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Digestión Anaerobia de Lodos y Residuos Agroindustriales

"Anaerobic Digestion of Sewage Sludge and Agro-industrial Wastes"

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DIGESTIÓN ANAEROBIA DE LODOS Y RESIDUOS AGROINDUSTRIALES

"Anaerobic digestion of sewage sludge and agro-industrial wastes"

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A mi familia

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Resumen

El incremento en la producción de lodos generados como consecuencia a la depuración de las aguas residuales urbanas plantea actualmente un problema asociado a la complejidad del residuo y a la aparición de una legislación medioambiental cada vez más restrictiva frente a la gestión y disposición de los mismos.

La composición de estos lodos, les convierte en una fuente de materia orgánica importante para el proceso de digestión anaerobia. Sin embargo, este proceso suele estar dificultado por diversos factores relativos a la naturaleza del substrato. El pre-tratamiento del residuo o la inclusión de un co-substrato pueden ser una opción válida para conseguir un mejor aprovechamiento del proceso de digestión anaerobia.

Entre los residuos orgánicos, también destacan por su volumen y complejidad los residuos procedentes del sector agroindustrial, ya que ofrecen múltiples posibilidades de valorización conjunta. La digestión anaerobia aplicada a este tipo de residuos es una opción adecuada de gestión y valorización económica gracias al aprovechamiento del biogás y a la estabilización de los mismos.

Este trabajo está enfocado al estudio de la digestión anaerobia de lodos de depuradora (primario y secundario) y de residuos agroindustriales (grasas y residuos de biorefinería). Mediante la co-digestión y la utilización de pre-tratamientos se pretende conseguir aumentar el rendimiento del sistema con el consecuente incremento en la producción de metano.

En el capítulo 4 y 5 de este trabajo se recogen los ensayos de digestión de lodos y del residuo obtenido de la trampa de grasa de la depuradora, conocido por sus siglas en inglés como FOG (fat oil and grease). Se evaluó el efecto sobre la producción de biogás y se determinó la concentración adecuada de co-sustrato. El proceso se estudió en condiciones de operación semi-continua. Los ensayos no mostraron un incremento en la producción especifica de metano de 304 L CH_4/Kg SV por efecto de la adición de FOG con un 0.2% (v/v), apreciándose un detrimento en el proceso con el incremento en la adición de FOG al 1.8% (v/v), junto con una menor producción especifica de metano (200 L CH_4/Kg SV). Siguiendo la hipótesis de una inhibición del proceso debido a la adsorción de los componentes de FOG se realizó un análisis de espectroscopia por infrarrojo.

En el capítulo 4 se evaluó también la digestión de una grasa obtenida del procesamiento de leche. En este caso se observó una operación estable del proceso bajo régimen semi-continuo con un

tiempo de retención hidráulica (TRH) de 40 días consiguiendo una producción específica de metano de 440 L CH_4/Kg SV.

Siguiendo una metodología similar, en el capítulo 6 se presentan los ensayos de co-digestión de lodos y un residuo agroindustrial con alto contenido en lípidos (grasa de descarte de carnicerías). Se obtuvo un incremento en la producción de biogás en ambas condiciones de temperatura evaluadas (mesófilo-termófilo) y a pesar de algunos episodios de inhibición, un funcionamiento estable. La producción específica de metano fue de 520 L CH_4/Kg SV para el sistema en mesófilo y 516 L CH_4/Kg SV para el sistema termófilo. Estos resultados fueron significativamente mayores que los resultados obtenidos en los reactores de digestión con lodo (163 L CH_4/Kg SV para el sistema mesófilo y 121 L CH_4/Kg SV en el sistema termófilo).

