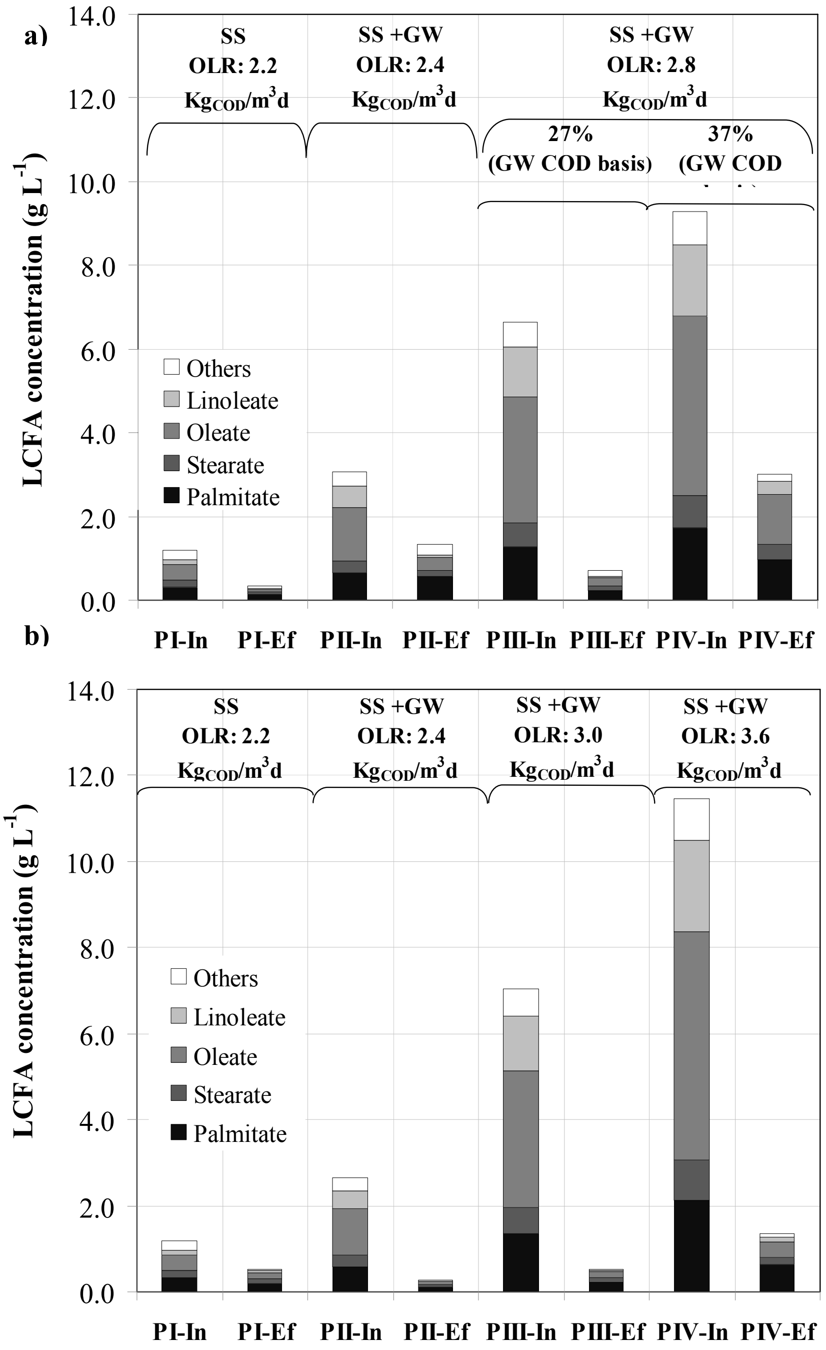

Figure 5. 3. *Influent and effluent LCFA concentrations in the thermophilic reactor (a); and the mesophilic reactor (b)*

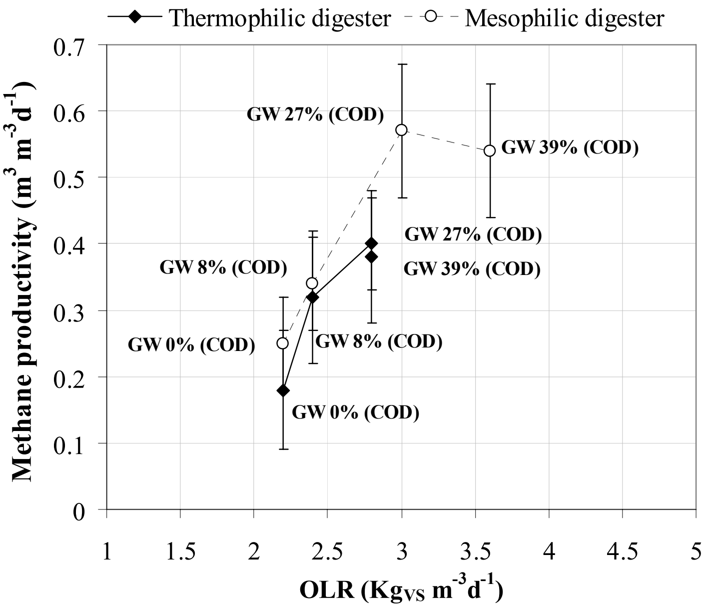

Figure 5. 4. *Methane production with different SS/GW ratios (on COD basis) as function of the OLR applied on the thermophilic and mesophilic reactors (mesophilic data from Silvestre et al. (2011*[1]*)).*

5.3.3 Biomass characterization

In order to understand the behaviour of the reactor, the biomass activity, once exposed to fats, was assessed by means of specific activity tests. Acetoclastic and hydrogenotrophic activities were performed at the end of periods PI, PIII and PIV. Figure 5.5 shows the evolution of net accumulated methane during the specific activity test over acetate and hydrogen as explained in section 5.2.4. Table 5.3 shows the lag-phase and the maximum slope estimated by the adjustment with the modified Gompertz equation.

The biomass sampled at the end of period PI has a good methanogenic hydrogenotrophic activity with a maximum slope of 367 $mg_{COD}{\cdot}g_{VS}^{-1}{\cdot}d^{-1}$, but presented a poor acetoclastic activity with a lag-phase of 13 days and a maximum slope of 28 $mg_{COD}{\cdot}g_{VS}^{-1}{\cdot}d^{-1}$ (Table 5.3). After 300 days in contact with the GW, the hydrogenotrophic activity at the end of PIII was similar to that of period PI, and even if the acetoclastic activity did not

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[1] Chapter 4

show a lag phase, the maximum slope was a bit lower than for period PI. A remarkable change in biomass activity was observed at the end of period PIV, when the system accumulated LCFAs (3.0 $g{\cdot}L^{-1}$). The acetoclastic activity increased significantly, while the slope of the hydrogenotrophic activity decreased by half compared to the previous periods (Table 5.3). These results are supported by the change in the methanogenic activity reflected in the mesophilic experiments (Silvestre *et al*., 2011[1]). In this case, and after 164 days in contact with fats, the biomass did not show any hydrogenotrophic activity and the acetoclastic activity showed an improvement with a maximum slope 6.3 times higher than the values obtained for the initial period without GW in the feed. This behaviour was also reported by Calveiro *et al*. (2001), showing that the acetoclastic methanogens of a mesophilic biomass had adapted to oleic acid, increasing their activity after an oleic organic shock, while the hydrogenotrophic activity was 8 times lower. In this sense, LCFA inhibition has been lately proved reversible with increasing consumptions of acetate and butyrate indicating such recovery.

Table 5. 3. *Average estimated parameters of the Modified Gompertz equation adjustment on the biomass specific activity test (λ and Rm), confidence interval (CI with α =0.05) and evaluation of the function fitting (r^2).*

Periods	**Substrate**[1]	***λ* - Lag phase**		***R_m* - Maximum slope**		**r^2**
		(days)	CI (95%)	($mg_{CODCH4}\ g_{VS}^{-1}\ d^{-1}$)	CI (95%)	
PI_c	Acetate	13	12-14	28	24-34	0.997
	H_2	0	-	367	289-613	0.980
$PIII_b$	Acetate	0	-	13	10-17	0.963
	H_2	0	-	257	301-445	0.991
PIV	Acetate	0	-	42	35-51	0.970
	H_2	0	-	141	92-310	0.880

[1] Acetate initial concentration = 10 mM, and H_2 initial concentration = 4.6 mM.

The increase of the acetoclastic activity and the concomitant decrease in hydrogenotrophic activity could be related to LCFA inhibition

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[1] Chapter 4

phenomena, to the fact that the LCFA degradation resulted in a 70% increase in acetate (Batstone *et al.*, 2002) or even because stress conditions favour the syntrophic conversion of acetate to methane (Petersen and Ahring, 1991). Montero *et al.* (2008) reported similar results: hydrogenotrophic methanogens were dominant in start-up conditions, but when OLR was gradually increased its population was displaced by the acetate-utilising methanogenic archaea.

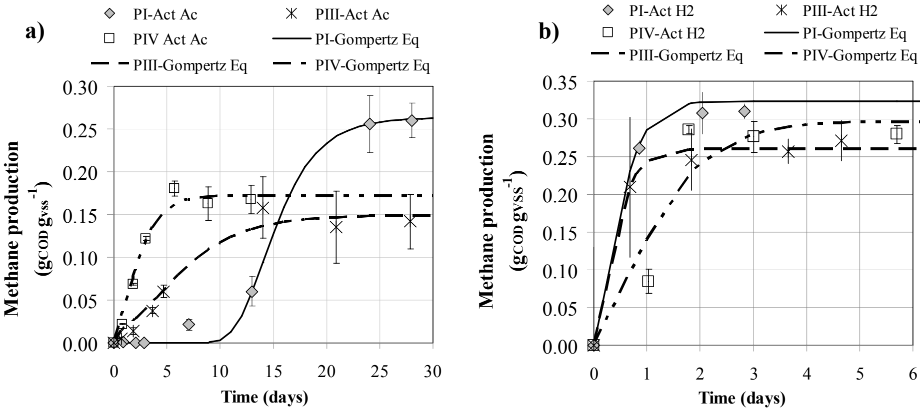

Figure 5. 5. *Accumulated methane production during the specific activity test over acetate (a) and hydrogen (b), of co-digestion the biomass sampled on periods PIc, PIIIb and PIV of the thermophilic operation.*

5.3.4 Dewatering properties of the digestate

The dewatering properties of the SS digestate, as stated before, have economic and energetic implications on a WWTP. Depending on its properties, the energy required for said dewatering and the cost of management could vary greatly. Figure 5.6 shows the filterability and centrifugability curves of the digestate for the different periods of the thermophilic and the mesophilic systems, running on the same conditions (Silvestre *et al.*, 2011[1]). Thermophilic digestates produced with SS digestion as the sole substrate (SS digestate) exhibit better dewaterability properties than the mesophilic ones. Oppositely, the mesophilic co-digestion digestate (GW-SS digestate) presented better dewaterability

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[1] Chapter 4

than the mesophilic SS digestate, while in the thermophilic range the dewaterability properties of the GW-SS digestate worsened (Figure 5.6).

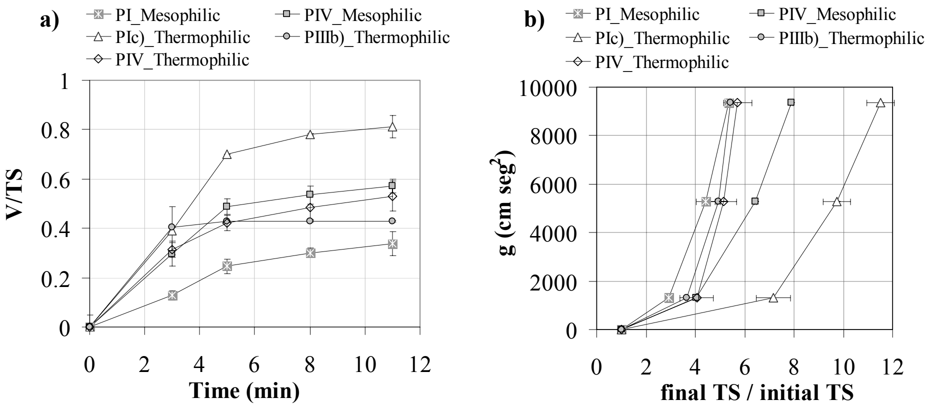

Figure 5. 6. *Dewatering properties of the thermophilic effluent, period PI_c (influent SS) and PIV (influent SS+GW) and mesophilic effluent, period PI (influent SS) and PIV (influent SS+GW) (data from Silvestre et al. (2011)[1]). a) Filterability test; and b) Centrifugability test.*

The presence of extracellular polymers (EPS) has been described as the major factor affecting dewatering properties (Novak *et al.*, 2003). Its occurrence depends on the nutrient balance of the substrate. An excess, or shortness, favours EPS production (Thaveesri *et al.*, 1994). For the mesophilic reactor, the addition of GW enhances the nutrient balance increasing the C/N ratio, thus explaining the improvement of the dewatering properties in comparison with an anaerobic digestion with only SS. These worse dewatering properties of the thermophilic GW-SS digestate are probably related to the presence of non-degraded LCFAs in the effluent that limit the dewatering.

5.4 CONCLUSIONS

The feasibility of thermophilic co-digestion of SS and GW has been proved to be viable. When GW was increased up to 12% or 27% (on COD basis) of the influent, OLR values increased from 2.2 to 2.4 and 2.8 $kg_{COD} \cdot m^{-3} \cdot d^{-1}$ repectively, and the methane yield increased 1.7 and 2.2

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[1] Chapter 4

times. An additional increase in GW resulted in an unstable reactor performance, LCFA accumulation in the effluent, and poor dewaterability of the digestate. Specific methanogenic activity tests have shown that grease waste addition improves the acetoclastic methanogenic activity, which suggests that the tolerance to LCFAs can be further enhanced by slowly increasing the feed of lipid-rich materials.

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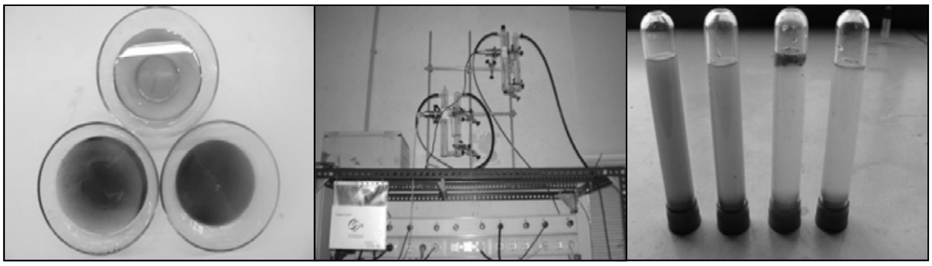

CHAPTER 6

ADDITION OF CRUDE GLYCERINE AS STRATEGY TO BALANCE THE C/N RATIO ON SEWAGE SLUDGE THERMOPHILIC AND MESOPHILIC ANAEROBIC CO-DIGESTION

The effect of adding crude glycerine (CGY) during continuous sewage sludge anaerobic digestion was investigated under thermophilic and mesophilic temperatures. Addition of CGY at thermophilic temperature range showed a negative impact on stability and performance of the process, even at low doses. The extreme pH values of CGY, together with the rapid release of VFA, causes SS alkalinity fail to control pH drop. On the contrary, at mesophilic temperature range the process performs steadily, with 148% increase in methane production when CGY represented 1% v/v of the influent (27% of influent COD). Further CGY percentages didn't show any added improvement; the biomass shift, due to a high C/N ratio, could explain this behaviour. Results suggested that CGY can be used as co-substrate of SS anaerobic digestion though, depending on the characteristics of CGY, and on operational conditions, different parameters should be taken into account to achieve a steady and consistent operation.

G. Silvestre, B. Fernández, A. Bonmatí. 2015. Addition of crude glycerine as strategy to balance the C/N ratio on sewage sludge thermophilic and mesophilic anaerobic co-digestion. Bioresource Technology, 193, 377-385.

6.1. INTRODUCTION

In recent years, renewable energy sources have received growing interest due to an increasing concern about global warming issues and stricter environmental legislations. Bio-fuel production –biodiesel and biogas– has risen considerably due to its environmental benefits as an alternative to fossil fuels, for its favourable energy balance, and its lower greenhouse gases emission rate (Ito *et al.*, 2005).

Biodiesel production in Europe, after a sudden growth at the beginning of 2000, is now stable at around 9,000,000 tons per year (EBB, 2010). Biodiesel is produced from vegetable oils through transesterification with methanol, which is catalyzed by KOH; but also from used cooking oil. Biodiesel production yields crude glycerine (CGY) as a by-product in a ratio of 10 kg per 100 kg of biodiesel produced (Chi *et al.*, 2007).

CGY comprises streams with variable amounts of components such as alcohols, water, inorganic salts coming from the catalysts, free fatty acids, un-reacted triglycerides, and methyl esters (Siles *et al.*, 2009). Although glycerine has more than 2,000 different applications, its purification is required in most of these industrial applications, which deems it economically unfeasible for most biodiesel plants (Siles *et al.*, 2009; Astals *et al.*, 2011). Alternatively, CGY can be handled as a substrate in biogas plants (Astals *et al.*, 2011).

Mono-substrate anaerobic digestion of CGY is limited due to its chemical composition (low N content and extreme pH), but it could make for an interesting co-substrate as it is easily biodegradable and has a high biogas potential. Moreover, it can be easily stored for long periods (Fountoulakis *et al.*, 2010). However, inhibition phenomena have been described depending on its specific composition and operational conditions (Astals *et al.*, 2011).

It is generally accepted that the optimal carbon to nitrogen ratio (C/N) of an anaerobic digestion process lies between 15 and 30 (Mshandete et al., 2004), while other authors point out that for methanogenic archea the optimal C/N ratio is 16-19 (Kivaisi and Mtila, 1998). CGY co-digestion with sewage sludge (SS) could be adequate since the high water content in SS might dilute the posible presence of toxic compounds of glycerine, while the SS brings in the essential micro/macro-nutrients for bacterial growth and CGY provides large amounts of carbon (Razaviarani and Rittmann, 2013). CGY dosage, used as co-substrate with nitrogen rich substrates, balances the C/N ratio because of its low nitrogen concentration (Robra *et al.*, 2010). Different studies show the benefits of

CGY addition during the anaerobic digestion of different organic wastes such as agro-wastes, cattle manure, the organic fraction of municipal solids wastes, and pig manure; though a strict control strategy is required to limit the risk of organic overloading (Robra *et al*., 2010; Astals *et al*., 2012).

SS co-digestion with organic wastes –such as fats or domestic wastes– has in recent years been proved to be an attractive strategy to optimize biogas production (Silvestre *et al*., 2011[1]; Silvestre *et al*., 2014[2]), and mesophilic CGY and SS co-digestion has lately raised a lot of interest (Fountoulakis *et al*., 2010, Razaviarani *et al*., 2013, Razaviarani *et al*., 2014, Jensen *et al*., 2014).

Currently, most wastewater treatment plant (WWTP) anaerobic digesters are operated at mesophilic temperatures (Silvestre *et al*., 2015[3]), but a growing concern to obtain pathogen-free biosolids as well as the need to improve biogas production, has increased the interest on thermophilic anaerobic digestion (Mata-Alvarez *et al*., 2014). Still, scarce information about SS anaerobic co-digestion is nowadays available.

SS co-digestion, besides optimizing biogas production, could lead to non-desired side effects such as changes on the dewatering properties of the digested sludge. These changes could have a direct effect on the economic performance of a WWTP – because if dewatering worsens, as described by Borowski and Szopa, (2007) and Silvestre *et al*., (2014)[2], energy requirements, as well as reagent consumption and management costs of the dewatered SS could rise.

The main objective of the present research was to study the effects of adding crude glycerine from biodiesel production to continuous sewage sludge anaerobic digestion, including the study of the optimal doses in terms of performance and stability parameters of the anaerobic process, and to monitor changes in specific activity of the anaerobic biomass, and variations in the dewatering properties of the anaerobic effluents. The study was carried out in thermophilic as well as in mesophilic temperature ranges.

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[1] Chapter 4

[2] Chapter 5

[3] Chapter 2

6.2. MATERIAL AND METHODS

6.2.1. Substrates and inocula

Three samples of crude glycerine (CGY_1, CGY_2, and CGY_3) from three different Spanish biodiesel factories were analyzed. The mixed sewage sludge (SS) used (70% primary sludge, and 30% activated sludge) was sampled from a municipal WWTP (Barcelona, Spain). The SS was sampled every second week, and those samples kept refrigerated at 4ºC, while glycerines were sampled just once and kept frozen. Figure 6.1 shows an illustration of the three glycerines used in the experiments.

Figure 6. 1. *Illustration of the three glycerines analyzed*

The mesophilic anaerobic inoculum used for the anaerobic biodegradability test was obtained from the anaerobic digester of the same WWTP, whereas the thermophilic inoculum was obtained from a lab-scale thermophilic reactor already under operation with SS and other co-substrates for more than two years.

6.2.2. Analytical methods

Total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), total chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), ammonia nitrogen (NH_4^+-N), total and partial alkalinity (TA, PA), sulphate (SO_4^{2-}-S), and phosphate (PO_4^{3-}-P) were determined according to Standard Methods (APHA, AWWA, WEF, 1995). Elemental composition (C, H, and N) was determined by catalytic oxidation combined with gas chromatography (LECO). Biogas composition (CH_4, H_2, CO_2) and volatile fatty acids (acetate, propionate, i-butyrate, n-butyrate, i-valerate, n-valerate, i-caproate and n-caproate acids) were determined by gas chromatography as described in Silvestre *et al.* (2011)[1].

6.2.3. Batch experiments

Two types of batch experiments were carried out, the anaerobic biodegradability test (ABT) and the specific activity test (SAT) following methods described in Silvestre *et al.* (2011)[2]. ABT tests were carried out to determinate the maximum methane potential and the anaerobic biodegradability of the different glycerine samples, at mesophilic (35ºC) and thermophilic (55ºC) temperatures. SAT tests were performed to evaluate the health of the biomass in the continuous reactors. A sample of biomass was taken at the end of each period and tested with four different substrates (10 mM acetic acid; 4.1 mM hydrogen; 6.0 mM propionate acid; a 6.2 mM i-butyrate and i-valerate acids 1:1 mixture). The maximum slope (R_m; $NL_{CH4}\cdot d^{-1}$) of the net accumulated methane curves and the observed lag phase (λ; days) were the parameters used to compare the different specific substrate activities. Net cumulative methane yields were expressed under normal conditions (0 ºC, 1 atm).

6.2.4. Continuous experiment

Two continuous stirred tank reactors (CSTR) with a working volume of 5.5 L and 5.0 L were operated with a hydraulic retention time (HRT) of 20 days at mesophilic and thermophilic temperatures, respectively. Reactor temperature was maintained by hot water recirculation through the double glass jacket. The content of the digester was continuously mixed by mechanical stirring at 25 rpm. The reactors were fed with a peristaltic pump and the effluents were collected in a glass reservoir via an overflow system. Biogas production was measured with a volumetric gas counter (Ritter Apparatebau GMBH&CoKG). A schematic diagram of the experimental set-up is showed in Silvestre *et al.* 2014[3]. The influent and effluent characteristics of both digesters were measured once a week, and the biogas composition twice a week. At the end of each co-digestion period, as previously mentioned, the inoculum was submitted to a SAT test and a dewatering test.

Performance of the continuous reactors was evaluated in terms of organic matter removal efficiency and gas yields. Previously, both digesters had been operated for nearly 2 years with SS and a mixture of SS and grease waste (Silvestre *et al.*, 2011[1]; Silvestre *et al.*, 2014[2]), their

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[1] Chapter 4

[2] Chapter 4

[3] Chapter 5

results being considered as reference values for comparison purposes. The initial periods of the thermophilic and mesophilic reactors were called P_TSS and P_MSS respectively, and corresponded to the sewage sludge mono-digestion (Silvestre *et al.*, 2011[1], Silvestre *et al.*, 2014[2]).

6.2.5. Dewatering test

Dewaterability properties of the digestates were assessed with the centrifugal settling test (CST) and the vacuum filtration test (VFT). Both tests were based on Standard Methods (APHA, AWWA, WEF, 1995).

6.3. RESULTS

6.3.1. Substrate characterization

Table 6.1 shows the characterization of the CGY and SS samples. The organic matter content of CGY, expressed as VS and COD, was more than 40-24 and 34-24 times higher than in the SS respectively, and the fact that close to 95-100% of the CGY's TS were VS must be noted. C/N ratio in the CGY samples was high, ranging between values as wide as 78 and 3,338. Since the optimal C/N ratio of an anaerobic digestion process is referred to be between 15 and 30 (Mashandete *et al.*, 2004), CGY anaerobic digestion must be limited, albeit it being an excellent co-substrate of waste streams with low carbon and high nitrogen content.

The extremely wide range in pH is also remarkable, with values between 3 and 13. In this sense, Hutňan *et al.* (2013) studied glycerines from different origins, and pH values were found to be between 4 and 13, depending on the biodiesel production process and the recovery process of CGY. Similarly, sulphate content also widely varies, as it depends upon the purification method applied in the biodiesel facility.

Table 6. 1.*Characteristics of crude glycerine (CGY) samples and sewage sludge (SS)*

Parameter	**Units**	**CGY_1**	**CGY_2**	**CGY_3**	**SS**
pH	-	5.5	12.6	3.3	6.8
TS	$g\ kg^{-1}$	926 ± 0	961 ± 6	781 ± 10	33 ± 5
VS	$g\ kg^{-1}$	924 ± 1	917 ± 10	744 ± 5	23 ± 5
COD	$g\ kg^{-1}$	1,517 ± 221	1,070 ± 135	1,397 ± 220	44 ± 8
C/N	$g\ g^{-1}$	588 ± 50	3,338 ± 10	78 ± 5	18 ± 2
SO_4^{2-}-S	$g\ kg^{-1}$	1.68 ± 0.03	2.73 ± 0.05	0.49 ± 0.03	0.02 ± 0.00
PO_4^{3-}-P	$mg\ kg^{-1}$	228 ± 20	101 ± 11	168 ± 20	5 ± 1
CH_4 yield$_{55°C}$	$NL_{CH4}\ kg_{VS}^{-1}$	303 ± 35	277 ± 30	475 ± 8	354 ±12
CH_4 yield$_{35°C}$	$NL_{CH4}\ kg_{VS}^{-1}$	349 ± 27	463 ± 38	490 ± 32	296 ± 6
AnBIO$_{55°C}$	%	60 ± 6	66 ± 8	70 ± 1	79 ± 3
AnBIO$_{35°C}$	%	65 ± 5	100 ± 2	98 ± 3	73 ± 1

Note: CGY: Crude Glycerine, SS: Sewage Sludge, AnBIO: Anaerobic Biodegradability

6.3.2. Methane yield

CGY showed different profiles and methane yields in batch tests depending on the origin of the glycerine and the range of temperature. The methane yield at mesophilic temperatures on all CGYs was higher than at the thermophilic range; and 1.2 - 1.7 times higher than the SS yield (296 $NL_{CH4}\cdot kg_{VS}^{-1}$) In contrast, at thermophilic ranges, the SS methane yield (354 $NL_{CH4}\cdot kg_{VS}^{-1}$) was higher than in the CGY samples, except for CGY_3 (Table 6.1).

CGY_1 showed the highest COD (1,517 $g_{COD}\cdot kg^{-1}$) with a slightly acidic pH (5.5), but presented the lowest methane yield at mesophilic range, though it was 1.2 folds higher than in thermophilic range. Compared with the other samples, CGY_1 showed the lowest anaerobic biodegradability, both at mesophilic and thermophilic temperatures – 65% and 60%, respectively.

CGY_2 and CGY_3, despite their differences in composition, presented similar mesophilic methane yields, 463 and 490 $NL_{CH4}\cdot kg_{VS}^{-1}$ respectively, and an anaerobic biodegradability close to 100% in both cases. On the contrary, at thermophilic range CGY_2 presented the lowest methane yield (277 $NL_{CH4}·kg_{VS}^{-1}$) and anaerobic biodegradability decreased to 66% and 70% for CGY_2 and CGY_3 respectively (Table 6.1). The high pH of CGY_2 (12.6) and the low pH of CGY_3 (3.3) showed the presence of residuals substance that could have an inhibitory effect at this range of temperature and explain this sharp decrease in anaerobic thermophilic biodegradability.

Although the methane yield of all de CGY samples was 2.6 to 4.7 times lower than the theoretical value of 1,295 m^3_{CH4} T^{-1} of pure glycerine (Amon *et al.*, 2006), the figures obtained were relatively close to those figures reported by Siles *et al.* (2009). The presence of impurities such as water, methyl ester, soap stock, methanol, and inorganic salts (sulphate, phosphate, etc.) is responsible for the decrease in methane yield as compared to pure glycerine.

Figure 6.2 shows the accumulated methane curves of the three CGY samples at mesophilic and thermophilic temperatures. Despite the differences in methane yield and anaerobic biodegradability, CGY_1 and CGY_2 showed similar methane production patterns at both temperatures with a two-step methane production curve. At thermophilic temperatures the production of methane almost stopped between days 5 to 9, with a sudden change in slope, and at mesophilic temperatures the change in slope is smooth without methane yield completely stopping (Figure 6.2). This fact could be related to partial inhibition caused by the accumulation of high amounts of VFA during the first day of operation which could be consumed thereafter, and/or to the presence of components with different degradation kinetics.

On the other hand, CGY_3 showed a completely different pattern. While at thermophilic temperatures no lag phase was observed –the methane is being produced in the first five days–; at mesophilic temperatures a lag phase of nearly five days was observed. However, methane yield at mesophilic temperatures reached a slightly higher final value than at the thermophilic range. Glycerine fermentation mainly produces 1.3-propanodiol and hydrogen (Jitrwung and Yorgeau, 2011), thus the long lag phase of CGY_3 at the mesophilic range could be attributed to an inhibition due to hydrogen, since the mesophilic inoculum has a low specific hydrogen activity (22 $mg_{COD\text{-}CH4}·g_{VS}^{-1}·d^{-1}$) in comparison with the thermophilic inoculum used (141 $mg_{COD\text{-}CH4}·g_{VS}^{-1}·d^{-1}$) (Silvestre *et al.*, 2011[1]; Silvestre *et al.*, 2014[1]).

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[1] Chapter 4

CGY$_2$ and CGY$_3$ were selected for the continuous co-digestion trials performed at thermophilic and mesophilic ranges, taking into account their methane yield and anaerobic biodegradability, in addition to their chemical composition (mainly the sulphate content).

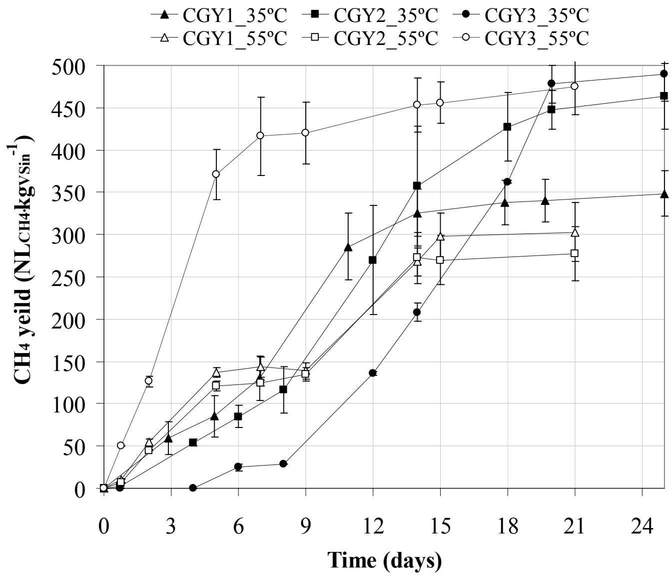

Figure 6. 2. *Accumulated methane production in the biodegradability test of the three samples of glycerine at mesophilic and thermophilic temperature ranges.*

6.3.3. Continuous experiment

6.3.3.1. Thermophilic reactor

Co-digestion of SS and CGY at thermophilic range was assessed with three different OLR and two glycerine samples (CGY$_2$ and CGY$_3$) throughout three periods of time (P$_T$I, P$_T$II, P$_T$III). At the end of the experiment, a fourth period (P$_T$IV), feeding the reactor only with SS, was also run in order to recover the reactor. Table 6.2 shows the average values for each period, as well as the initial period (P$_T$SS) of this reactor, corresponding to sewage sludge mono-digestion, the performance being as described in Silvestre *et al.* (2014)[2].

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[1] Chapter 5

During the first period (P_TI), CGY_3 was added at a ratio of 1.6% v/v, representing 34% of the influent COD, with an OLR of 2.4 $kg_{COD}\cdot m^{-3}\cdot d^{-1}$ and a HRT of 22 days. The low pH of CGY_3 (pH 3.3) brought about a fast alkalinity consumption in the digester with a concomitant decrease in pH, with values lower than 7, in less than 10 days (Figure 6.3c) The instability of the process was also shown by a 69% to 54% decrease in methane content of the biogas (Figure 6.3a) and an increase in volatile fatty acids (VFA) concentrations above 1 $g\cdot L^{-1}$ (Figure 6.3b) Even though the methane production was stable during this period (Figure 6.3a), but considering that VFA consumption at thermophilic temperatures is slow (Silvestre *et al.*, 2014)[2], CGY_3 was replaced with CGY_2, which is characterized by a high pH (Table 6.1).

The co-digestion experiment with CGY_2 was carried out in two different periods of time with different OLR (P_TII and P_TIII). During period P_TII, the OLR applied was 2.5 $kg_{COD}\cdot m^{-3}\cdot d^{-1}$ whilst CGY_2 represented 1.5% v/v of the feed and 39% of the influent COD, similar to the operational conditions of period P_TI (Table 6.2). Methane production and total alkalinity showed a trend similar to period P_TI, though the methane content of the biogas suffered from a gradual increase back to 64% v/v (Figures 6.3a and 6.3c) However, VFA concentrations continued to increase and pH decreased to values close to 6.7 - 6.9 (Figures 6.3b and 6.3c).

Period P_TIII commenced with an OLR of 3.6 $kg_{COD}\cdot m^{-3}\cdot d^{-1}$ due to a new 2% w/w increment on glycerine, representing 43% of the influent COD. This increment in OLR initially improved methane production from 0.21 to 0.54 $m^3\cdot m^{-3}\cdot d^{-1}$ (P_TIIIa). Nevertheless, the significant daily variation in methane production together with the VFA increment indicated an unbalanced process (Figures 6.3a and 6.3b). Total VFA increased to maximum values close to 7 $g\cdot L^{-1}$ in the second part of this period (P_TIIIb) in parallel with a decrease in pH below 6.5 (Figure 6.3c). At that point, methane production suddenly decreased to values below 0.25 $Nm_{CH4}^3\cdot m^{-3}\cdot d^{-1}$, remaining rather stable while VFA concentrations slowly decreased.

Finally, and in order to recover the system (P_TIV), the addition of glycerine was interrupted and the OLR was reduced to 2.3 $kg_{COD}\cdot m^{-3}\cdot d^{-1}$, being SS the sole substrate. As can be seen in Figure 6.2b, VFA were consumed fast, reaching values below 0.1 $g\cdot L^{-1}$ in less than 25 days, and methane production increased to 0.32 $Nm^3\cdot m^{-3}\cdot d^{-1}$. Although this last period was operated similarly to the initial period P_TSS (Silvestre *et al.*, 2014)[1], methane production was two times higher. This phenomenon can be attributed to the growth of active biomass induced by the extra carbon source provided by the glycerol, which is able to degrade higher sewage sludge quantities and thus, to attain a higher methane production rate (Fountoulakis *et al.*, 2010; Ma *et al.*, 2007).

Evaluation and monitoring of reactor stability

Methane production was improved with the addition of CGY (Table 6.2); nevertheless, CGY co-digestion periods showed undesired instability, leading to the necessary establishment of key parameters, their inhibitory threshold and the development of a robust control system to avoid reactor failure. In this regard, the stability of the anaerobic co-digestion process was evaluated monitoring VFA concentrations, pH, alkalinity ratios (IA/PA ratio) and methane composition.

As it is well known, the accumulation of intermediate products, such as VFA, is a process unbalance indicator. Figure 6.3b shows the different VFA profiles throughout the different co-digestion periods. Propionic acid increased progressively from the beginning of CGY co-digestion, reaching its highest concentrations (> 5.7 $g\cdot L^{-1}$) when the pH was below 6.5 and the reactor was close to failure (period P_TIIIb). This behaviour agrees with the null propionic activity of the initial anaerobic biomass of the reactor. On the other hand, acetic content showed a rather stable profile with values below 1.0 $g\cdot L^{-1}$ throughout the entire reactor operation. Other VFA such as i-butyric, i-valeric or n-caproic acids showed a similar trend: their concentrations during period P_TIIIa increased up to 0.2, 0.3 and 0.5 $g\cdot L^{-1}$, respectively, to later decrease once pH values dropped below 6.8. The remaining VFA (n-butyric, n-valeric and i-caproic acid) showed values below 0.05 $g\cdot L^{-1}$ throughout all the experimental periods.

Marchaim and Krause (1992) proposed the use of the propionate/acetate (P/A) ratio as control parameter. In this sense, Hill *et al.* (1987) established the anaerobic system failure at a P/A ratio greater than 1.4, and Ferrer *et al.* (2010) fixed this ratio at 2. But in the present study, the failure of the system occurred when the P/A ratio was close to 16. Alternatively, Nielsen *et al.* (2007) proposed propionic acid as a more realistic control parameter to predict process failure. However, in this case, it was not a useful parameter as high propionic acid concentrations did not curb methane production (Figure 6.3a). Methane production was only hampered when propionic acid reached the extremely high

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[1] Chapter 5

concentration of 6 g·L^{-1} at the end of PTIIIa (Figure 6.3b). The behaviour of n-caproic acid, which is rarely observed in anaerobic digestion processes, is also interesting; it began to accumulate when total concentration of VFA reached 6 g·L^{-1} (Figure 6.3b) and its accumulation was coincident with a clear instability of the anaerobic process.

Another index to follow the performance of a reactor is the total concentration of VFA. Results obtained agree with different authors that showed a strong anaerobic inhibition when total VFA concentrations range between 5-6 g·L^{-1} due to a pH drop below 7 (Amon *et al.*, 2006) , although the system didn't fully collapse till total VFA concentration exceeded 10-12 g·L^{-1} (Nielsen *et al.*, 2007).