Posteriormente en el capítulo 7 se realizó la evaluación del proceso digestión de lodos considerando la aplicación de dos pre-tratamientos diferentes: ultrasonidos (7.1) y microondas (7.2). En ambos casos los resultados mostraron un aumento en la producción de biogás. En el caso del pre-tratamiento con ultrasonidos se observó un aumento del 14% sobre una producción especifica de 233 L CH_4/Kg SV obtenida para el lodo sin pre-tratamiento y un 30% de incremento con el tratamiento al lodo secundario (sobre un valor base de 229 L CH_4/Kg SV). El pre-tratamiento con microondas de lodo secundario a diferentes energías, mostró un aumento en la solubilización de la materia orgánica y un aumento en la producción especifica de metano del 25% al 46% (rango de energía aplicado de 448-2700 kJ/L) sobre un valor de producción especifica del lodo secundario sin tratamiento de 303 L CH_4/Kg SV.

Por último se exploró la posibilidad de operar con un reactor anaerobio de flujo ascendente enfocado al tratamiento de un residuo agroindustrial procedente de una "biorefinería verde". Actualmente, el continuo desarrollo del concepto de biorefinería plantea también un reto interesante para la gestión de los residuos producidos en este tipo de nueva industria. Dichos residuos conocidos como "residuos agroindustriales de biorefinería" tienen un gran potencial de aprovechamiento para la producción de biogás mediante digestión anaerobia. El efluente utilizado es conocido como Jugo marrón y deriva de la fermentación del jugo verde tras la obtención de proteínas. En los ensayos de digestión anaerobia en discontinuo se alcanzaron buenos resultados de potencial de biogás (500.2 L CH_4/Kg SV). Estos resultados se recogen en el capítulo 8, que si bien comprenden una metodología distinta a la utilizada en los anteriores capítulos de este trabajo de tesis, supone la puerta hacia una nueva línea de trabajo en el grupo de investigación.

Abstract

The increase in sludge production from the biological treatment of urban wastewater treatment is currently becoming an issue of great concern due the complexity of the residue and the emergence of an increasingly restrictive regulation for the management and disposal of this type of wastes.

The sludge composition make them an important source of organic matter for the anaerobic digestion process. However, this process is often affected by several factors regarding the nature of the substrate. The pretreatment of the waste or the addition of a co-substrate may be a valid option for enhancing the anaerobic digestion process. Among organic wastes, the agro-industrial residues can offer multiple possibilities for combined valorization due to their high volumetric production and complexity. The Digestion of this type of organic wastes is an appropriate management alternative presenting high feasibility thanks to the utilization of biogas and waste stabilization.

This work is focused on the study of anaerobic digestion of sewage sludge (primary and secondary) and agro-industrial wastes (fats and biorefinery residues). Through the application of co-digestion and different pre-treatments is intended to achieve the stability of the digestion system and increase methane production.

In Chapters 4 and 5 of this work, it was tested the digestion and co-digestion of sludge and the fatty residue obtained from the grease trap of the wastewater treatment plant, which is known as FOG (fat oil grease). It was assessed the effect on biogas production and established the suitable content of co-substrate. Subsequently, the process was studied under semi-continuous conditions to evaluate the stability of operation. The experiments did not show an increase in biogas production (304 L CH_4/Kg VS) with the addition of FOG at 0.2% v/v, but presented a detriment in performance with the increase in FOG addition up to 1.8% (v/v) showing a lower methane yield (200 L CH_4/Kg VS). The inhibition process hypothesis of adsorption of FOG components was studied with the use of infrared spectroscopy analysis.

The digestion of an agro-industrial residue with high lipid content (fat obtained from processing milk) was also evaluated in chapter 4. Experiments showed stable performance under semi-continuous operation with an HRT of 40 days, achieving a methane yield of (440 L CH_4/Kg VS).

Following a similar methodology, the co-digestion experiments of sludge and an agro-industrial waste with high lipid content (fat discard butchers) are presented in Chapter 6. Results showed

an increase in biogas production for both temperatures tested (mesophilic-thermophilic). Despite some episodes of inhibition, the stable operation of the reactor was achieved. Methane yields were 520 L CH_4/Kg VS for the mesophilic system and 516 L CH_4/Kg VS, for the thermophilic system. These results were significantly higher than those obtained from the reactor digesting sludge (163 L CH_4/Kg VS for the mesophilic system and 121 L CH_4/Kg VS for the thermophilic system)