The alkalinity index (IA/PA ratio) is also used to evaluate reactor stability (Ferrer *et al.*, 2010; Astals *et al.*, 2012). This ratio shows to what extent acid concentration, estimated by intermediate alkalinity (IA), exceeds the buffer capacity provided by HCO_3^-, estimated by PA. An IA/PA ratio of 0.4 is said to be the upper limit of a stable reactor operation. As can be seen in Figure 6.3c, the IA/PA ratio was above this value throughout the entire reactor operation except on period P_TIV, where it decreased down to 0.3 showing a complete recovery of the reactor.

Biogas composition, specifically methane percentage, is also used to assess anaerobic digestion performance, but it also has some limitations. During period P_TI, CH_4 content decreased concomitant with the slight acidification of the thermophilic reactor. The low pH of the reactor (Figure 6.3c) might have promoted CO_2 stripping from the liquid, altering the relative amount of methane in the gas. However, this parameter was of no use during the instability occurred in period P_TIII, since CH_4 content was quite stable throughout this period (Figure 6.3a).

As can be seen, none of these parameters can stand alone for process control but rather the combination of all of them. Besides, the inhibitory threshold values are hard to define as they depend on many factors such as operational parameters, biomass activity and adaptation, the history of the reactor feeds, etc. In this regard, a control system should include different parameters and adaptive threshold values in order to properly control the operation.

Table 6. 2. *Summary of the average operating performance of the thermophilic reactor during each period (error margins represent standard deviations*

Parameter	**Units**	**P_TSS (56 days)**	**P_TI (10 days)**	**P_TII (10 days)**	**P_TIII$_a$ (29 days)**	**P_TIII$_b$ (15 days)**	**P_TIV (36 days)**
T^{a1}	ºC	56 ± 1	56 ± 1	56 ± 0	56 ± 6	55 ± 0	55 ± 1
HRT^1	Days	20 ± 2	22 ± 2	20 ± 1	18 ± 3	19 ± 1	21 ± 2
OLR_{COD}^1	$kg_{COD}\, m^{-3}d^{-1}$	2.2 ± 0.2	2.4 ± 0.1	2.5 ± 0.0	3.6 ± 0.6	3.4 ± 0.5	2.3 ± 0.5
OLR_{VS}^1	$kg_{VS}\, m^{-3}d^{-1}$	1.2 ± 0.1	1.2 ± 0.0	1.1 ± 0.0	1.2 ± 0.1	1.3 ± 0.2	1.1 ± 0.3
CGY	-	-	CGY3	CGY2	CGY2	CGY2	-
$CGY_{addition}$	% v/v	-	1.6	1.5	2.0	3.0	-
$CGY_{addition}$	% VS_{in}	-	32 ± 2	37 ±2	51 ± 7	51 ± 7	-
$CGY_{addition}$	% COD_{in}	-	34 ± 2	39 ± 1	43 ± 0	43 ± 0	-
C/N inlet	$g \cdot g^{-1}$	10	61	11	11	11	10
$COD_{removal}^2$	%	56 ± 3	50 ± 4	52 ± 0	58 ± 3	54 ± 3	38 ± 2
$VS_{removal}^2$	%	50 ± 11	68 ± 3	64 ± 5	73 ± 2	60 ± 4	46 ± 12
$CH_{4\ production}^1$	$Nm^3m^{-3}d^{-1}$	0.18 ± 0.10	0.23 ± 0.01	0.25 ± 0.04	0.53 ± 0.14	0.33 ± 0.15	0.32 ± 0.16
$CH_{4\ yield}^1$	$Nm^3 \cdot T_{VSinlet}^{-1}$	0.26 ± 0.45	0.18 ± 0.01	0.20 ± 0.03	0.39 ± 0.04	0.24 ± 0.09	0.21 ± 0.08
% CH_4^3	%v/v (biogas)	66 ± 4	57 ± 4	59 ± 2	64 ± 1	64 ± 1	65 ± 2
α	1%TMP_{VS}/CH_{4yield}	0.73	0.51	0.57	1.11	0.68	0.59
TA^3	$g_{CaCO3}\, L^{-1}$	5.1 ± 0.4	3.3 ± 0.6	2.8 ± 0.1	3.2 ± 0.3	3.6 ± 0.2	3.5 ± 0.5
PA^3	$g_{CaCO3}\, L^{-1}$	2.7 ± 0.2	2.0 ± 0.8	1.1 ± 0.3	1.0 ± 0.1	0.7 ± 0.4	2.1 ± 1.0
IA/PA	$g \cdot g^{-1}$	0.3 ± 0.2	0.5 ± 0.1	0.6 ± 0.1	0.7 ± 0.0	0.8 ± 0.0	0.4 ± 0.2
VFA	$g_{acetate\ eq}\, L^{-1}$	0.12 ± 0.0	0.25-0.93	0.93-1.64	5.35-6.25	4.78-7.14	4.78-7.14
pH^3	-	7.4 ± 0.2	7.1 ± 0.2	6.8 ± 0.1	6.9 ± 0.1	6.4 ± 0.2	7.3 ± 0.4

Note: Average and S.D of the daily (^{1}), weekly (^{2}) or twice week (^{3}) samples of each period

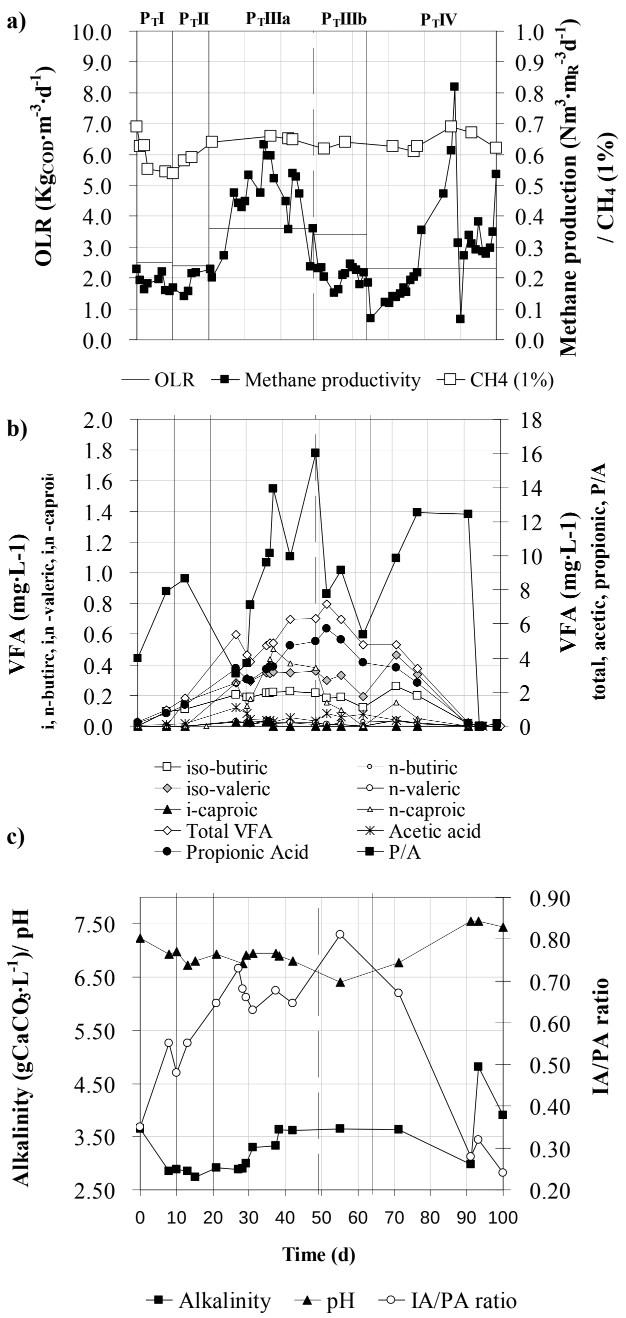

***Figure 6. 3**. Evolution of the operational parameters of the thermophilic co-digestion of SS and CGY. a) OLR, CH_4 production and CH_4 %, b) VFA and P/A ratio, c) Alkalinity, pH and IA/PA ratio.*

6.3.3.2. Mesophilic reactor

Two different mixtures of SS and CGY_3 were assessed in the mesophilic reactor. The operation of the reactor was divided in two periods (P_MI, P_MII). During periods P_MI and P_MII, CGY was 1.2 and 2.4% v/v of the feed, equivalent to 27% and 31% of the influent COD, respectively. HRT was kept constant at 20 days for the entire experiment; as a consequence, the addition of glycerine increased the OLR to 3.0 (P_MI) and 3.2 $kg_{COD}·m^{-3}·d^{-1}$ (P_MII). The only changes were therefore the influent C/N ratio and VS content: the addition of CGY_3 increased C/N ratio to 50 and 87, as well as VS content to 35 and 44 $g_{VS}·L^{-1}$, in P_MI and P_MII, respectively.

Reactor stability evaluation

The main parameters corresponding to each period, as well as to the mesophilic SS mono-digestion (Silvestre *et al.*, 2011)[1] are shown in Table 6.3. The removal efficiency of the organic matter remained similar in periods P_MI and P_MII, but it was higher (57% in P_MSS) when compared to the SS mono-digestion (35% in P_MSS). This increment was related to the high biodegradability of CGY_3 at mesophilic temperatures.

Methane production was 2.5 times higher in P_MI than in the SS mono-digestion (P_MSS), as Figure 6.4 illustrates. The methane content of the biogas decreased, being 7% lower in period P_MI than in period P_MSS. This biogas change in composition is explained by the composition of the wastes: SS has high protein related compounds that lead to a biogas richer in methane, compared to the biogas produced from alcohols like glycerol.

Volumetric methane production increased slightly (0.66 $Nm^3·m^{-3}·d^{-1}$), but the methane yield decreased from 325 $NL_{CH4}·kg_{VS}^{-1}$, in P_MI, to 275 $NL_{CH4}·kg_{VS}^{-1}$ in P_MII. These data indicated that part of the added glycerine in the latest period was not degraded, though an overloading was dismissed since VFA concentration was low throughout all periods. A low N concentration of the P_MII influent limited the growth of the microorganisms since C/N ratio was far from being optimum (Mshandete *et al.*, 2004). In this regard, Bouallagui *et al.* (2009) reported an increase of the biogas production yield (+8.1%) when decreasing C/N ratio from 34 to 28.

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[1] Chapter 4

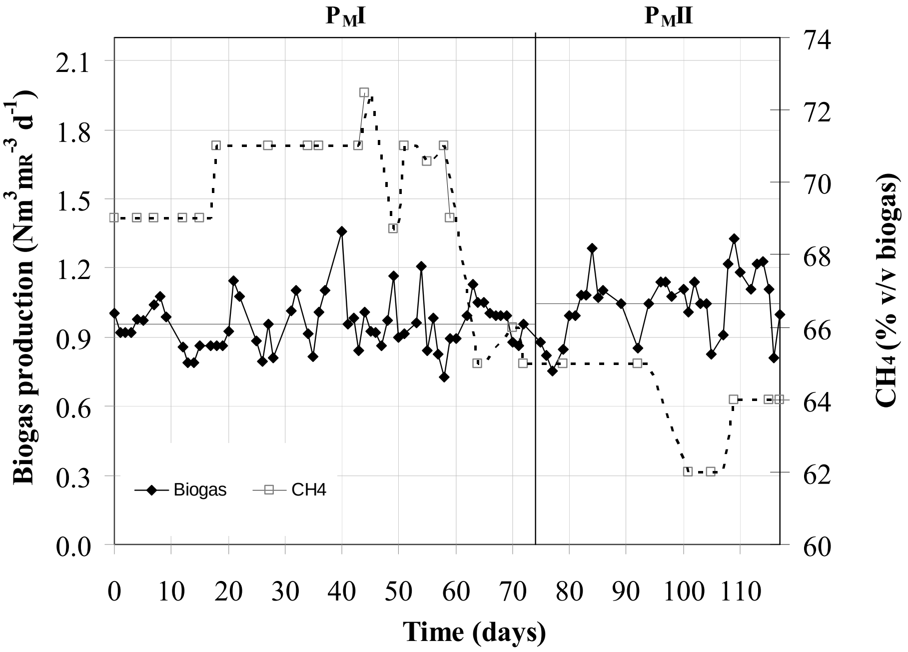

Figure 6. 4. *Biogas production rate and methane % during SS-CGY anaerobic co-digestion operation at mesophilic temperatures*

The addition of 1.2% v/v of glycerine (P_MI) resulted in the most efficient process in terms of performance with an increment in methane production of +148%. These results were in accordance with Fountoulakis *et al.* (2010) and Razaviarani *et al.* (2013) who obtained a maximum biogas production during co-digestion with 1.0%v/v and 1.1% of glycerine, respectively, showing an increase of the daily methane production of +113% and +87%, respectively. However, Razaviarani *et al.* (2013) showed a considerable decrease in the methane yield and organic matter removal efficiency when the glycerine added was higher than 2% v/v of the feed mixture. Likewise, Fountoulakis *et al.* (2010) found an unstable process with high VFA concentration in the effluent when the glycerine content was 3% v/v.

The relative quantity of CGY to be added is highly dependent on the characteristics of the main waste. CGY has a high degradation rate and, as a consequence, high amounts of VFA are delivered rapidly to the medium (Mata-Alvarez *et al.*, 2000). For this reason, the alkalinity content of the substrate is crucial to avoid a pH drop that could adversely affect, in a first instance, the methanogenic activity. In the SS mesophilic co-digestion experiment, the buffer capacity was enough when VFA concentration was negligible. On the contrary, in the thermophilic anaerobic co-digestion experiment, alkalinity was not enough by itself, and the high rate of VFA generation causes VFA accumulation and as a consequence a pH drop occurred (Figure 6.3b).

Table 6. 3. *Summary of the average operating performance of the mesophilic reactor during each period (error margins represents standard deviation)*

Parameter	**Units**	**P_MSS (113 days)**	**P_MI (72 days)**	**P_MII (42 days)**
T^a	°C	34 ± 1	36 ± 1	36 ± 1
HRT^1	Days	20 ± 3	20 ± 2	20 ± 1
$OLR_{COD}{}^1$	$kg_{COD}\,m^{-3}d^{-1}$	2.2 ± 0.6	3.0 ± 0.3	3.2 ± 0.4
$OLR_{VS}{}^1$	$kg_{VS}\,m^{-3}d^{-1}$	1.5 ± 0.6	1.2 ± 0.1	1.6 ± 0.4
CGY	-	-	CGY_3	CGY_3
$CGY_{addition}$	% v/v	-	1.2	2.4
$CGY_{addition}$	% VS_{in}	-	39 ± 9	55 ± 11
$CGY_{addition}$	% COD_{in}	-	27 ± 4	31± 7
C/N inlet	$g \cdot g^{-1}$	10	50	87
$COD_{removal}{}^2$	%	35 ± 13	57 ± 5	57 ± 5
$VS_{removal}{}^2$	%	36 ± 7	57 ± 3	64 ± 4
$CH_{4\,production}{}^1$	$Nm^3m^{-3}d^{-1}$	0.25 ± 0.07	0.62 ± 0.07	0.66 ± 0.09
$CH_{4\,yield1}$	$Nm^3\,t_{VSinlet}{}^{-1}$	249 ± 80	325 ± 52	275 ± 53
% $CH_4{}^3$	%v/v (biogas)	72 ± 3	65 ± 1	63 ± 1
α	1% CH_{4yield}/TMP_{VS}	0.84	1.09	0.91
TA^3	$g_{CaCO3}\,L^{-1}$	5.7 ± 1.1	3.5 ± 0.1	3.6 ± 0.3
PA^3	$g_{CaCO3}\,L^{-1}$	3.6 ± 0.9	2.8 ± 0.2	2.7 ± 0.1
IA/PA	$g \cdot g^{-1}$	0.31 ± 0.1	0.22 ± 0.1	0.12 ± 0.1
VFA^2	$g_{acetate\ eq}\,L^{-1}$	< 100	< 100	< 100
pH^3	-	7.8 ± 0.4	7.6 ± 0.2	7.2 ± 0.2

Note: Average and S.D of the daily (^{1}), weekly (^{2}) or twice week (^{3}) samples of each period

Animal slurries accept higher ratios of glycerine than SS, due to a higher buffer capacity and nitrogen content. Robra *et al.* (2010) obtained the best performance from cattle slurry anaerobic co-digestion with glycerine, when the glycerine represented 10% of mixture. Astals *et al.* (2012) reported a +400% increase in biogas production during pig manure anaerobic co-digestion with 4% v/v of glycerine; however, the glycerine used had a C/N of 48, much lower to that of the CGYs used in this study.

The same author studied in batch anaerobic tests different mixtures of pig manure and glycerine, obtaining the best biogas yield with an 80% pig manure and 20% glycerine mixture, though in this case the C/N ratio was even lower (C/N: 23) (Astals *et al.*, 2013). Similarly, Amon *et al.* (2006) obtained their optimal methane yield when 6% of glycerine was added to an anaerobic co-digestion fed with pig manure and maize silage.

Another positive effect observed during SS and CGY mesophilic co-digestion is the synergy effect over biogas production. Methane production in the mesophilic periods P_MI and P_MII was 1.01 and 0.91 of the theoretical value calculated considering the methane potential of CGY_3 and SS obtained in the ABT (α), figures higher than the one calculated for period P_MSS (SS feed alone) (Table 6.3). Contrary, at thermophilic range the synergic effect was not observed; α was 0.51-0.57 for period P_TI and P_TII, respectivaly (Table 6.2). Period P_TIII showed higher α value, but it was not relevant as the system was not in steady state conditions (Figure 6.3). Fountoulakis *et al.* (2010) and Ma *et al.* (2007) reported similar results and hypothesised that the increase in active biomass due to a more balanced composition of the substrate is responsible of this synergic effect.

6.3.4. Biomass adaptation assessment

In order to assess the effect of crude glycerine addition over mesophilic specific activity of the mesophilic anaerobic biomass, specific substrate activity tests were performed using the initial inoculum (In-P_M0) and the digestate sampled at the end of each co-digestion period of the continuous mesophilic trial (In-P_MI and In-P_MII). Acetate, hydrogen, propionate and a mixture of butyrate/valerate were the substrates used to study said specific activity. The maximum slope (R_m) of the net accumulated methane curves (Figure 6.4) and the observed lag phase (λ) were the parameters used to compare the different specific substrate activities (Table 6.4).

In-P_MI showed a specific acetate activity similar to In-P_M0 (initial inoculum), while In-P_MII showed a specific acetate activity (98 mg $_{COD\text{-}CH4}\cdot g_{VSS}^{-1}d^{-1}$) lower than In-$P_M$I. The lower activity of the acetoclastic methanogenic population found in In-P_MII could be related to the high C/N ratio of the mixture applied during period P_MII (87) which curbs the growth of anaerobic biomass. Likewise, the H_2 activity test of In-P_MI and In-P_MII showed a higher maximum slope (R_m) compared to the initial inoculum (In_M0); R_m of In-P_MI was 113 $mg_{COD\text{-}CH4}\cdot g_{VSS}^{-1}d^{-1}$ and 75 $mg_{COD\text{-}CH4}\cdot g_{VSS}^{-1}d^{-1}$ for In-P_MII (Table 6.4). This shift towards hydrogenotrophic methanogenesis was also reported by Tokumoto and

Tanake (2012a) and Tokumoto and Kashiwagi (2012b). The authors also observed an increase in CGY biodegradability when mixed with sewage sludge. The presence of monosaccharides, specifically glucose, coming from the cell-wall of the SS, was hypothesised to be responsible of such biodegradation stimulation (Tokumoto and Tanake, 2012a).

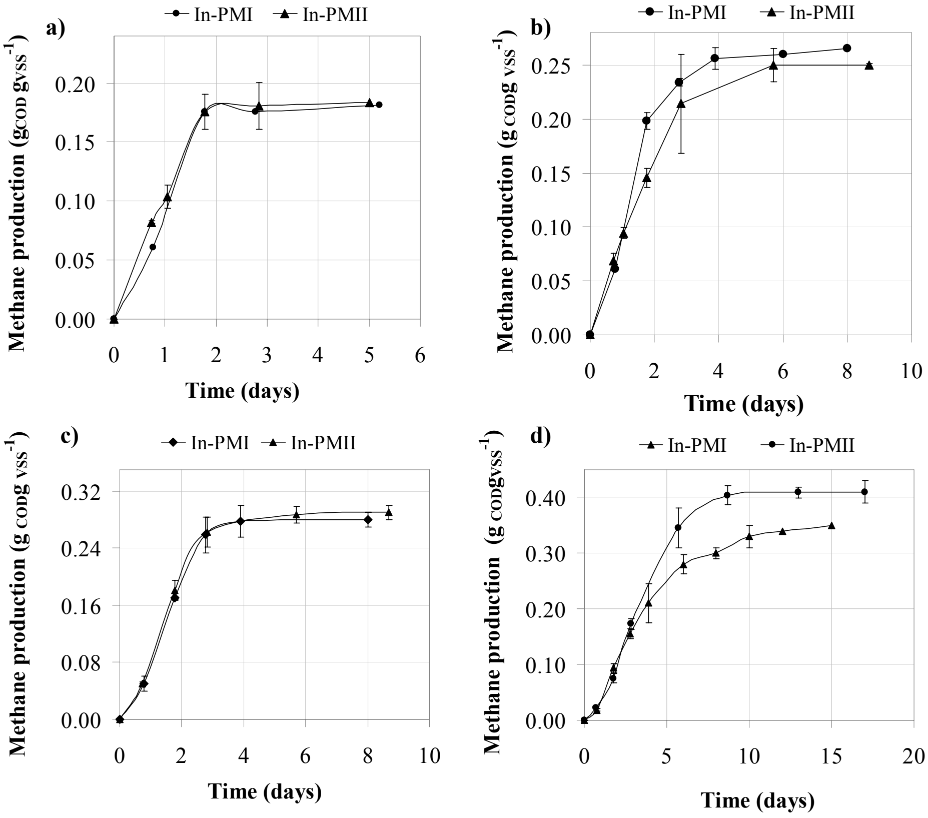

Figure 6. 5. *Evolution of accumulated methane during specific activity tests over (a) acetate, (b) hydrogen, (c) propionic and (d) butyric/valerate.*

Propionate and butyrate/valerate activity tests of In-P_MI and In-P_MII showed similar profiles, no lag phase and a similar R_m. On the contrary In-P_M0 showed a lower R_m and a 3 day lag phase for propionate activity.

This specific activity tests showed that the addition of CGY induces an increase in specific biomass activity compared to the mono-digestion of

SS and the co-digestion of SS and trapped grease waste, performed in the same reactor and with the same SS, reported in Silvestre *et al.* (2011)[1]. CGY degradation resulted in more H_2 and propionate production and, as a consequence, hydrogen and propionate activity in the biomass is promoted. Similarly, a higher specific activity of butyrate/valerate degraders in In-P_MII, and to a lesser extent in InP_MI, could be explained by a higher C/N of the co-digestion mixture, which induced an increase of butyrate yield and as a consequence promoted this specific activity as suggested by Fu *et al.* (2012).

Table 6. 4. *Specific activities of the mesophilic inocula over hydrogen, acetate, propionate, and butyrate/valerate Note: maximum slope (R_m) is expressed in $mg_{COD\text{-}CH4}\ g_{VSS}\cdot d^{-1}$ and lag phase (λ) is expressed in days (d).*

Inoculum	**C/N feed**	**Acetate**		**Hydrogen**		**Propionate**		**Butyrate/ valerate**	
		R_m	λ	R_m	λ	R_m	λ	R_m	λ
In-SS[*]	10	21 ± 1	0.4	30 ± 1	1	-	-	60 ± 6	1
In-P_M0	14	132 ± 33	0	22 ± 5	0	14 ± 4	3	63 ± 4	0
In-P_MI	50	131 ± 15	0	113 ± 4	0	97 ± 7	0	57 ± 1	0
In-P_MII	87	98 ± 3	0	75 ± 16	0	97 ± 8	0	63 ± 6	0

6.3.5. Dewatering properties

Sludge dewaterability was measured with the filterability and centrifugability test of the digested samples collected at the end of each mesophilic co-digestion period P_MI and P_MII, as well as the initial effluent (P_M0), and the SS used as influent. Figure 6.6 shows the filterability and centrifugability curves of the different samples. As can be seen, dewaterability was worse in the mesophilic SS-CGY digestates (P_MI and P_MII) than in the initial effluent (P_M0), and in raw SS.

Dewatering properties have been related to many factors, such as particle size, specific surface area, density, bound water, and pH, among others; but the concentration of extracellular polymeric substances (EPS) is widely accepted as the main factor. EPS are metabolic products of

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[1] Chapter 4

microorganisms that influence the aggregation of bacterial cells in flocs or biofilms (Laspidou and Rittmann, 2002).

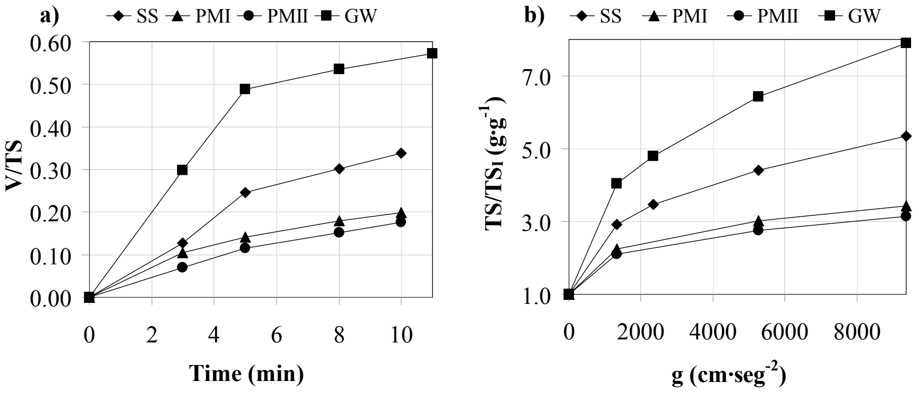

Figure 6. 6. *Dewatering properties of the mesophilic effluent: (a) filterability test; and (b) centrifugability test (SS: effluent of SS anaerobic mono-digestion; GW: initial effluent that corresponds to the SS-GWco-digestion period;* P_M*I: effluent obtained at the end of* P_M*I;* P_M*II: effluent obtained at the end of* P_M*II).*

Houghton and Stephenson (2002) reported that EPS composition varied after sludge digestion and was also affected by the composition of the feed, attributing an excess in EPS production to acidogenic bacteria. These bacteria can modify the cellular growth pathway towards EPS production, for example, when there is insufficient nitrogen for protein synthesis in a medium with a high C/N ratio, which is exactly the case in the CGY co-digestion periods, or when there is an increase in the food to microorganism ratio (Sheng *et al.*, 2010).

The great increase in C/N ratio, when adding CGY to the SS anaerobic digester, underpins the hypothesis that a higher EPS production is caused and hence an increased resistance to dewatering of SS-CGY digestates. This fact should be taken into account as worsening the dewatering properties of the digestate could have dramatic effects over WWTP economics.

6.4. CONCLUSIONS

Thermophilic anaerobic co-digestion of SS with CGY showed great instability. The extreme pH values of CGYs together with a swift release of VFA, causes VFA accumulation and a drop in pH. The process, at mesophilic range, performs steadily with an increase in methane production of 148% (CGY 1% v/v). Further CGY addition does not show any improvement; the biomass shift due to a high C/N ratio could explain this behaviour. Results suggested that CGY can be used as co-substrate in SS anaerobic digestion but, depending on CGY characteristics and operational temperature, different parameters should be taken into account to operate steadily.

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CHAPTER 7

OPTIMISATION OF SEWAGE SLUDGE ANAEROBIC DIGESTION THROUGH CO-DIGESTION WITH OFMSW: EFFECT OF COLLECTION SYSTEM AND PARTICLE SIZE

The effect of organic fraction of municipal solid waste (OFMSW) loading rate and particulate size on the sewage sludge (SS) mesophilic anaerobic co-digestion was assessed in continuous stirred tank reactor at hydraulic retention time of 20 days. The SS-OFMSW mixture composed by 51% of the COD fed (%54 volatile solids fed (inlet-VS)), at OLR of 3.1 $kg_{COD}\cdot m^{-3}\cdot d^{-1}$ showed the highest increment on the methane production and yield +200% and +59% respectivaly, under stable conditions. The effect of the particulate size was assessed with the same mixture and same operational conditions but reducing the OFMSW particulate size from 20 mm to 8 mm with the aim to improve the hydrolysis step, but the results showed any influence in the OFMSW particulate size range analyzed. In addition, specific biomass activity was assessed at the end of each co-digestion period. Results showed that OFMSW promoted β-oxidation syntrophic acetogens and acetoclastic methanogens activity; although the last increase of the OFMSW percentage (from 47% to 54% inlet-VS) affected negatively the specific substrate activity, but not inhibitory effect was observed. Therefore, the results obtain in the continuous experiment could be related with some inhibitory or toxic effect and not due to hydrolysis limitation. The specific biomass activity test was demostrated to be an interesting tool to evaluate and control the co-digestion process, especially when conventional parameters did not explain the behaviour of the biological system.

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7.1. INTRODUCTION

Nowadays, management of organic waste is receiving a renewed interest due to the global warming issue and more stringent environmental legislation framework. Two of the organic wastes produced in more quantities are the organic fraction of municipal solid waste (OFMSW) and the sewage sludge (SS) from wastewater treatment plants (WWTPs). Mesophilic anaerobic digestion is a well-established technology and extensively applied to SS treatment, typically in continuously stirred tank reactor (CSTR). Although currently biogas production is usually maximized in many WWTPs, by applying codigestion or pretreatments strategies, it is still necessary to study and to develop strategies to improve the biogas yield and balance its energy demands.

The anaerobic co-treatment of organic wastes, so called co-digestion, is not often found in SS treatment facilities even it is a common practice with agro-industrial wastes (Mata-Alvarez *et al*., 2015, Long *et al*., 2012). The objective is to increase gas yield based on an improved composition of the influents, since the co-substrate usually are complentary to the major waste in most of cases, or due to increased organic loading rate without changing the retention time. In the SS and OFMSW case, SS provides adequate micro/macronutrients, alkalinity and moisture content, but a low carbon-nitrogen (C/N) ratio and methane yield that are balance with OFMSW, which is characterized by its high solid concentration and high C/N ratio. The benefits on the increase of the biogas production has been reported by several researches using, mainly, temperature phased anaerobic digestion configuration (Gómez *et al*., 2006; Stroot *et al*., 2001; Habiba *et al*., 2009; Heo *et al*., 2005).

Although the benefits of SS-OFMSW codigestion, their full-scale application shows several limitations: complex regulatory framework, conditioning step (particulate size reduction, inert separation, etc.), high variability of the OFMSW characteristics that depend on several factors as management practices (as collection frequency or inert content (IP)) and inhibitory effects (volatile fatty acids, metals, long chain fatty acids, ammonia, etc.) of the degradation process (Iacovidou *et al*., 2012). At lest, a homogenization and particle size reduction of the OFMSW as conditioning step is need for the SS-OFMSW codigestion case; so, these operations usually are performed in parallel, being mechanical pretreatments as a rotory drum, screw press, disc screen shredder, or piston press treatment the most analyzed at full-scale (Ariunbaater *et al*., 2014). Mechanical pretreatments showed the advantages of moderate energy consumption and other advantages, as no odoor generation, besides easy implementation. In addition, the mechanical pretreatment of OFMSW showed an increase on biogas yields between 20-40% as compared to the untreated substrates (Ariunbaater *et al.*, 2014).

In this context, few studies found in literature have dealt with the effect of OFMSW particulate size onto the SS anaerobic co-digestion, but the main conclusions pointed out that the reduction of particle sice increased both the kinetics and methane yield of the co-treatment process (Sharma *et al.*, 1988; Izumi *et al.*, 2010). Izumi *et al.* (2010) reported a methane yield increase of +28% when the mean particulate size (MPS) decreased from 0.89 to 0.72 mm, but when the MPS was decreased below 0.51 mm, the methane yield decreased due to the high VFA accumulation in the anaerobic digester. Esposito *et al.* (2011) modelled the OFMSW particulate size effect during the anaerobic co-digestion with SS, within a MPS range between 50-2.5 mm. The results showed a direct correlation between OFMSW particulate size, the organic loading rate (OLR) and the rate of disintegration and acidification.

Biological process as continuous anaerobic systems are commonly evaluated in terms of gas yield and organic matter removal and related to the corresponding operational conditions (McMahon *et al.*, 2004), as feedstock composition. But changes in the feed also induce changes in the microorganisms involved in the process (Demirel & Yenigün, 2006), that might be quantified as a change in quantity or in specific activity. The study of this population dynamic by means of specific activity tests of the main anaerobic microbes was proposed in this work as a new tool to select the procedure of co-substrates addition or the change in particle size of substrates along the SS-OFMSW co-digestion.

The aim of this work was to study the effect of OFMSW and SS co-digestion on the biogas yield and on the stability of the process (VFA profile and concentration, alkalinity ratio, biogas composition, etc.). In addition, the performance of the process was analyzed by means of the specific substrate activity of the anaerobic biomass along the co-digestion implementation. Besides, the effect of the OFMSW particulate size on the co-digestion process was analysed.

7.2 MATERIAL AND METHODS

7.2.1. Experimental design

In the first step, the physic-chemical characterisation, the maximum methane potential (MP_{VS}; $NL_{CH4}·kg_{VS}^{-1}$) and anaerobic biodegradability (AB; %COD) at 35ºC, of representative samples of SS and three different OFMSW samples were determined by anaerobic biodegradability tests (ABT), using these values as criteria to select the OFMSW to be co-digested with SS.

In a second step, continuous experiment with lab-scale digester was carried out along 262 days increasing gradually the OLR by adding higher OFMSW amounts, but maintaining a fixed the hydraulic retention time (HRT) at 20 days. Within this approach, three mixtures SS-OFMSW were tested, corresponding to periods PI, PII and PIII. Since SS was twice a month collected in the WWTP and the corresponding VS content was variable, it was decided not to fix the SS amount, but maintaining a constant volatile solids (VS) concentration (25 $g_{VS}·kg^{-1}$) in the influent during the co-digestion experiments. The reference values for comparison purposes during the continuous experiments were obtained by operating the digesters solely with SS with an HRT of 20 days, which was called period P_{SS} (Silvestre *et al.*, 2011)[1].

Finally, in a third step, the effect of particle size of OFMSW was studied: the digester was operated in the best conditions found in the second step but the OFMSW particulate size was reduced from 20mm to 8mm. The effect of the particulate size was evaluated firstly in batch (ABT test) and then continuously (CSTR configuration), along 87 days (period PIV).

The continuous experiments of the second and third steps were evaluated by means of volumetric methane production ($Nm_{CH4}·m^{-1}d^{-1}$) and methane yield (MY_{VS}; $NL_{CH4}·kg_{VS}^{-1}$), besides common control parameters as organic matter (volatile solids (VS) and chemical oxygen demand (COD)) removal efficiency, VFA concentration and profile, alkalinity ratio and biogas composition. It was defined as "stable condition" that condition at which the organic matter removal is above 60% COD, the total VFA concentration is lower than 100 $mg·L^{-1}$, the alkalinity ratio is below 0.3 and methane content is above 60% v/v biogas. In addition, the specific activity tests were performed with inocula

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[1] Chapter 4

sampled at the end of each SS-OFMSW co-digestion period to evaluate the evolution of main anaerobic populations.

7.2.2 Waste collection and characterization

Fresh SS samples were collected in a WWTP (Barcelona, Spain) from the incoming flow of its mesophilic digester, and represented the bigger quantity of the co-digestion mixtures in weigh basis. The SS was composed by a mixture of 70% primary sludge and 30% waste actived sludge. During continuous experiment, SS was sampled and characterized every two weeks, and kept in the fridge at 4ºC till being used.

The three OFMSW samples ($OFMSW_1$, $OFMSW_2$ and $OFMSW_3$) were taken from different municipal solid waste treatment facilities (Barcelona, Spain) with different collection system (Table 7.1): $OFMSW_1$ was collected from a door-to-door collection system, while the $OFMSW_2$ and $OFMSW_3$ were collected from surface containers. $OFMSW_1$ contained fruit (mainly orange peels), vegetable waste, with an inert content of 2% weigh, while $OFMSW_2$ and $OFMSW_3$ were composed by a mixture, mainly garden waste. Illustrations of the OFMSWs samples used in this study are showed in the Figure 7.1. All samples were shredded with an industrial meat mincer till a particle size of 20 mm, previously to their characterization (Figure 7.2). All OFMSW samples were kept frozen till being used.

The mesophilic anaerobic inoculum used for anaerobic biodegradability test was obtained from the anaerobic digester of the same WWTP as the collected SS, while the mesophilic inoculum for continuous experiments came from own lab-scale reactors, previously operated during 150 days with several SS-glycerol mixtures.