The assessment of sludge digestion with the application of two different pre-treatments is presented in Chapter 7: sonication (7.1) and microwave (7.2) pre-treatment. In both cases, the results showed an increase in biogas production. With regard to ultrasound pre-treatment, the results showed a 14% increase on methane yield, based on an initial value of 233 L CH_4/Kg VS which was obtained from the digestion of sludge, and a 30% increase when applied to the treatment of waste activated sludge (considering a comparison value of 229 L CH_4/Kg VS). The application of microwave pre-treatment at different energies on waste activated sludge showed an increase in organic matter solubilization and in methane yield from 25% to 46% (with the energy applied in a range of 448-2700 kJ/L) taking as basis the methane yield value of 303 L CH_4/Kg VS for untreated waste activated sludge.

Finally, the operating performance of an up-flow anaerobic reactor treating an agro-industrial waste from a "green biorefinery" was studied. Nowadays, the development of technologies associated with the biorefinery concept presents an interesting challenge regarding the management of waste produced by this new industry. Such waste, known as "agro-industrial biorefinery waste", has a great potential to be used for the production of biogas by anaerobic digestion. The effluent used in this study is known as brown juice and derived from the fermentation of green juice after obtaining proteins. Digestion batch tests showed good results of biogas potential (500.2 L CH_4 / Kg SV). These results are presented in Chapter 8 of this work, but comprise a different methodology to the conventional used in previous chapters of this work, and open possibilities to a new line of work in the research group.

Abreviaturas/Abbreviations

La siguiente lista de abreviaturas está relacionada a la parte experimental de este trabajo

BMP	Biochemical Methane Potential
COD	Chemical Oxygen Demand
CST	Capillary Suction Time
DTG	Derivative Thermogravimetry
FID	Flame Ionisation Detector
FOG	Fat Oil and Grease
FTIR	Fourier Transform Infrared Spectroscopy
GC	Gas Chromatography
HCA	Hierarchical Cluster Analysis
HRT	Hydraulic Retention Time
LCFA	Long Chain Fatty Acids
ORL	Organic Loading Rate
PCA	Principal Component Analysis
PS	Primary Sludge
PSD	Particle Size Distribution

SMP	Specific Methane Potential
SRF	Specific Resistance to Filtration
SS	Sewage Sludge
SSA	Specific Surface Area
STP	Standard Temperature Pressure
TCD	Thermal Conductivity Detector
TG	Thermogravimetry
TOC	Total Organic Carbon
TS	Total Solid
VFA	Volatile Fatty Acids
VS	Volatile Solids
WAS	Waste Activated Sludge -secondary sludge
WWTP	Waste Water Treatment Plant

Capítulo/Chapter 1

1. Introducción

1.1 Planteamiento de la situación actual

La creciente industrialización y las mejoras obtenidas en la calidad de vida en los últimos tiempos, ha conllevado al incremento de forma considerable del volumen de residuos generados. De aquí surge la preocupación y necesidad de aplicar sistemas adecuados de gestión de residuos para evitar impactos negativos sobre los ecosistemas, la biodiversidad y la salud humana.

La gestión y disposición del lodo que se genera como resultado de las distintas etapas de depuración de las aguas residuales, es un problema de creciente importancia, ya que dicha gestión, representa cerca de un 50% de los actuales costes de operación en las plantas de tratamiento (Appels et al., 2008).

Durante los últimos 20 años, la aplicación de la Directiva 91/271/ CEE relativa al tratamiento de aguas residuales urbanas obligó a los antiguos miembros de la Unión Europea (UE) a mejorar la recolección y el tratamiento de dichas aguas.

La cantidad de lodo de depuradora generado para algunos países de la Unión Europea está reflejada en la Tabla 1.1.

Tabla 1.1 Datos de producción de lodo en la UE

País	Producción de lodo 10^3 tm m.s/año							
	2004	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**
Bélgica	116	113	128	129	140	-	176	-
Bulgaria	58	42	38	40	43	39	50	52
República Checa	179	172	203	216	220	207	196	218
Dinamarca	-	-	-	140	108	108	141	-
Alemania	2261	2170	2049	2035	1982	1958	1780	-
Estonia	-	30	28	29	22	22	-	18
Grecia	83	117	126	134	136	152	-	147
España	1092	1121	1065	1153	1156	1205	1205	-
Francia	1060	-	-	-	1087	-	966	-
Croacia	-	-	-	-	-	30	30	31
Italia	-	1056	-	-	-	-	1103	-
Lituania	-	66	71	76	54	50	51	52
Luxemburgo	14	13	15	16	13	-	10	-
Hungría	161	261	238	205	172	149	170	168
Holanda	354	359	373	353	353	350	351	-
Austria	305	-	255	-	254	-	263	-
Polonia	476	486	501	533	567	563	527	519
Rumania	-	68	226	100	79	120	82	114
Eslovenia	10	14	19	21	20	27	30	26
Eslovaquia	53	56	55	55	58	59	55	59
Finlandia	150	148	150	147	144	149	-	-
Suecia	210	210	207	217	214	212	204	-
Reino Unido	1721	1771	1809	1825	1814	1761	1419	-
Suiza	205	-	210	-	210	210	-	-

p.e. Población equivalente (http://epp.eurostat.ec.europa.eu)

La producción de lodos varía significativamente entre los diferentes países. La mayor producción de lodos se observa en Alemania, Reino Unido, España, Francia e Italia. Estos países contribuyeron en casi un 73% del total de lodos producidos en la UE.

En España, el volumen de generación de dicho residuo se ha incrementado considerablemente en los últimos años (Figura 1.1). Según los datos del Registro Nacional de Lodos, hacia 2012 se producían anualmente alrededor de 1 129 000 toneladas m.s.

Se espera que esta cantidad vaya en aumento debido no solo al incremento en el número de estaciones depuradoras de aguas residuales construidas, sino también al aumento de la población y de los hábitos de consumo.

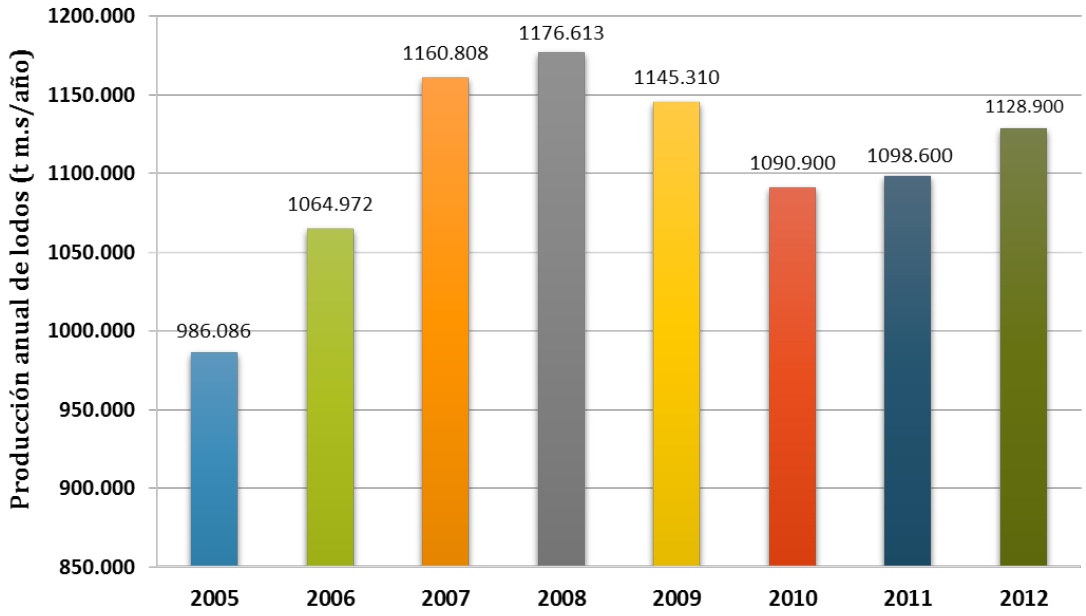

Figura 1.1 Evolución de la producción anual de lodos

Conforme a lo que establece la Ley 22/2011, de 28 de julio, de residuos y suelos contaminados, las estaciones depuradoras de aguas residuales, como productoras de lodos, deben asegurar la correcta gestión de dichos residuos. La orientación de su gestión debe realizarse respetando los principios de la política de residuos relativos a la protección del medio ambiente y la salud humana y aplicando la jerarquía en las opciones de gestión, priorizando la prevención sobre el reciclado y otros tipos de valorización, incluida la energética, quedando en último lugar el depósito en vertedero.