Figure 7. 1. *Illustration of the three OFMSW analyzed (a) $OFMSW_1$, b) $OFMSW_2$, c) $OFMSW_3$*

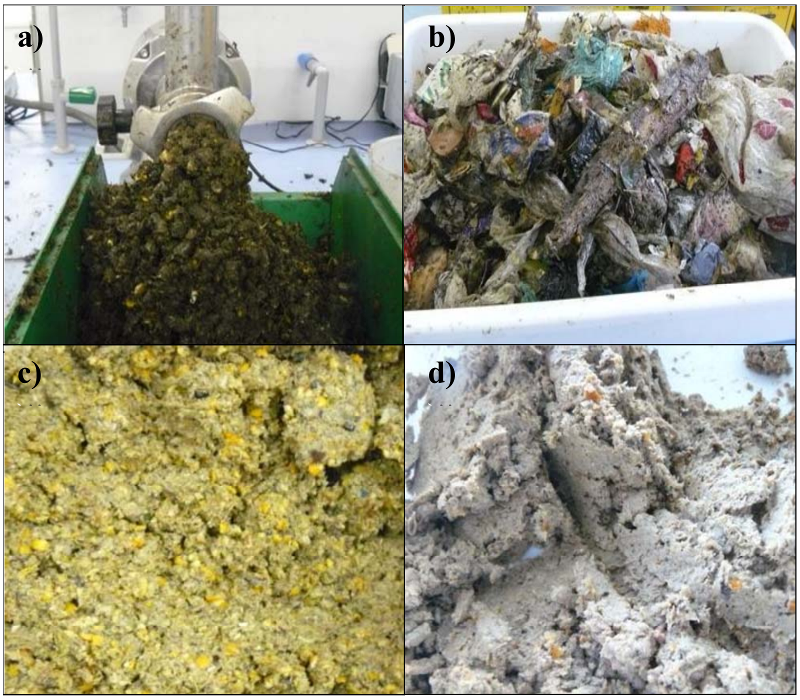

Figure 7. 2. *Illustration of a) reduction particulate size in a meet mincer; b)inerts content on the OFMSW samples; c) OFMSW$_1$ after the particulate size was reduced to 20mm; d) OFMSW$_1$ after the particulate size was reduced to 8mm;*

Total solids (TS), VS, total suspended solids (TSS), volatile suspended solids (VSS), total Kjeldahl nitrogen (TKN), total ammonia nitrogen (NH_4^+-N), total and partial alkalinity (TA, PA), sulphate (SO_4^{2-}-S) and phosphate (PO_4^{2-}-P) were determined following the Standard Methods (APHA, AWWA, WEF, 1995). Total COD (CODt) was determined following COD solid methodology (Noguerol-Arias *et al*., 2012). The C-N ratio was calculated by determining the elemental composition (carbon (C), nitrogen (N) and hydrogen (H)), by catalytic oxidation combined with gas chromatography (LECO instruments).The methane and H_2 composition of biogas, and volatile fatty acids (VFA) (acetate, propionate, i-butyrate, n-butyrate, i-valerate, n-valerate, i-caproate and n-caproate acids) concentrations were determined by gas chromatography (TCD and FID detectors, respectively), as described elsewhere (Silvestre *et al*., 2011)[1].

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[1] Chapter 4

7.2.3 Batch tests: biodegradability and activity assays

Two different anaerobic batch test were carried out: ABT, following Field *et al.* (1988) and Silvestre *et al.* (2011)[1], to determine the AB (%initial COD) and the MP_{VS} ($NL_{CH4}\cdot kg_{VS}^{-1}$) of collected wastes, and specific activity test (SAT) assays of inocula collected in the continuous system.

ABT was carry out in duplicate in glass vials of 1.2 L of capacity, with a media volume of 0.5 L composed by a mixture of inoculum (5 $g_{VSS}\cdot L^{-1}$) and substrate (5 $g_{COD}\cdot L^{-1}$) at mesophilic temperature. The SAT were done in duplicate on vials of 0.12 L of capacity, filled with 0.05 L of liquid media composed by a mixture of inoculum (5 $g_{VSS}\cdot L^{-1}$) and a specific substrate: acetic acid (10 mM), hydrogen (4.1 mM), propionate acid (6.0 mM) and a mixture 1:1 of iso-butyrate: iso-valerate acids (6.2 mM). The inocula for SAT were stored at 35ºC during 2 days before running the tests in order to ensure the degradation of residual organic matter. Besides this, all media contained macro and micronutrients and bicarbonate (1 $g_{NaHCO3}\cdot g_{COD}^{-1}$), and bubbled with N_2 gas to remove the oxygen. Blanks, or media without substrate were also prepared.

Regarding SAT assays, the activity over a specific substrate was defined as the maximum slope of net produced methane rate per VSS-inoculum, following Soto *et al.* (1993) calculations, which allowed to calculate the lag phase (λ; days) and the maximu slope (Rm, $g_{COD-CH4}\cdot g_{VSS}^{-1}\cdot d^{-1}$).

In both types of assays, the mean methane production from blanks was subtracted to the mean methane production of vials to calculate the net methane volume, which was expressed under normal conditions (0 ºC, 1 atm). The experiment was considered finished when the difference between consecutive measurements of the daily methane production was lower or equal to 5%.

7.2.4 Continuous digesters set-up

The continuous experiment was carried out in a jacket glass cylindrical tank of 5.5L of working volume, provided with a mechanical stirrer and a volumetric gas flow meter (*Ritter Apparatebau GMBH & CO KG*) as described elsewhere (Silvestre *et al.*, 2014)[2]. The HRT was fixed in 20 days since this is the common value in SS digestion in WWTP (Silvestre

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[1] Chapter 4

[2] Chapter 5

et al., 2015)[1]. Reactors were fed with SS twice a day with a temporized peristaltic pump while the OFMSW was fed manually once a day. The SS was sampled in the same WWTP along the trial: the ratio primary and secondary was mantained in same proportion and COD of the SS was kept 45 $g_{COD}·m^{-3}$ by addition of deionised wate, to minimize the change in the sludge composition. The analytical parameters of the effluent and influent were measured once a week (TS, VS, COD, NH_4^+-N, VFA, alkalinity ratio, pH), except the biogas composition that was measured twice a week, and the control parameters were calculated with week mean values. Those intervals with operational problems, such us obstructions, pumps miscalibration, temperature drop, etc., were not taken into account in the calculations.

Theoretical methane potential (TMP) of the different mixtures assessed in the continuous experiment was estimated from the MP_{VS} of solely substrates, obtained by means of ABT experiments, and the proportion of each substrate in the mixture. The ratio between the MY_{VS} obtained in continuous experiment and TMP (α) was calculated for each period. It was defined that when $\alpha > 1$, a synergist degradation of mixtures is observed.

7.3. RESULTS AND DISCUSSION

7.3.1 Co-substrate selection

The composition of the different substrates is shown in Table 7.1. The SS was characterized by a low C-N ratio, VS and COD concentration than the OFMSW samples. Therefore, the addition of OFMSW during SS anaerobic digestion improved the nutrients balance and might increasing the metabolic activity of the biomass (Macias-Corral *et al.*, 2008), since the sampled OFMSW showed C-N ratio between 14-21 and an organic N-related compounds content of 82-86% TKN. The C/N ratio of the OFMSW samples was within the optimal range 15-30 for the growth and activity of anaerobic biomass in general (Mshandete *et al.*, 2004; Kayhanian and Hardy, 1994) and also inside the optimal range 16-19 suggested by Kivaisi and Mtila (1998) to reach an optimal methanogenic performance.

Another important factor when improving the methane yield of SS anaerobic degradation is methane content. In this case, the COD/SO_4^{2-} ratio in the OFMSW samples was higher than the reported values (706-1,474 $g_{COD}·g_{SO4}^{-1}$) at which a substrate competition between methanogens

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[1] Chapter 2

and sulphate reducing bacteria usually begins (Choi and Rim, 1994); so little depletion of acetate by sulphate reducing bacteria was expected.

The main difference among the three OFMSW was the IP, which could result in hydrodynamic problems inside the reactor or blockage of the pumping system (Krupp *et al.*, 2005). A source collection system is strongly recommended when anaerobic digestion wants to be implemented to treat OFMSW, although it might increase considerable the cost of waste management (Bolzonella *et al.*, 2006). Regarding the sampled OFMSW, those collected from surface containers system ($OFMSW_2$ and $OFMSW_3$) showed higher IP content that the sample collected from the door-to-door system ($OFMSW_1$) (Table 7.1).

Table 7. 1. *Characterization of OFMSW samples and sewage sludge SS. Note: ^{1}IP: impurities content (weight basis); ^{2}AB: anaerobic biodegradability; $^3MP_{VS}$: maximum methane potential on basis volatile solids*

Parameters	**Units**	**$OFMSW_1$**	**$OFMSW_2$**	**$OFMSW_3$**	**SS**
Collection system		door-to-door collection system	surface containers	surface containers	-
TS	$g \cdot kg^{-1}$	259 ± 8	283 ± 9	422 ± 13	33 ± 5
VS	$g\ kg^{-1}$	247 ± 5	220 ± 5	300 ± 6	23 ± 4
COD	$g\ kg^{-1}$	385 ± 12	393 ± 12	641 ± 19	44 ± 8
C-N	$g\ g^{-1}$	17 ± 2	14 ± 1	21 ± 4	10 ± 2
TKN	$mg\ kg^{-1}$	6026 ±60	7503 ± 225	7331 ± 733	2000 ± 294
NH_4^+-N	$mg\ kg^{-1}$	870 ±4	1180 ± 6	1344 ± 7	841 ± 109
Norg	% NKT	86	84	82	58
SO_4^{2-}-S	$mg\ kg^{-1}$	545 ± 6	308 ± 4	435 ± 5	19 ± 0
COD/SO_4^{2-}-S	$g_{COD} \cdot g_{SO4}^{2-}$	706	1276	1474	2316
^{1}IP	%	2	18	11	-
^{2}AB	(%COD)	90 ± 10	73 ± 11	81 ± 14	67 ± 1
$^3MP_{VS}$	$NL_{CH4} \cdot kg_{VS}^{-1}$	460 ± 20	366 ± 55	412 ± 3	296 ± 6

Regarding biodegradability, SS has a biodegradability of 67% CODt and a MP_{VS} of 298 $NL_{CH4} \cdot kg_{VS}^{-1}$. The accumulated net methane curves (Figure 7.3a) of the three OFMSW with a particle size of 20 mm showed similar trends: no lag phase was observed and moreover, the 80% MP_{VS}

was reached within in the first 5 days of experiment. The MP_{VS} were 1.5, 1.2 and 1.5 higher in $OFMSW_1$, $OFMSW_2$ and $OFMSW_3$, respectively, than in the SS (Table 7.1), while AB of $OFMSW_1$ was the highest of the three samples, 90%.

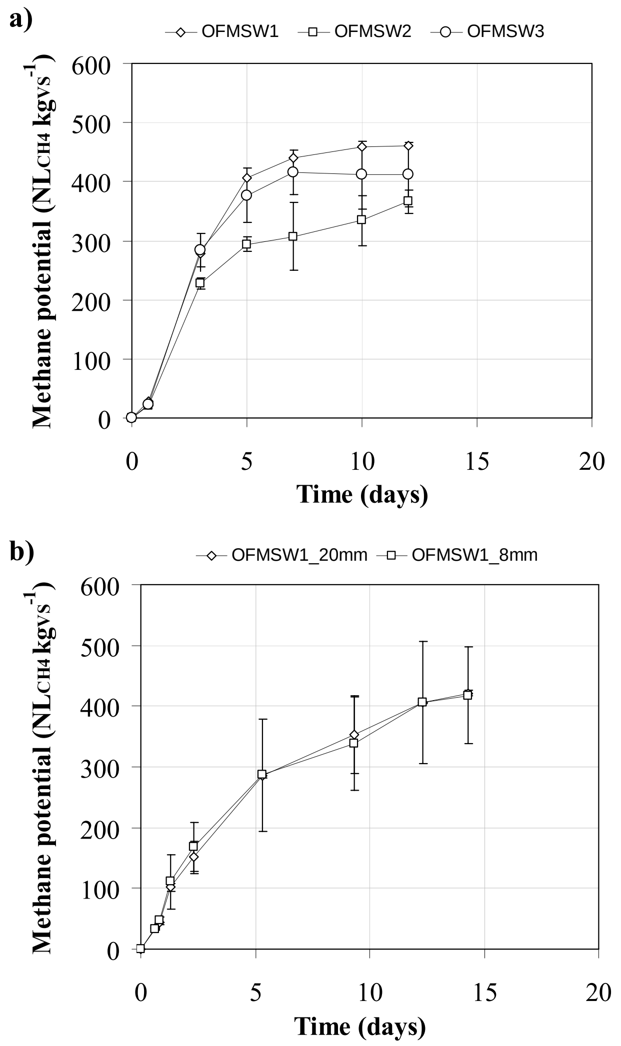

Figure 7. 3. *(a) Evolution of maximum methane potential on VS basis (MP_{VS}), and (b) $OFMSW_1$ sample crushed at 8 mm and 20 mm particulate size.*

Based on the experimental characterisation and ABT data, the door-to-door collected $OFMSW_1$ was selected for co-digestion purposes: higher MP_{VS} and anaerobic biodegradability, probably due to its lower IP content (Table 7.1). As secondary criteria, the anaerobic digesters of WWTP facilities are designed to manage SS with TS content between 3-6% (Silvestre *et al.*, 2015)[1] and no IP, so $OFMSW_1$ also fitted better general operational parameters of WWTP, as well as represented lower investment for implementation.

7.3.2 Continuous experiment

Effect OFMSW loading

The results obtained during the SS-$OFMSW_1$ co-digestion were compared with the mesophilic anaerobic digestion with SS alone period (P_{SS}), which was defined by a HRT of 20 days and OLR of 2.2 $kg_{COD}·m^{-3}·d^{-1}$. At the end of P_{SS}, the organic removal, the methane production and the MY_{VS} were 35% CODt, 0.25 $NL_{CH4}·L^{-1}·d^{-1}$ and 249 $NL_{CH4}·kg_{VS}^{-1}$, respectively (Table 7.2), while the SAT of the biomass in this period (In_{SS}) is shown in Table 7.3 and were used for comparison purposes between different digester´s performance with SS mixtures. The In_{SS} results were extensively described and discussed in Silvestre et *al.* (2011)[2].

Three mixtures SS-OFMSW were studied in mesophilic continuous co-digestion: $OFMSW_1$ represented the 23% (PI), 44% (PII) and 51% (PIII) on inlet COD basis. The starting OLR (2.2 $kg_{COD}·m^{-3}·d^{-1}$) was progressively increased till 2.3 $kg_{COD}·m^{-3}·d^{-1}$, 2.8 $kg_{COD}·m^{-3}·d^{-1}$ and 3.1 $kg_{COD}·m^{-3}·d^{-1}$ along the periods PI, PII and PIII, respectively. The operational conditions and control parameters are shown in Table 7.2.

The volumetric methane production (Figure 7.4) increased when increasing the amount of $OFMSW_1$, till 0.39 (PI), 0.65 (PII) and 0.75 (PIII) $Nm^3_{CH4}·m^{-3}·d^{-1}$. The MP_{VS} also increased +25%, +54% and +59% with PI to PIII. In all mixtures analyzed, the VFA concentration remained stable (<100 $mg·L^{-1}$), alkalinity ratio showed adequate values and NH_4^+-N concentration was closer to the optimal concentration of 2.1-3.1 $gN·L^{-1}$ (Procházka *et al.*, 2012).

The decrement of CH_4 content in the biogas, as $OFMSW_1$ quantities increased, cannot be attributed to instability process. The higher carbohydrates concentration of $OFMSW_1$ in comparison with SS explained the decrease of the methane content of the biogas .These results

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[1] Chapter5

[2] Chapter 4

are similar to those previously compiled from literature dealing with SS-OFMSW co-digestion. Heo *et al*. (2003) showed a decreased in the methane composition from 85% to 50% when a mixture of food waste (FW) was increased from 10% (VS basis) to 50% during SS co-digestion. Dai *et al*. (2012) reported similar results, reducing the methane composition from 65% to 54% during SS-FW anaerobic co-digestion process.

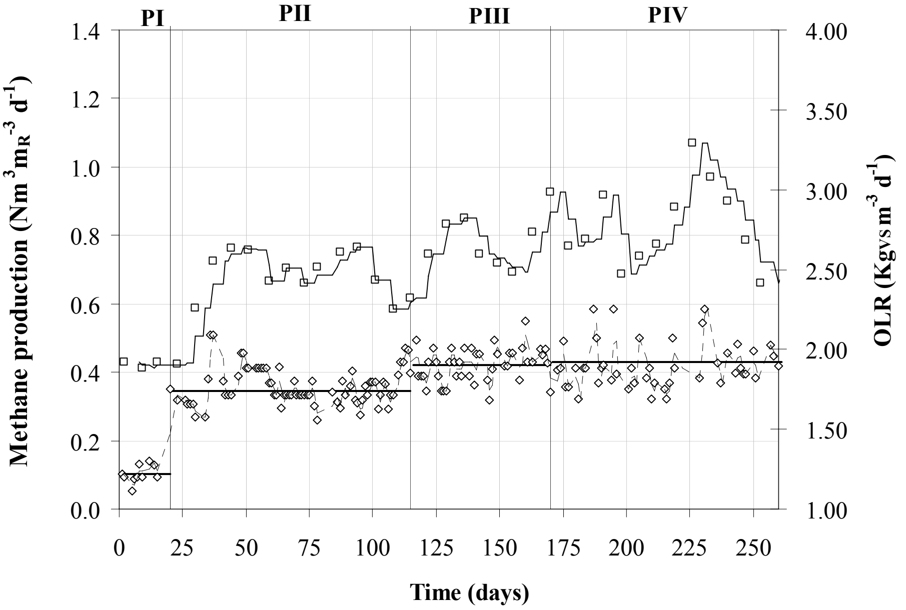

Figure 7. 4. *Evolution of the organic loading rate (OLR) (daily values) and volumetric methane production (weekly average). Notation: square-VMP, rhombus-OLR.*

TMP of the different SS-OFMSW$_I$ mixtures used in the continuous experiment was estimated from the MY$_{VS}$ of SS and OFMSW$_I$ in batch experiments, and the wastes proportion of each mixture. The MY$_{VS}$ obtained in the continuous experiment with SS alone, PI, PII and PIII corresponded with α values of 0.84, 1.00, 1.25 and 1.39, respectively, which suggested a synergism effect mainly during periods PII and PIII.