La gestión de lodos de depuradoras de aguas residuales, tiene con respecto a otros tipos de residuos la peculiaridad de que ciertos usos y posibilidades de reciclaje están regulados por normas específicas, algunas de carácter agronómico al existir la posibilidad de utilizarlos como abonos y enmiendas orgánicas en los suelos. En este sentido cabe mencionar la Directiva 86/278/CE, relativa a la protección del medio ambiente y en particular de los suelos en la utilización de los lodos con fines agrícolas. Esta Directiva regula las condiciones orientadas a evitar el posible efecto nocivo sobre las aguas, el suelo, la vegetación, los animales y la salud humana (PNLD 2007-2015). La citada directiva se incorporó a la legislación española mediante el Real Decreto 1310/1990. En él se designa al Ministerio de Agricultura, Pesca y Alimentación (actual MAGRMA) como el organismo competente en materia de aplicación y control.

Todas las orientaciones sobre la gestión de lodos se recogen en el Plan Nacional Integrado de Residuos (PNIR) (MAGRAMA, 2014a), así como en la legislación enumerada a continuación:

Relativas a la aplicación en la agricultura

- Directiva 86/278/CEE del Consejo, de 12 de junio de 1986, relativa a la protección del medio ambiente y, en particular, de los suelos, en la utilización de los lodos de depuradora en agricultura.
- Reglamento (CEE) 2003/2003 del Parlamento Europeo y del Consejo de 13 de octubre de 2003 relativo a los abonos.
- Real Decreto 1310/1990, de 29 de octubre, por el que se regula la utilización de los lodos de depuradora en el sector agrario.
- Orden de 26 de octubre de 1993, sobre utilización de lodos de depuración en el sector agrario y orden AAA/1072/2013, de 7 de junio, sobre utilización de lodos de depuración en el sector agrario
- II Plan Nacional de Lodos de depuradora de Aguas Residuales (2007-2015).
- Real Decreto 261/1996, de 16 de febrero, sobre protección de las aguas contra la contaminación producida por los nitratos procedentes de fuentes agrarias.

Relativas a la gestión de residuos

- Directiva 2008/98/CEE del Parlamento Europeo y del Consejo, de 19 de noviembre de 2008.
- Directiva 1999/31/CEE del Consejo, de 26 de abril 1999, relativa al vertido de residuos.
- Decisión del consejo de 19 de diciembre de 2002 por la que se establecen los criterios y procedimientos de admisión de residuos en los vertederos.
- Directiva 2000/76/CE of del Parlamento Europeo y del Consejo, de 4 Diciembre 2000 sobre la incineración de residuos.
- Ley 22/2011, de 28 de julio, de residuos y suelos contaminados.
- Real Decreto 1481/2001, de 27 de diciembre, por el que se regula la eliminación de residuos mediante depósito en vertedero.
- Real Decreto 653/2003, de 30 de mayo, Real Decreto 815/2013, de 18 de octubre, por el que se aprueba el Reglamento de emisiones industriales y de desarrollo de la Ley 16/2002, de 1 de julio, de prevención y control integrados de la contaminación, sobre incineración de residuos.

Los lodos deben someterse a algún tipo de tratamiento con el fin de estabilizarlos, reducir su volumen, mejorar sus características y reducir los problemas de salud asociados. Existe una tendencia general para reducirlos, reciclarlos y reutilizarlos de una forma respetuosa con el medio ambiente (MAGRAMA, 2014b).

La digestión anaerobia ha demostrado jugar un papel importante en la gestión del lodo, además de ser utilizada como método de estabilización, al mismo tiempo, es un proceso de generación de bioenergía, debido a la producción de biogás. El término biogás incluye una mezcla de gases producidos a lo largo de las múltiples etapas del proceso de descomposición de la materia orgánica, su alta concentración en metano le proporciona características combustibles ideales para su aprovechamiento energético como electricidad y calor mediante motores de cogeneración, calderas y turbinas.

La digestión anaerobia es considerada una parte importante y esencial de una EDAR moderna, debido a su capacidad para transformar la materia orgánica, reducir la cantidad de sólidos finales en el lodo, destruir la mayor parte de los patógenos presentes y evitar posibles problemas de olor asociados con la materia putrescible residual. Los lodos primarios tradicionalmente han sido tratados por esta vía, sin embargo, cuando se trata de lodos secundarios llamados también lodos activados, las opciones de tratamiento dependen de muchos factores ya que contienen sustancias poliméricas extracelulares y células microbianas que dificultan el proceso de digestión. Es por ello que en la última década, se han propuesto y ensayado diferentes tecnologías para facilitar el proceso de digestión y aumentar su productividad, tales como los pre-tratamientos de lodos activados y la co-digestión con otros substratos.

La co-digestión tiene como objetivo la dilución del potencial tóxico de algunos compuestos, mejorar del balance de nutrientes, obtener efectos sinérgicos entre los microorganismos, modular la carga orgánica biodegradable y el incremento en el rendimiento de la producción de biogás (Sosnowski et al., 2003). La producción de biogás mediante co-digestión anaerobia de residuos ha comenzado su desarrollo en España siguiendo los pasos de otros países europeos como Dinamarca, Alemania, Suecia o Austria. El creciente interés y desarrollo de esta alternativa se debe a la existencia en España de una gran cantidad y diversidad de residuos orgánicos biodegradables que son susceptibles de ser empleados para la producción de biogás. La disposición en vertedero de todos estos residuos es cada día más problemática y costosa debido a la normativa europea (Directiva 2008/98/CE) que limita la entrada de materiales orgánicos. Por ello, la producción de biogás constituye una alternativa de valorización cada vez más extendida.

Los residuos agroindustriales constituyen habitualmente materias primas o sustratos empleados en la producción de biogás. Estos residuos suelen variar considerablemente en su composición, homogeneidad o biodegradabilidad. Los residuos agroindustriales presentan un gran potencial para aumentar el rendimiento de metano, tal y como se ha demostrado en varios estudios (Martín-González et al., 2010; Suto et al., 2006; Kabouris et al., 2009; Tezel et al., 2009). En ellos se detallan los beneficios obtenidos de la co-digestión de estos residuos bajo condiciones mesofílicas y termofílicas que resultan en un aumento significativo de la producción de biogás. La adición de grasas y otros residuos agroindustriales se ha estudiado en diferentes procesos de co-digestión (Cuetos et al., 2008; Luostarinen et al., 2009; Lansing et al., 2010;) con resultados exitosos.

Los subproductos y residuos que forman el grupo de las materias primas agroindustriales son los que provienen de la agricultura, pesca y ganadería, de la industria alimentaria y de otras industrias similares, tales como: industrias de biodiesel, bioetanol y biorrefinerías.

Los residuos de alto contenido en lípidos como las grasas y los aceites se producen en cantidades considerables cada año a partir de las industrias de procesamiento de alimentos, los mataderos, las industrias de aceites comestibles, almazaras y la industria de productos lácteos (Cirne et al., 2007). Todos estos residuos, suelen ser problemáticos ya que causan problemas operacionales en digestores anaeróbios debido a la obstrucción, además de causar problemas de transferencia de masa, flotación de biomasa, etc. (Pereira et al., 2004). Sin embargo, son sustratos atractivos para la co-digestión debido al incremento en la producción de metano obtenido cuando se compara con proteínas o carbohidratos.