Table 7. 2. *Operation and process parameters of the mesophilic continuous SS /OFMSW1 codigestion.(Increments were calculated regarding period PSS)*

Parameters	**Units**	$\mathbf{P_{SS}}$	**Period I**	**Period II**	**Period III**	**Period IV**
$\mathbf{OFMSW_{1addition}}$	$\%VS_{in}$	0	32 ± 7	47 ± 4	54 ± 2	54 ± 6
$\mathbf{OFMSW_{1addition}}$	$\%COD_{in}$	0	23 ± 5	44 ± 12	51 ± 13	52 ± 14
$\mathbf{HRT^1}$	days	20 ± 3	20 ± 1	20 ± 1	22± 1	21± 2
$\mathbf{OLR_{COD}}^1$	$kg_{COD}\cdot m^{-3}d^{-1}$	2.2 ± 0.7	2.3 ± 0.2	2.8 ± 0.30	3.1 ± 0.1	3.0 ± 0.3
$\mathbf{OLR_{VS}}^1$	$kg_{VS}\cdot m^{-3}d^{-1}$	1.5 ± 0.6	1.3 ± 0.1	1.7 ± 0.2	1.9 ± 0.1	1.7 ± 0.2
$\mathbf{CH_{4production}}^1$	$Nm^3\cdot m^{-3}d^{-1}$	0.25 ± 0.07	0.39 ± 0.01	0.65 ± 0.06	0.75 ± 0.07	0.77 ± 0.12
$\mathbf{CH_{4production}}$ ***incr***	*%* $CH_{4production}$ *-SS*	-	*56*	*160*	*200*	*208*
$\mathbf{CH_{4yield}}^1$	$NL_{CH4}\cdot kg_{VS}^{-1}$	249 ± 80	311 ± 22	383 ± 45	395 ± 41	420 ± 27
$\mathbf{CH_{4yield}}$ ***incr***	*%* CH_{4yield} *-SS*	-	*25*	*54*	*59*	*69*
$^4\alpha$	*%* CH_{4yield}	*0.084*	*1.0*	*1.250*	*1.390*	*1.490*
% $\mathbf{CH_4}^3$	%v/v (biogas)	72 ± 3	66 ± 0	64 ± 1	64 ± 2	64 ± 5
$\mathbf{VFA^2}$	$mg\cdot L^{-1}$	<100	<100	<100	<100	<100
$\mathbf{NH_4^+\text{-}N^2}$	$mgNH_4^+\cdot L^{-1}$	700 ± 100	-	1060 ± 81	1077 ± 93	1114 ± 271
$\mathbf{TA^3}$	$g\ CaCO_3\ L^{-1}$	5.7 ± 1.1	3.8 ± 0.0	4,3 ± 0,4	3.8 ± 0.5	3.7 ± 0.2
$\mathbf{PA^3}$	$g\ CaCO_3\ L^{-1}$	3.6 ± 0.9	2.9 ± 0.1	3.4 ± 0.4	3.3 ± 0.4	3.0 ± 0.3
$\mathbf{pH^3}$	-	7.8 ± 0.4	7.3 ± 0.0	7.5 ± 0.2	7.8 ± 0.2	7.5 ± 0.2
$\mathbf{COD_{removal}}^2$	%	35 ± 13	50 ± 4	58 ± 23	68 ± 6	73 ± 9
$\mathbf{VS_{removal}}^2$	%	36 ± 7	57 ± 2	67 ± 6	70 ± 4	76 ± 9
$VS_{removal\ incr}$	*%VS removal-SS*	-	*58*	*86*	*161*	*111*

Note: Average and S.D of the daily (^{1}), weekly (^{2}) or twice week (^{3}) samples of each period

This synergism phenomena had been previously referred during the co-digestion of different wastes (Jianzheng and Ajay, 2011; Li *et al.*, 2009; Macias-Corral *et al.*, 2008; Pagés *et al.*, 2011), mainly explained due to micronutrients balance, promoting higher concentration and activity of the anaerobic biomass, and as a consequence, higher removal rates and gas production. The observed improvement of activity was shown within the SAT tests (Table 7.3 and Figure 7.5), by means of the maximum slope and lag phase (λ). The SSA was assessed with inocula taken at the end of periods PII (In2), and PIII (In3). The results were compared with the activity of the biomass at the starting point of period (In0) and with the activity of the inoculum collected during P_{SS} (InSS).

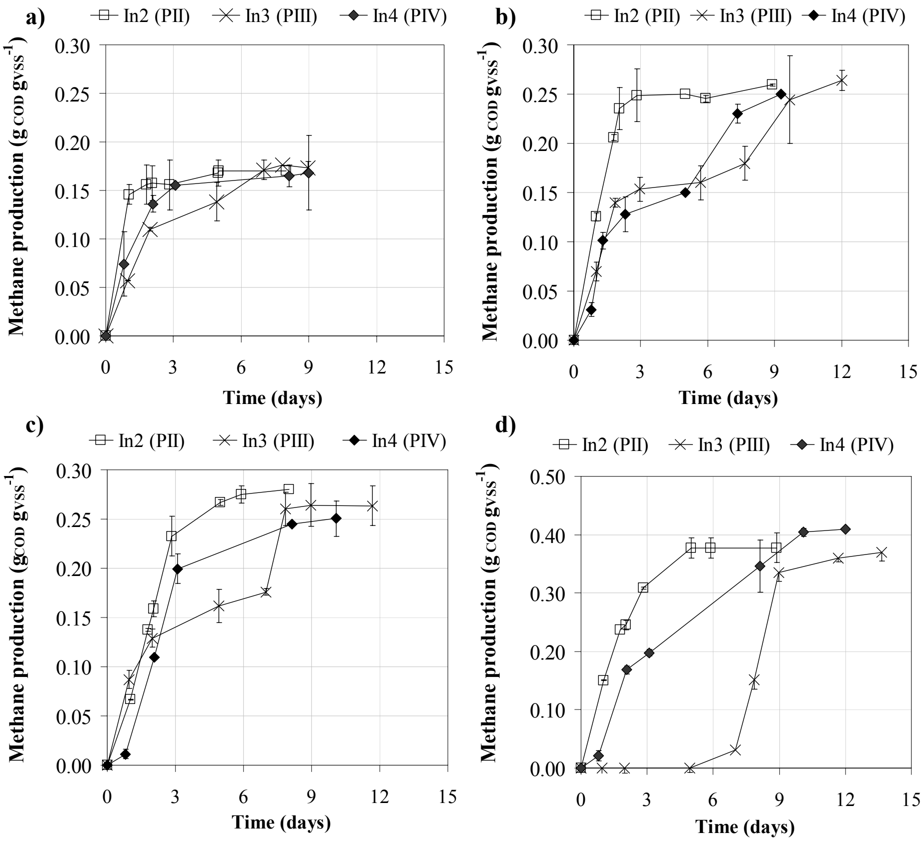

Figure 7. 5. *Methane curves obtained in the specific activity test: (a) acetate acid, (b) hydrogen, (c) propionate acid and (d) butyrate and valerate acids.*

The SAT of In2 was +1.5, +1.5 and +1.7 times the specific activity over acetic, hydrogen and butyric/valeric than that of the starting inoculum (In0) respectivaly. The adjustment of the C-N ratio after the $\mathrm{OFMSW_1}$ addition, from a high C/N (80) during glycerol co-digestion, as mention before the reactor was fed with SS-glycerine, promoted higher activity of the anaerobic biomass. Also, the inoculum In2 showed the highest activity over acetic, hydrogen and butyric/valeric, in comparison with the activities obtained during other SS co-digestion mixtures, for example with trapped grease waste (Silvestre *et al.*, 2011)[1].

Table 7. 3. *Specific activities of the mesophilic inocula over acetate, hydrogen, propionate, and butyrate/valerate Note: maximum slope* (R_m) *is expressed in* $\mathrm{mg_{COD\text{-}CH4}\ g_{VSS}{\cdot}d^{-1}}$ *and lag phase* (λ) *is expressed in days (d). * obtained while co-digesting SS and glycerol*

		Period				
Parameters	**Units**	**P0**[1]	**P_{SS}** [2]	**PII**	**PIII**	**PIV**
OLR	$\mathrm{kg_{COD}{\cdot}m^{-3}d^{-1}}$	-	2.2	2.9	3.2 2	3.3
$\mathrm{OFMSW_1}$	$\mathrm{\%VS_{inlet}}$	-	0	47	54	54
Inocula	*abbreviation*	*In0*	*InSS*	*In2*	*In3*	*In4*
Rm_Acetate	$\mathrm{mg_{DQO\text{-}CH4}{\cdot}g_{VSS}^{-1}{\cdot}d^{-1}}$	98	21	144	56	64
λ_Acetate	days	0	0.4	0	0	0
Rm_H_2	$\mathrm{mg_{DQO\text{-}CH4}{\cdot}g_{VSS}^{-1}{\cdot}d^{-1}}$	75	22	116	75	75
λ _H_2	days	0	1	0	0	0
Rm_Propionate	$\mathrm{mg_{DQO\text{-}CH4}{\cdot}g_{VSS}^{-1}{\cdot}d^{-1}}$	97	-	61	65	67
λ _Propionate	days	0	-	1	0	0
Rm_Butyrate/valerate	$\mathrm{mg_{DQO\text{-}CH4}{\cdot}g_{VSS}^{-1}{\cdot}d^{-1}}$	63	60	109	154	84
λ _Butyrate/valerate	days	0	1	0	5	0

Regarding the specific activities over butyrate/valerate and acetate, the $\mathrm{OFMSW_1}$ was composed by high content of carbohydrates, being butyrate and acetate acid the main products of their degradation (Elbeshbishy & Nakhla, 2012). This could explain the high

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[1] Chapter 4

butyrate/valerate and acetate activity found during the SS-OFMSW co-digestion. Only the specific propionate activity showed lower value, in contrast with the starting inoculum In0 which showed a good propionate activity because the glycerol addition might improved this specific activity since 1,3-propanodiol and hydrogen are the main products of glycerol fermentation (Ekman & Börjesson, 2011, Jitrwung R. & Yorgeau V., 2011).

The In3 showed huge differences in comparison with the specific activities of In2. In3 showed lower activity, mainly the specific acetic activity which decreased in -2.6 and -1.8 times the value obtained with In2 and In0, respectively. The maximum slope of the specific hydrogen activity was -1.5 lower than the obtained with In2, but similar to the value that showed In0. Specific butyric/valeric activity reached the higher maximum slope (154 $mg_{COD\text{-}CH4}\cdot g^{-1}{}_{VSS}\cdot d^{-1}$) but showed a lag phase of 5 days. Only the specific propionic activity of the In3 showed similar maximum slope than the In2.

The continuous experiment did not show an inhibitory effect of the biomass, in terms of methane production, methane yield or VFA acids accumulation, although the specific substrate tests showed an important decreased in the biomass activity. Although no VFA accumulation was detected, the higher OLR applied in PIII than in PII, could produce stress in the biomass activity, especially in the acetoclastic methanogens, reducing their specific activity.

Another possibility is that some specific toxic components might reduce the biomass activity. The $OFMSW_1$ showed high quantities of fruits peels, especially orange peels which contain essential oils that have being referenced as bactericide (Mizuki *et al.*, 1990, Lane *et al.*, 1984). Considering that the orange peel typically contains 5.4 $g\cdot kg^{-1}$ (Braddock *et al.*, 1986) and the $OFMSW_1$ contained a 24.9% of peel orange on wet weight basis, the essential oils loading rate was estimated closer to 2.0 $mg\cdot L^{-1}d^{-1}$ in PI, 4.8 $mg\cdot L^{-1}d^{-1}$ in PII and 6.2 $mg\cdot L^{-1}d^{-1}$ in PIII. Lane *et al.* (1984) suggested than the limit of essential oil loading are 7.5 $mg\cdot L^{-1}\cdot d^{-1}$, while Mizuki et al. (1990) found a significant inhibition of methane fermentation of citrus unshu peel at loading above 200 $mg\cdot L^{-1}\cdot d^{-1}$ when added directly commercial limonene. Moreover, Mizuki *et al.* (1999) suggested than a lower essential oil concentration, below the limit inhibitory load, resulted in a change in the microbial population and in gas production greater than of the digestion with no peel oil, and this could be related to the high specific activity detected in In2.

Effect of particulate size

The effect of the particulate size of the $OFMSW_1$ was assessed reducing the particulate size from 20 mm till 8 mm. Previously, the ABT test at 35 ºC with particle size 20 mm or 8 mm showed the same biodegradability and final MP_{VS} values (Figure 7.3b).

The continuous digestion of SS-$OFMSW_1$ with reduced particle size corresponded with period PIV (Table 7.2): The methane production and methane yield, increasing slightly, +3% and +6% respectively, in comparison with the period PIII. Also COD and VS removal increased in +7-9%. Although the objective of the OFMSW reduction size was to accelerate the hydrolysis of the OFMSW, the results suggested than the reduction from 20 to 8 mm was not significantly effective, probably because the HRT of 20 days was long enough to degrade $OFMSW_1$ regardless of the particulate size. Regarding the effect of particle size on SAT, the specific activity of In4 was similar to In3 (Table 7.3), except on the reduction of the lag phase of the butyrate-valerate specific activity which was null.

Esposito *et al.* (2011) showed that higher OLR were needed to induce a digester failure with higher OFMSW particulate size with a MPS range of 50.0-2.5 mm, due to the slower disintegration and acidification occurring for higher OFMSW particle sizes, but also larger particles resulted in the decrease of the overall digestion efficiencies in terms of COD removal, which is in accordance with the obtained results obtained in this work. Besides this, although Izumi *et al.* (2010) improved the MY_{VS} reducing the particulate size, they worked with a relatively small MPS range (0.9 to 0.3 mm), they also found that there was a certain particle size that induced VFA accumulation in the anaerobic digester. These results are not in accordance with the results obtained in the present study since no VFA accumulation was observed in period PIV, suggesting that other factors could influence the OFMSW anaerobic digestion like the source and composition of the waste, or specific activity of the anaerobic biomass.

7.4. CONCLUSIONS

The SS-OFMSW mixture of 51% inlet-COD (54% inlet-VS) of OFMSW, at OLR 3.1 $kg_{COD}\cdot m^{-3}\cdot d^{-1}$ (1.9 $kg_{VS}\cdot m^{-3}\cdot d^{-1}$) and HRT of 20 days showed the best conditions digestion performed, obtaining +200% and +59% of methane production and yield in comparison with the solely SS digestion under these conditions. Further reduction of the particle size of the OFMSW from 20 to 8 mm did not improve significantly the gas yield nor production rate. The evolution of specific activities was assessed and used as a feasible tool to explain and manage the system responses, especially when conventional control parameters were not sufficient to explain the performance description.

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CHAPTER 8

PROMOTING THERMOPHILIC SPECIFIC ANAEROBIC BIOMASS ACTIVITY THROUGH SEWAGE SLUDGE ANAEROBIC CO-DIGESTION WITH ORGANIC FRACTION OF MUNICIPAL SOLID WASTE

Thermophilic sewage sludge co-digestion with organic fraction of municipal solid waste was assessed in continuous experiment aiming to obtain the most adequate operational parameters, as well as, analyzing the effect of the OFMSW addition in the specific biomass activity. The maximum methane yield was obtained when the OFMSW was the 53% of the COD fed (48%-volatile solids fed) (organic loading rate of 2.2 $kg_{VS}·m^{-3}·d$ and 20 days of hydraulic retention time). In addition, this mixture promoted the activity of the saturated fatty acid oxidizers and acetoclastics methanogens populations. Therefore, the OFMSW co-digestion with sewage sludge could be an interesting strategy to increase the methane production and to increase the thermophilic biomass activity

G. Silvestre, A. Bonmatí, B. Fernández. Promoting thermophilic specific anaerobic biomass activity through sewage sludge anaerobic co-digestion with organic fraction of municipal solid waste. Submitted to a peer review Journal

8.1. INTRODUCTION

The management of the municipal solid waste (MSW) minimizing the impact over the environment is one of the most important challenges than the modern society has to face. Each European citizen generates 588 kg of MSW per year that must be managed and treated to avoid environmental and health problems (Eurostat, 2014). European policies tackle the problem through strict legislation, like the Directive 2006/12/EC (CEC, 2006), which requires that each State Member elaborates a national strategy to reduce the fate of biodegradable waste into landfills.

The current trend in MSW management is based, in most of the European States, on the source separation of the biodegradable fraction, called organic fraction of the municipal solid waste (OFMSW), before its treatment by biological processes like composting or anaerobic digestion (AD). AD is usually found in large facilities, since AD shows several benefits like the stabilization of the organic matter and generation of renewable energy that can partially balance the energy demand of the treatment plant, reducing the environmental impact of these installations (Mata-Alvarez, 1992). Nevertheless, the high investment cost of such installations reduces their feasibility to large cities. Thus, OFMSW strategy with other organic waste like sewage sludge (SS) generated in wastewater treatment plants (WWTPs) could be an interesting approach for cities where large facilities are not feasible. Furthermore, it could be a good strategy to optimize WWTPs anaerobic digesters, since they usually work below their organic loading rate design, just by increasing the waste flow treated which might improve the biogas production and therefore, improve the economic feasibility of the WWTP itself (Pavan *et al.*, 2007, Silvestre *et al.*, 2015a[1]).

AD is a biological process which depends strongly on the working temperature, being the optimal temperature range for the anaerobic biomass activity 30-38ºC, mesophilic range, and 50-57ºC, thermophilic range. The mesophilic AD is most widespread in industrial applications, mainly due to its lower heat requirement. Several studies about the mesophilic SS-OFMSW anaerobic can be found in the literature including full scale examples (Bolzonella *et al.*, 2006, Krupp *et al.*, 2005,), as well as Silvestre *et al.* 2015c[2] chapter 7 of this thesis.

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[1] Chapter 2

[2] Chapter 7

Although the thermophilic AD is characterized by higher organic matter removal efficiency and higher biogas production than the mesophilic AD (Gavala *et al.*, 2003); thermophilic anaerobic SS-OFMSW has been studied in a lesser extent (Kim *et al.*, 2011, Sosnowoski *et al.*, 2008). Moreover, it has been reported that the thermophilic digestion reduces the pathogen content in digestates (Dohányos *et al.*, 2004), and it is recommended prior to nutrients recycle (Environmental DG, EU, 2000). But, the main drawback of thermophilic AD is its higher sensitivity to inhibitory and toxic compounds (Silvestre *et al.*, 2014[1], Silvestre *et al.*, 2015b[2], Hidaka *et al.*, 2013). Guo *et al.* (2014) showed lower richness and evenness of bacterial species in thermophilic than in mesophilic biomass. Silvestre *et al.* (2014)[1] reported lower specific biomass activity in thermophilic AD compared with mesophilic AD running with the same operational conditions. Besides, the addition of OFMSW could bring into the reactor inorganic impurities, as well as toxics and inhibitors compounds, depending on several factors; collection system and frequency, sorting method, climate, and cultural practices (Forster-Carneiro *et al.*, 2008), that could reduce the specific biomass activity, as was showed in Silvestre *et al.* 2015c[3].

The aim of this work was to study the thermophilic SS-OFMSW anaerobic digestion, evaluating the biogas yield, the stability of the process and the dynamics of the specific substrate activity of the anaerobic biomass under different SS-OFMSW mixtures and organic loading rates. In addition, a comparative analysis with the SS-OFMSW mesophilic performance (Silvestre *et al.* 2015c[2]) was carried out.

8.2. MATERIAL AND METHODS

8.2.1. Substrates

Three OFMSW samples ($OFMSW_1$, $OFMSW_2$ and $OFMSW_3$) were collected from different MSW facilities located in Catalonia (Spain). The composition and characterization of each OFMSW samples are widely described in Silvestre *et al.* 2015c[3].

SS (70% primary sludge and 30% waste activated sludge) was collected from a WWTP located in Barcelona (Spain) every two weeks and kept at 4ºC before used.

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[1] Chapter 5

[2] Chapter 6

[3] Chapter 7

8.2.2 Experimental design and set-up

SS and OFMSW anaerobic experiment was studied by means of batch test, anaerobic biodegradability test (ABT), and continuous experiments.

ABT test was carried out following Field *et al.* (1988) and Silvestre *et al.* (2011)[1], were performed to determine the anaerobic biodegradability (AB; % initial COD) and the maximum methane potential on volatile solids basis (MP_{VS}; $NL_{CH4}\cdot kg_{VS}^{-1}$). ABT results were used to select the OFMSW to be used in the continuous experiment.

The continuous experiment was carried out in a continuous stirred tank reactor (CSTR) with a working volume of 5.0 L. A detailed description of the set up is described in Silvestre *et al.* (2014)[2]. SS were fed twice per day with a peristaltic pump and the OFMSW was fed manually once per day. Reactor operation lasted 150 days (Figure 8.2) and was divided into three periods (PI, PII, PIII), that were evaluated in terms of volumetric methane production ($Nm^3\cdot m^{-3}\cdot d^{-1}$) and yield ($MY_{VS}$; $NL_{CH4}\cdot kg_{VS}^{-1}$), besides common control parameters as organic matter (volatile solids (VS), chemical oxygen demand (COD)) removal efficiency, volatile fatty acids (VFA) concentration and profile, alkalinity ratio and biogas composition. These data were compared with reference values corresponding to the operation of the same reactor fed only with SS at a hydraulic retention time (HRT) of 20 days (Silvestre *et al.*, 2014)[2]; this reference period was called P_TSS.