Por otro lado, las tecnologías de pre-tratamiento para lodos activados generados por las plantas de tratamiento de aguas, se han enfocado en la disrupción de las estructuras de flóculos y membranas celulares en lodos secundarios a través de fuerzas externas, tales como mecánicas (Baier & Schmidheiny, 1997), química (Chiu et al., 1997), ultrasonidos (Hogan et al., 2004 y Wood et al., 2009) , enzimática (Barjenbruch & Kopplow, 2003) , métodos térmicos y combinaciones (Valo et al., 2004 y Doğan & Sanín, 2009) para aumentar el material orgánico soluble y disponible en la digestión anaerobia y mejorar la productividad de la misma.

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Capítulo/Chapter 2

2. Antecedentes y objetivos

2.1 Lodos de depuradora

Los lodos de depuradora se definen como los lodos generados en el proceso de depuración del agua residual doméstica o urbana. Son una mezcla heterogénea compleja de microorganismos, material inorgánico y productos orgánicos no digeridos; como papel, residuos vegetales, aceites y material fecal (Degremont, 1979).

El proceso de depuración de aguas residuales comprende comúnmente un tratamiento para eliminar alrededor del 50-60% de los sólidos suspendidos y el 30-40% de la DBO (Appels et al., 2008). El **lodo primario** es producido durante los procesos de tratamiento primario de las aguas residuales, esto ocurre después de las rejas de desbaste, desarenado, tanque de sedimentación primario. La composición del lodo depende de las características del área de recogida de las aguas. El lodo primario contiene generalmente una gran cantidad de materia orgánica, vegetales, frutas, papel, etc.

La eliminación de la materia orgánica disuelta y los nutrientes de las aguas residuales tiene lugar durante el tratamiento biológico del agua. Normalmente se caracteriza por la interacción de distintos tipos de bacterias y microorganismos, que requieren oxígeno consumen materia orgánica. El lodo resultante se llama **lodo activado o secundario**. Normalmente este lodo está en forma de flóculos que contienen biomasa viva y muerta además de partes minerales y orgánicas adsorbidas y almacenadas. La biomasa genera sustancias que son una compleja mezcla de biopolímeros los cuales comprenden polisacáridos, proteínas, ácidos nucleicos, sustancias húmicas, lípidos, etc. Algunos compuestos específicos de estas sustancias poliméricas extracelulares son reconocidas por su carácter recalcitrante para los procesos anaerobios.

El comportamiento de sedimentación de los flóculos en los lodos activados es de gran importancia para el funcionamiento de una planta de tratamiento biológico. Los flóculos deben ser eliminados para separar la biomasa del agua tratada, y el volumen requerido de lodo activado pueda ser bombeado de nuevo al reactor biológico.

A causa de las características físico-químicas de los procesos de depuración, los lodos tienden a acumular una serie de contaminantes, tales como metales, patógenos, nutrientes y compuestos orgánicos (caracterización general en Tabla 2.1). Dichos contaminantes pueden impedir que se cumpla con los requisitos ambientales y legales, para su aplicación directa al suelo, por ello, están obligados a someterse a tratamientos biológicos (aerobios o

anaerobios), térmicos (secado o pasteurización), químicos (encalado) o de almacenamiento prolongado.

Tabla 2.1 Características generales de los lodos de depuradora

Parámetro	Lodo Primario	Lodo Secundario
Sólidos Totales (%)	5–9	0,8–1,2
Sólidos Volátiles (%)[a]	60–80	59–68
Nitrógeno (%)[a]	1,5–4	2,4–5,0
Fósforo (%)[a]	0,8–2,8	0,5–0,7
Celulosa (%)[a]	8–15	7–9,7
Hierro (g/kg)	2–4	–
pH	5,0–8,0	6,5–8,0
Grasa (%)	7–35	5–12
Proteína (%)	20–30	32–41
Alcalinidad (mg/L de $CaCO_3$)	500–1500	580–1100
Ácidos orgánicos (mg/L de acetato)	200–2000	1100–1700
Contenido Energético (kJ/kg TS)	23 000–2 900	19 000–23 000

[a] *Porcentaje sobre sólidos totales, (Fuente Tyagi & Lo, 2013)*

Entre los distintos métodos, la digestión anaerobia, es una de las técnicas más aplicadas entre los estados miembros de la UE (Spinosa, 2011, Cao & Pawloski, 2012). Es una de las tecnologías más eficientes para la estabilización y gestión de los lodos; ya que, reduce el contenido final en sólidos, destruye la mayoría de patógenos presentes, reduciendo los problemas de olor asociados y optimiza los costes en las plantas depuradoras a través de la producción de biogás gracias a su uso como fuente de energía.