In addition, specific activity test (SAT) were performed with the inocula sampled at the end of each period (In1-55 (PI), In2-55 (PII), In3-55(PIII)) to evaluate the evolution of main anaerobic populations, including the SAT of the starting inoculums (In0-55), and that of the reference period (InSS-55). Acetic acid (10 mM), hydrogen (4.1 mM), propionic acid (6.0 mM) and a mixture 1:1 of iso-butyric and iso-valeric acids (6.2 mM) were used as substrates. SAT was performed as described elsewhere (Silvestre *et al.*, 2011)[1]. The maximum slope (R_m, $g_{COD-CH4}\cdot g_{VSS}^{-1}\cdot d^{-1}$) and the lag phase (λ; days) were calculated to compare the different SAT experiments.

8.2.3 Analytical methods

Total solids (TS), VS, COD, ammonia nitrogen (NH_4^+-N), alkalinity and pH were measured once a week in the effluent and influent,

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[1] Chapter 4

[2] Chapter 5

following the Standard Methods (APHA, AWWA, WEF, 1995). VFA (acetic, propionic, i-butyric, n-butyric, i-valeric, n-valeric, i-caproic and n-caproic acids) were measured in the effluent once per week by gas chromatography (TCD and FID, respectively) following the methodology described in Silvestre *et al.* (2011)[1]. Biogas composition (CH_4, CO_2 and H_2) was analysed twice per week with a gas chromatograph (CO-300 Varian, USA) equipped with a packed column and a TCD detector as described elsewhere (Silvestre *et al.*, 2011)[1].

8.3. RESULTS

8.3.1 Biochemical methane potential test

MP_{VS} of all the OFMSW samples was between 425-470 $NL_{CH4}\cdot kg_{VS}^{-1}$ (Table 8.1), while the SS was a 20-33% lower. Figure 8.1 shows the cumulative methane evolution of the three OFMSW samples; as can be seen, 80% of the MP_{VS} was reached in the first five days in all the cases, and none of them present lag phase. Based on the produced methane, the calculated biodegradability of $OFMSW_1$ was the highest (87% COD), while the $OFMSW_2$ showed the lowest (68% COD), similar than SS (67% COD).

Table 8. 1. *Maximum methane potential (MP_{VS}) and anaerobic biodegradability (AB) of the different organic fraction of the municipal solid waste samples and sewage sludge at thermophilic and mesophilic temperatures*

	MP_{VS} (NL_{CH4} kg_{VS}^{-1})			**AB (%COD)**	
Temperature	**55ºC**	**35ºC***	**Increment thermo/meso**	**55ºC**	**35ºC***
SS	354 ± 12	298 ± 6	+19%	79 ± 3	67 ± 1
$OFMSW_1$	425 ± 6	460 ± 20	-8%	87 ± 1	90 ± 10
$OFMSW_2$	444 ± 16	366 ± 55	+21%	68 ± 8	73 ± 11
$OFMSW_3$	470 ± 50	412 ± 3	+14%	78 ± 2	81 ± 14

*Data from Silvestre et al. 2015[1]

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[1] Chapter 4

[3] Chapter 5

The same OFMSW samples were analyzed in mesophilic temperatures (Silvestre *et al.*, 2015c[2]). Although AB values were rather similar at both temperatures, the MP_{VS} of $OFMSW_2$ and $OFMSW_3$ were +21% and +14 higher in thermophilic than in mesophilic temperature range, respectively (Table 8.1). Conversely, $OFMSW_1$ showed similar values at both temperature ranges. Moreover, $OFMSW_2$ showed the lowest MP_{VS} value at mesophilic range and the highest at thermophilic range. This different behaviour could be attributable to its chemical composition (Converti *et al.*, 1999; Wongwilaiwaun *et al.*, 2010) and/or the presence of inhibitor compounds.

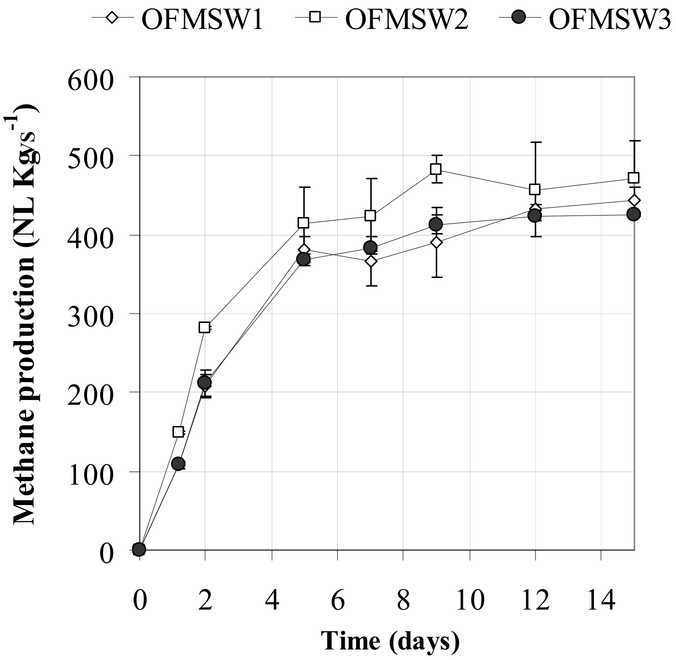

Figure 8. 1. *Maximum methane production on VS basis (MP_{VS}) during the batch test*

8.3.2 Continuous experiment

$OFMSW_1$ was selected to perform the continuous co-digestion experiment. Three different periods (PI, PII, PIII) with three different OLR and two different SS and $OFMSW_1$ mixtures were studied during 150 days of continuous operation (Table 8.2). HRT was maintained at 20 days during the whole operation of the reactor. As mentioned before, results were compared with the period where SS was anaerobically digested alone, called period P_TSS, whose operational conditions were: OLR 1.2 $kg_{VS}·m^{-3}·d^{-1}$, HRT of 20 days and SS influent with 22 $g_{VS}·L^{-1}$(Silvestre *et al.*, 2014)[3].

Table 8. 2. *Average operation performance of the different periods of the thermophilic SS-OFMSWco-digestion experiment*

Parameters	**Units**	**P_TSS**	**PI**	**PII**	**PIII**
$OFMSW_{1addition}$	% VS_{in}	-	29 ± 3	47± 5	48 ± 11
$OFMSW_{1addition}$	% COD_{in}	-	25 ± 5	44± 9	53 ± 11
HRT^1	days	20 ± 2	21 ± 2	21 ± 2	23 ± 7
$OLR_{COD}{}^1$	$kg_{COD}\ m^{-3}d^{-1}$	2.2 ± 0.2	2.3 ± 0.3	3.0 ± 0.3	3.3 ± 0.6
$OLR_{VS}{}^1$	$kg_{VS}\ m^{-3}d^{-1}$	1.2 ± 0.1	1.2 ± 0.0	1.8 ± 0.2	2.0 ± 0.3
$CH_{4production}{}^1$	$Nm^3m^{-3}d^{-1}$	0.18 ± 0.09	0.51 ± 0.08	0.73 ± 0.07	0.88 ± 0.11
Increment VMP	*% P_{SS}-55*	-	*183*	*305*	*389*
$CH_{4yield}{}^1$	$NL_{CH4}\ kg_{VS}{}^{-1}$	260 ± 45	428 ± 58	393 ± 67	441 ± 43
Increment MY_{VS}	*% P_{SS}*	-	*65*	*51*	*70*
CH_4 content^3	%v/v biogas	66 ± 4	65 ± 2	63 ± 3	63 ± 2
pH^3	-	7.4 ± 0.2	7.5 ± 0.1	7.5 ± 0.6	7.3 ± 0.1
TA^3	g $CaCO_3$ L^{-1}	5.1 ± 0.4	3.5 ± 0.4	3.7 ± 0.3	3.0 ± 0.8
PA^3	g $CaCO_3$ L^{-1}	2.7 ± 0.2	2.9 ± 0.2	3.0 ± 0.3	2.1 ± 0.7
NH_4^+-N^3	mg L^{-1}	534 ± 87	918 ± 47	1018 ± 93	885 ± 90
VFA^2	mg L^{-1}	< 200	< 100	< 100	< 100
$COD_{removal}{}^2$	%$inlet_{COD}$	56 ± 3	64 ± 4	70 ± 6	75 ± 7
Incr. $COD_{removal}$	*% P_{SS}*	-	*14*	*25*	*34*
$VS_{removal}{}^2$	%inlet VS	50 ± 11	62 ± 4	71 ± 9	72 ± 7
Incr. $VS_{removal}$	*% P_{SS}*	-	*24*	*42*	*44*

Note: Average and S.D of the daily ([1]), weekly ([2]) or twice week ([3]) samples of each period

The two first periods (PI, PII) of co-digestion were completed and performed with a SS with 35 $g_{COD}·L^{-1}$ (18 $g_{VS}·L^{-1}$). Table 8.2 shows the main parameters of the different periods analyzed in thermophilic anaerobic co-digestion experiment. In period PI, the $OFMSW_1$ accounted the 25% COD inlet (29% VS inlet) resulting in same OLR than in the reference period P_TSS (2.2 $kg_{COD}·m^{-3}·d^{-1}$). In period PII, the OLR was increased till 3.0 $kg_{COD}·m^{-3}·d^{-1}$ by increasing the $OFMSW_1$ amount, reaching a 44% COD inlet (47% VS inlet). Finally in period PIII, similar SS-OFMSW mixture (49:51 COD basis) as in PII was fed but in this case, the SS had a concentration of 45 $g_{COD}·L^{-1}$ (22 $g_{VS}·L^{-1}$), and in consequence the OLR was pushed up to 3.3 $kg_{COD}·m^{-3}d^{-1}$ (2.0 $kg_{VS}·m^{-3}·d^{-1}$).

Methane composition on the biogas decreased from 66% (SS anaerobic digestion) to 65% (PI) and 63% v/v (PII and PIII, respectively). This result agrees with the results obtained in mesophilic co-digestion (Silvestre *et al.*, 2015c[1]) and is also coherent with the carbohydrate content of the $OFMSW_1$. No unbalance or failure signs were detected in these periods in terms of VFA concentration, alkalinity or pH. Total ammonia concentration increased in parallel with the OLR, but the process was not inhibited since NH_4^+-N concentration was below the levels described as inhibitors the anaerobic biomass (Procházka *et al.*, 2012; Angelidaki & Ahring, 1993).

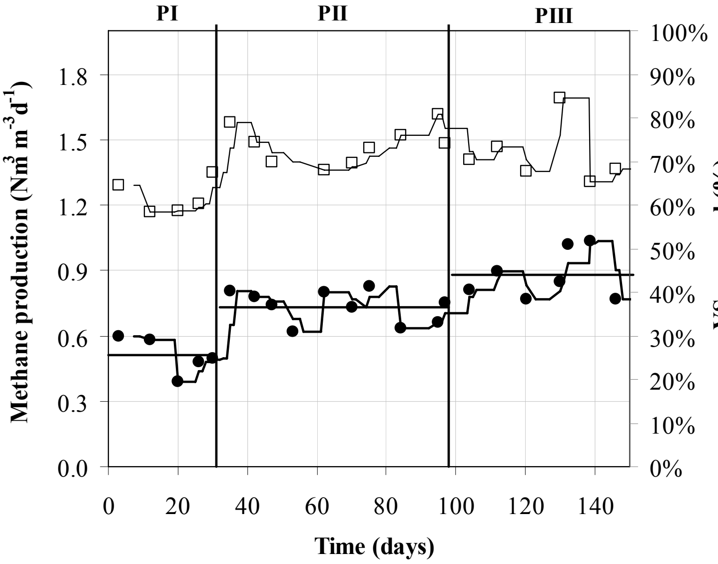

Figure 8. 2. *Volumetric methane production and volatile solids removal efficiency during the thermophilic SS -$OFMSW_1$ co-digestion experiment (average of one week of the operation). Notation: circles-methane production, squares-OLR.*

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[1] Chapter 7

Figure 8.2 shows the evolution of the methane production together with the VS removal efficiency through the different co-digestion periods. During the co-digestion periods, the mean volumetric methane production increased on +183%, +305% and +389% in PI, PII and PIII respectively, in comparison with the methane production obtained during P_TSS (Silvestre *et al.*, 2014[1]). In accordance with the methane production, the COD and VS removal were higher in the co-digestion periods than P_TSS, especially in the last period PIII where the COD and VS removal were 75% and 72%, respectively.

The highest methane yield (441 $NL_{CH4}·kg_{VS}^{-1}$) was recorded in period PIII, being +70% higher than the methane yield obtained during the SS anaerobic digestion alone. The PI showed +65% higher methane yield than the P_T-SS, although the OLR applied in both periods was similar.

The methane production obtained during the thermophilic and mesophilic anaerobic co-digestion experiments were compared and they are shown in Figure 8.3a. A lineal correlation for biogas production and %VS $OFMSW_1$ inlet, obtained in the mesophilic (Silvestre *et al.*, 2015c[2]) and thermophilic performance (with similar OLR of 1.2-2.1 $kg_{VS}·m^{-3}·d^{-1}$) was obtained. As can be seen, the thermophilic co-digestion correlation has a higher slope and showed higher production than the mesophilic, mainly in the periods where the $OFMSW_1$ was in high concentration (PII and PIII).

Figure 8.3b shows a comparison between the methane yield (MY_{VS}) of the continuous periods and the theoretical methane potential (TMP) in mesophilic and thermophilic temperatures. TMP was calculated taking into account the MP_{VS} of SS and $OFMSW_1$ and the percentages of SS and $OFMSW_1$ in each of the feed mixture. During the SS mono-digestion MY_{SV} reached the 84% and 73% of the TMP in mesophilic and thermophilic temperatures, respectively. During co-digestion periods, the MY_{SV} was 100% (PI-35), 125% (PII-35) and 139% (PIII-35) of the TMP at mesophilic range, and 120% (PI), 110% (PII) and 121% (PIII) in thermophilic range, suggesting that the introduction of OFMSW has a synergistic effect over SS anaerobic digestion. This effect has been previously reported by different authors and the balance of macro and micronutrient has been hypothesised as the reason for this synergism, the increase of the organic matter (COD and VS) removal rates and as a

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[1] Chapter 5

[2] Chapter 7

consequence of the production of CH_4 (Jianzheng and Ajay, 2011; Li *et al.*, 2009; Macias-Corral *et al.*, 2008; Pagés *et al.*, 2011).

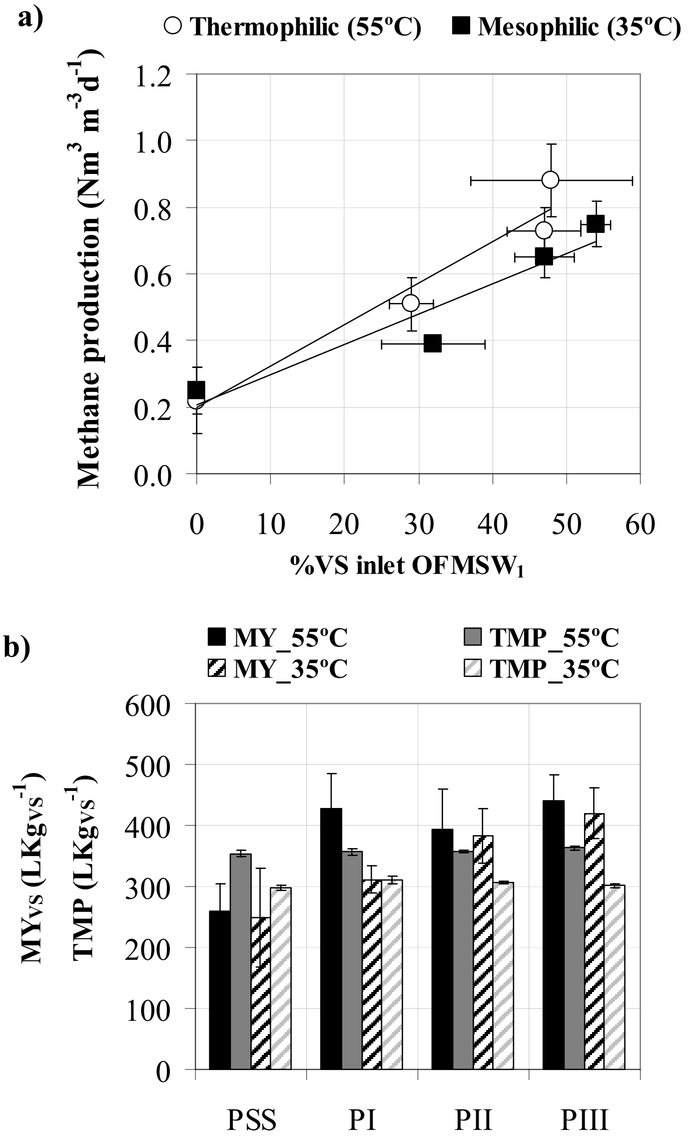

Figure 8. 3. *Comparison between mesophilic and thermophilic periods with OFMSW*$_1$*: a) methane production (*$Nm_{CH4}^3 \cdot m^{-3} \cdot d^{-1}$*) versus the percentage of the VS of the feed provided by the OFMSW; b) methane yield (*MY_{VS}*) and the theoretical methane production (TMP).*

Therefore, the expected improvement at thermophilic temperature was observed in this work, since the thermophilic anaerobic digestion is characterized by accelerated biochemical reactions and higher growth rate of microorganisms, resulting in higher methanogenic activity (Zábranská *et al.*, 2000). Some authors observed no significant differences between thermophilic and mesophilic conditions under the same experimental conditions if the HRT was ≥ 20 days, although the benefits of the thermophilic anaerobic digestion at lower HRT are more evident (Gavala *et al.*, 2003; Ferrer *et al.*, 2010). The effect of addition of OFMSW during thermophilic anaerobic co-digestion is scary analyzed, but Cavinato *et al.* (2013) showed higher pronounced increase on methane yield during OFMSW-SS co-digestion during mesophilic than thermophilic temperatures.

On the other hand, some authors reported that the anaerobic performance depends on the inocula source, the start-up and the history of the digesters (MacMahon *et al.*, 2004; Lin *et al.*, 2012). The community structure in thermophilic and mesophilic anaerobic communities could be different and in consequence, the response under different SS-OFMSW mixtures and OLR could deviate.

8.3.3 Specific substrate activity

The specific activity tests were carried out to analyze the effect of OFMSW addition during SS anaerobic digestion on the specific biomass (Figure 8.4). The starting inoculum (In0-55) of the thermophilic digester came from a thermophilic SS and grease waste (GW) co-digester (Silvestre *et al.*, 2014[1]). The inocula sampled along the SS-$OFMSW_1$ co-digestion represented the biomass at the end of PI (InI-55), PII (InII-55) and PIII (InIII-55) periods. The inoculum collected in the reference period P_TSS was called In-SS, and represented the inoculum obtained at the end of the SS anaerobic mono-digestion (Silvestre *et al.*, 2014)[1].

Specific propionate and butyrate-valerate acid activities were null when evaluating the specific activity of the inoculum taken in the SS mono-digestion (In-SS) and also in the SS-GW co-digestion experiment (In0-55) (Table 8.3). Thus, SS mono-digestion and SS:GW co-digestion lead to a population with low propionic and butyric/valeric degradation capability. On the other hand, hydrogen specific activity was much higher than the acetic specific activity suggesting that the starting inocula had highly active hydrogenotrophic methanogens compared with the acetate-utilizing methanogens.

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[1] Chapter 5

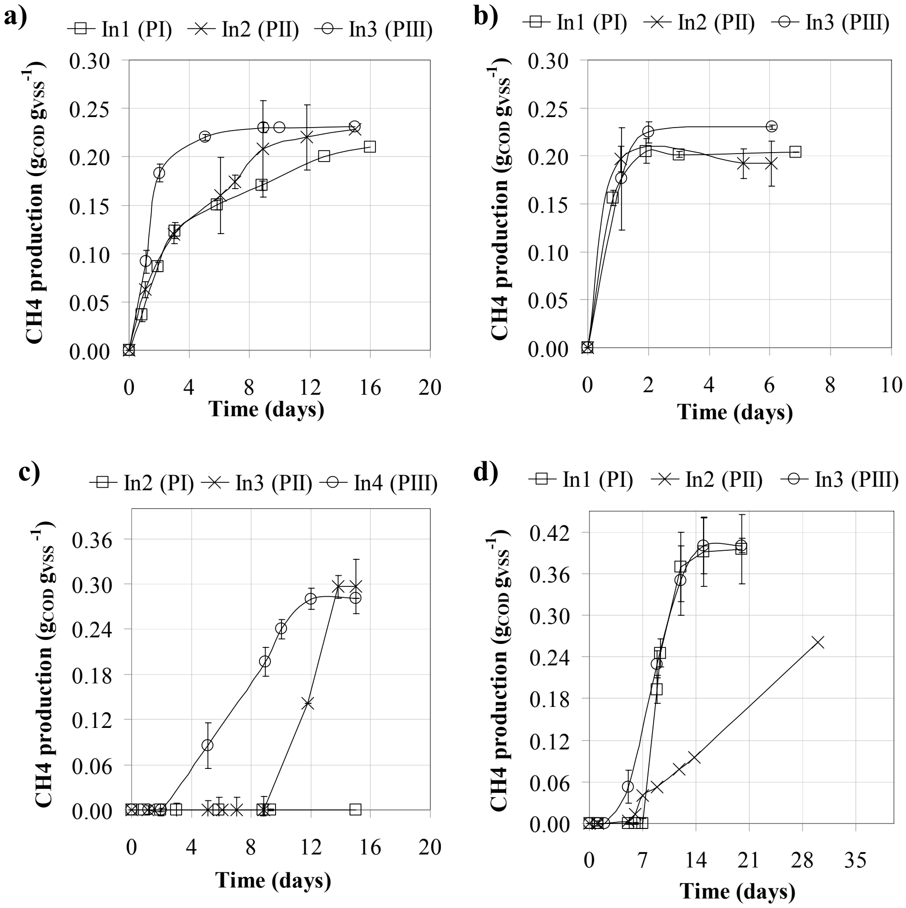

Figure 8. 4. *Specific substrate activity of thermophilic inocula, along the operational periods, with a) acetate, b) hydrogen, c) propionate and d) butyrate and valerate as substrate.*

The specific activity of the anaerobic biomass was changing during the thermophilic co-digestion periods with OFMSW$_1$. Specific acetogenic activity of inocula InI-55 and InII-55 were similar to the initial specific activity (In0-55), but the InIII-55 showed a two-fold increase. Regarding the specific hydrogen activity, InI-55 showed a similar activity to In0-55, but InII-55 and InIII-55 showed a 1.3 increase with respect InI-55. Although the specific hydrogenotrophic activity stayed higher than the specific acetate, the OLR increase along co-digestion periods reduced considerable the specific activity differences between them. The results were in accordance with Montero *et al.* (2007) who showed that hydrogenotrophic methanogens were dominant in the start-up conditions, but this population was displaced by the acetate-utilising methanogens populations once the OLR was increased gradually.

Table 8. 3. *Lag phase (λ) and maximum slope (R_m) obtained in the specific activity tests with the different inocula at thermophilic temperature.*

	Period	P_{SS}-55	P0-55*	P1	P2	P3
Substrate	**Inoculum**	**InSS**	**In0-55**	**In1-55**	**In2-55**	**In3-55**
Acetate	R_m $(mg_{CODCH4}\ g_{VSS}^{-1}d^{-1})$	28	42	42	36	91
	λ (d)	8	0	0	0	0
Hydrogen	R_m $(mg_{CODCH4}\ g_{VSS}^{-1}d^{-1})$	367	141	141	190	182
	λ (d)	0	0	0	0	0
Propionate	R_m $(mg_{CODCH4}\ g_{VSS}^{-1}d^{-1})$	-	-	-	-	28
	λ (d)	-	-	-	-	2
Butyrate /Valerate	R_m $(mg_{CODCH4}\ g_{VSS}^{-1}d^{-1})$	-	-	89	18	34
	λ (d)	-	-	7	5	2

The specific butyrate/valerate activity was detected in InI-55, showing a lag phase of 7 days and an activity of 89 $mg_{COD}{\cdot}g_{VSS}^{-1}{\cdot}d^{-1}$. In the successive periods, the lag phase was reduced till 5 days in InII-55 and 2 days in InII-55 (Table 8.3). Contrary, the specific propionic activity was not detected till the last co-digestion period (PIII) were InIII-55 presented an activity of 28 $g_{COD}{\cdot}g_{VSS}^{-1}{\cdot}d^{-1}$.

It appears that the introduction of complex waste as OFMSW promotes the anaerobic biomass activity, and specifically the activity of VFA degraders. Carbohydrates are the main component in the OFMSW and the proteins in the sewage sludge, so the co-digestion of both substrates could improve the anaerobic process in terms of methane yield and specific substrate activity (Elbeshbishy & Nakhla, 2012). In the co-digestion of SS with grase waste (Silvestre *et al.*, 2014[1]), the high long chain fatty acids concentration could explain the differences between the specific substrate activities, and therefore, suggest that the composition of the substrate also affected the specific activities of the anaerobic biomass.

Finally, in order to show the effect of temperature over the specific activity, the specific activity at the different OLR and temperatures (thermophilic and mesophilic range) are depicted in Figure 8.5. As can be seen, SS-OFMSW at thermophilic range, showed the higher hydrogenotrophic methanogen activity, being between +25% to +78% higher than mesophilic hydrogenotrophics. The thermophilic acetic activity was almost constant through all the thermophilic periods, with a slight increase in period PIII (Figure 8.5a). On the other hand, at mesophilic range, acetate activity showed high variations along the periods, but its mean value was similar than thermophilic one. This changes on the acetic activity showed that the addition of OFMSW could lead to certain instability at mesophilic range (period PIII-35; Silvestre *et al.*, 2015c[2]). Since the OFMSW_1 used in the co-digestion was characterized by the presence of antimicrobial compounds of the vegetable and fruit presents in the OFMSW_1, such as flavour substances (terpens and limonene) or antioxidants (poliphenols), that had been described as cytotoxic due to its lipophylic nature and specifically toxic in anaerobic digesters (Bakkali *et al.*, 2008; Moufida *et al.*, 2003; Wikandari *et al.*, 2014). Furthermore, this substance have different inhibitory threshold depending on the temperature range. In this regard, d-Limonene has been described to cause inhibition at 400 µL/L concentration under mesophilic conditions and between 450 and 900 µL/L under thermophilic conditions (Wikandari *at al.*, 2014). The higher resistance of the thermophilic biomass to the limonene inhibition could be related with the thermophilic microorganism exhibit reduced membrane fluidity and in addition, the essential oils evaporation effect is more pronounced, but against the essential oil diffusion is also higher (Ruiz *et al.*, 2014).

The propionic and butyric-valeric activity was higher at mesophilic range than in thermophilic through the different operational periods, specially the propionic activity, showing that this possible inhibition is not severe and the mesophilic reactor performs all right (Silvestre *et al.*,

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[1] Chapter 5

[2] Chapter 7

2015c[2]). Nonetheless, the addition of OFMSW in the thermophilic SS anaerobic digestion improves the activity of the syntrophic propionate-oxidizing bacteria and saturated fatty acid oxidizer population, reducing the differences between the mesophilic and thermophilic biomasses.

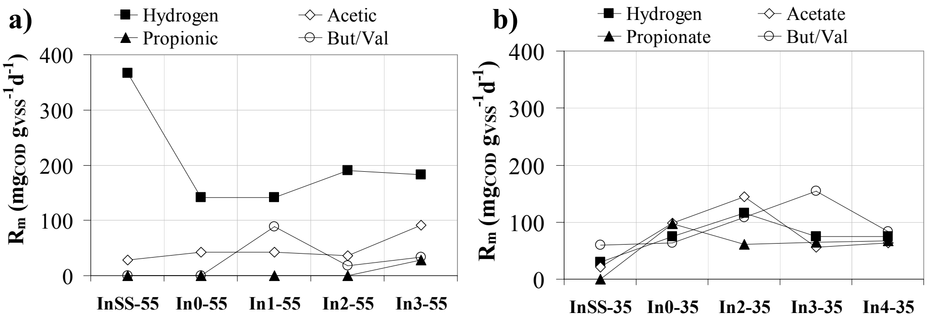

Figure 8. 5. *Comparison of specific substrate activities of the different inocula at thermophilic and mesophilic range. Notation: InSS-55: inoculum from thermophilic SS mono-digestion period; In0-55: starting inoculum of the thermophilic trial; In1-55; In2-55, In3-55: inocula from thermophilic SS-OFMSW trial; InSS-35: from mesophilic SS mono-digestion period; In0-35: starting inoculum of the mesophilic SS-OFMSW trial; In2-35, In3-35, In4-35: inocula from mesophilic SS-OFMSW trial (Silvestre et al., 2015c[1])*

8.4. CONCLUSIONS

Sewage sludge co-digestion with OFMSW at thermophilic range showed a good performance, with a maximum volumetric methane production of $0.88\ Nm^3\cdot m^{-3}\cdot d^{-1}$, and a methane yield of $441\ NL_{CH4}\cdot kg_{VS}^{-1}$ when 47% of the feed COD (48%-VS inlet) were supplied by the OFMSW. The specific activity tests showed that the initial biomass play an important role during the anaerobic co-digestion performance, and suggested than the feed composition, the organic loading rate, and the temperature range have a great influence on the specific substrate activity. At thermophilic range the OFMSW addition promoted the activity of the saturated fatty acid oxidizers and acetoclastics methanogens populations.

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[1] Chapter 7

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CHAPTER 9

GENERAL CONCLUSIONS AND SUGGESTION FOR FURTHER RESEARCH

This chapter reports the main conclusions obtained. Suggestions for further research related to sewage sludge anaerobic co-digstion are also presented.

9.1 FINAL CONCLUSIONS

In this thesis, sewage sludge anaerobic co-digestion with different organic wastes coming from various origins and with different compositions was proposed as a strategy to improve the energy balance of a WWTP. This work was focused on the development of two different operational strategies (co-digestion and temperature) to optimize the sewage sludge co-digestion process maximizing biogas production and avoiding inhibitory episodes.

Three co-substrates selected were: grease waste, crude glycerol and the organic fraction of municipal solid waste. It can be concluded that the addition of the different organic wastes studied, during SS anaerobic co-digestion, respectively increased methane productivity and yield –at mesophilic and thermophilic temperature ranges– between 2.2 and 5 times, and between 1.1 and 1.7 times, in comparison with sewage sludge anaerobic mono-digestion. Co-digestion with the OFMSW showed the highest methane yield either at mesophilic and thermophilic temperature ranges, compared with the other wastes object of this research.

Besides the effect on methane yield, which proved to be a stability index for the process throughout the continuous co-digestion trials, the specific substrate activity of the biomass along the different performance periods with the different organic wastes was assessed as a tool to determine adaptation and performance of the entire process.

Moreover, the addition of these co-substrates showed an increase in specific biomass activities, them being more pronounced when using the OFMSW as co-substrate. These specific substrate activities were promoted in function of the composition of each different co-substrate, thus adding grease waste promoted acetate and β-oxidation syntrophic acetogenic activity, while adding crude glycerol resulted in an improvement of hydrogen and propionate activity. Adding the OFMSW promoted the activity of saturated fatty acid oxidizers and acetoclastic methanogenic populations. In addition, the evolution of each particular substrate activities throughout the different co-digestion periods has been proved to be a feasible tool to analyse the performance of anaerobic digestion.

Regarding operational temperatures, sewage sludge anaerobic co-digestion with grease waste and crude glycerol was more robust and stable in terms of performance under mesophilic than thermophilic temperature conditions; it being less affected by the presence of inhibitory components such as LCFAs or the extreme pH of CGY. However, sewage sludge co-digestion with the OFMSW showed a better performance and a higher increase in methane production in thermophilic than in mesophilic anaerobic digestion.

As a general conclusion, it can be said that sewage sludge co-digestion is a suitable strategy to increase methane production and to optimize the energy balance of a WWTP. But, depending on the composition of the organic waste and the operational temperature range selected, different operational strategies should be implemented. These results could be implemented and put into practice as part of the sewage sludge co-digestion process of WWTPs, ultimately aiming to improve the energy balance, attain energy self-sufficient operations in a WWTP, and perhaps even net energy producing WWTPs.

From the overall results, major conclusions by chapter were reached which are briefly described below:

In **chapter 2**, the analysis of the sewage sludge anaerobic digestion process, as a source of energy in a WWTP, showed that renewable energy in form of biogas can supply between 39% and 76% of the total energy demand of the entire plant. Energy production strongly depends on the concentration of organic matter in the initial wastewater, while the WWTP energy consumption mainly depends on the active sludge system performance. Although these results suggest that anaerobic digestion is a good technology to recover energy from wastewater, it is necessary to apply some strategies to increase energy recovery: strategies such as co-digestion. From a WWTP management point of view, energy optimisation might be accomplished including not only the sludge line, but also the wastewater treatment line.

In **chapter 3**, a literature survey about sewage sludge co-digestion was carried out. The main conclusions reached in this review were that an increase in the organic loading rate during the co-digestion process is the parameter more widely studied showing a clear influence over biogas production. Nevertheless, other specific operational strategies, such as temperature changes, where applied to achieve stability throughout the process and to avoid inhibitory or failure episodes. Comprehensive management solutions for the different available organic wastes, according to each different organic waste origin and composition, have never been thoroughly studied.

In **chapter 4** grease waste (GW) was proved to be a suitable co-substrate for anaerobic digestion of sewage sludge under mesophilic temperature conditions. When GW addition reached up to 26% of the COD fed (with 20 days of HRT and 3.0 $kg_{COD}·m^{-3}·d^{-1}$ of OLR), methane productivity and yield raised 2.3 and 1.5 times in comparison with sewage sludge mono-digestion. Specific biomass activity tests for this experiment confirmed that the anaerobic biomass had adapted to GW. Acetate and β-oxidation syntrophic acetogenic activities of the adapted inoculum were 6.3 and 11.2 times higher than in the initial inoculum. These results suggest that biomass acclimatization achieved by slowly increasing the grease waste dose is a good strategy to enhance fat degradation and reduce the inhibitory effect of LCFAs.

In **chapter 5**, sewage sludge and grease waste co-digestion was assessed under thermophilic temperature conditions. The maximum increase in methane productivity and yield was 2.2 and 1.1 times higher, compared to sewage sludge mono-digestion, when grease waste addition reached up to 27% of the COD fed (with 20 days of HRT and 2.8 $kg_{COD}\cdot m^{-3}\cdot d^{-1}$ of OLR). Additional GW dosage resulted in an unstable reactor performance, LCFA accumulation in the effluent, and poor dewaterability properties in the digestate. Specific methanogenic activity tests have shown that grease waste addition improves acetoclastic methanogenic activity, which suggests that tolerance to LCFAs can be further enhanced with a slow increase in lipid-rich materials.

In **chapter 6**, crude glycerol (CGY) was analyzed as a co-substrate for sewage sludge anaerobic co-digestion. Results showed greater differences between operation under mesophilic and thermophilic conditions. Thermophilic anaerobic SS-CGY co-digestion showed great instability due to the extreme pH of glycerol and the swift glycerol hydrolysis step that leads to VFA accumulation. The same process, under mesophilic conditions, performs steadily, with a respective increase in methane productivity and yield 2.5 and 1.3 times higher, compared with sewage sludge mono-digestion, the optimum CGY dose being of 27% of the COD inlet (with 20 days of HRT and 3.0 $kg_{COD}\cdot m^{-3}d^{-1}$ of OLR). Further CGY addition didn´t show any improvement; with a biomass shift due to a high C/N ratio probably explaining this behaviour. Results suggest that CGY can be used as co-substrate in SS anaerobic digestion but, depending on the CGY characteristics and the operational temperature, different parameters should be taken into account to perform steadily: such as pH and alkalinity of the co-digestion mixture.

Regarding **chapter 7,** the organic fraction of municipal solid waste (OFMSW) was proved to be a suitable co-substrate under mesophilic temperature conditions. When the OFMSW addition reached up to 51% of COD inlet, methane productivity and yield were 3.1 and 1.6 folds higher compared to sewage sludge mono-digestion (with 22 days of HRT and 3.1 $kg_{COD}\cdot m^{-3}\cdot d^{-1}$of OLR). Besides this, reduction of the OFMSW particulate size, from 20 to 8 mm, was assessed. It was concluded that said reduction did not significantly improve either gas yield or production rates. Once again, it was concluded that the evolution of specific activities was assessed and used as a feasible tool to explain and manage the system's response, especially when conventional control parameters were not enough to explain the performance of the reactor.

In **chapter 8**, sewage sludge co-digestion with the OFMSW under thermophilic temperature conditions showed a good performance, with methane productivity and yield respectively 5 and 1.7 folds higher compared to SS anaerobic mono-digestion, when the OFMSW addition reached up to 33% of the COD inlet (with 23 days of HRT and 3.3 $kg_{COD}\cdot m^{-3}\cdot d^{-1}$of OLR). Specific activity tests showed that the initial biomass plays an important role in the anaerobic co-digestion performance, and suggests that the composition of the feed, the organic loading rate, and the temperature range have a great influence on specific substrate activities. In fact, under thermophilic temperature conditions, adding the OFMSW promoted the activity of saturated fatty acid oxidizers and acetoclastic methanogenic populations.

9.2. SUGGESTIONS FOR FURTHER RESEARCH

Although this thesis describes sewage sludge co-digestion as an effective strategy to boost methane production, there is still further research to be done. For example:

- Study instability events such as hydraulic and organic overloading shocks, and develop suitable recovery strategies
- Analyse the effect over the process of changes in composition of the co-substrate , determining its impact on the stability of the process and on methane production
- Study sewage sludge co-digestion as a strategy to balance the differences in quality and quantity of the sewage sludge organic loading rate; analysing synergetic effects of co-digestion.
- Develop control strategies to limit the impact of seasonal variations in the composition of the co-substrate and other instability events
- Integration of specific activity assays and/or microbial ecology tools as routine parameters, for a better understanding of the process and the optimization of biogas production
- New digester configurations, particularly the implementation of TPAD or a combination of different temperatures depending on the co-digestion mixture.