Además del acondicionamiento del residuo para cumplir con los requisitos legales, estos tratamientos tienen como objetivo reducir el contenido de agua en el lodo crudo y transformar la materia orgánica altamente putrescible en un residuo estable o inerte.

2.2 Residuos agroindustriales

La agroindustria se define como el conjunto de actividades de manufacturación mediante las cuales se elaboran materias primas y productos derivados del sector agrícola. Por tanto se refiere a la transformación de productos procedentes de la agricultura, la ganadería, la actividad forestal y la pesca (FAO, 1997).

Es evidente que las actividades acogidas a esta definición forman un grupo muy variado: desde la extracción de las materias primas hasta la producción de productos manufacturados como textiles, transformados alimentarios, papel, biocombustibles y otros productos con alto valor añadido.

Entre estos tipos de materias primas agroindustriales merece mencionar por su potencial en la producción de biogás las siguientes:

- De origen animal: estiércoles, purines y gallinaza.
- De origen vegetal: hierba, hoja de remolacha, paja, trigo, cultivos energéticos, etc.
- De la Industria: residuos provenientes de la industria de biodiesel, bioetanol y biorrefinerías.
- Otros residuos de la cadena alimentaria: residuos de comida y aceites de gastronomía.

Pese a su importante contribución, la agroindustria puede tener también efectos perjudiciales para el medio ambiente, generando impactos ambientales de todo tipo: vertidos perjudiciales en los medios hídricos o edáficos, emisiones tóxicas en el aire y la producción de importantes volúmenes de residuos.

2.2.1 Residuos agroindustriales con alto contenido en lípidos (grasas y aceites)

Los lípidos son un grupo de biomoléculas que se caracterizan por ser poco o nada solubles en agua y, por el contrario, muy solubles en disolventes orgánicos no polares. Las grasas son compuestos orgánicos heterogéneos encontrados en plantas y animales, las cuales forman una categoría de lípidos con una estructura química y propiedades físicas específicas.

Químicamente las grasas son ésteres de ácidos grasos y glicerina:

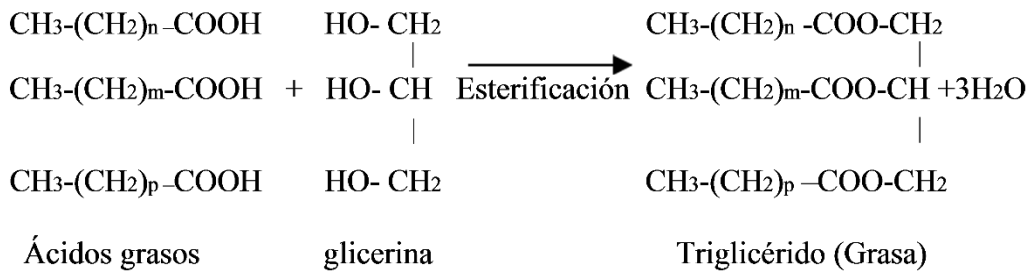

Figura 2.1 Composición de las grasas

La mayor parte de las grasas son triglicéridos y n, m, p son números que van generalmente del 10 al 18 (Figura 2.1), aunque hay excepciones como en el caso de la mantequilla que cuenta con ácidos grasos de 2 al 8 (Gunstone F. 1967).

Las opciones de disposición y tratamiento de las grasas pueden incluir soluciones tales como: aplicación directa a la tierra, depósito en vertederos, compostaje, fabricación industrial de lubricantes o jabones, incineración, digestión anaerobia, o producción de biodiesel (Wiltsee, 1998; Rohm, 2005; Chung et al., 2010).

Las grasas y aceites son considerados como residuos de alto contenido en lípidos y son producidos generalmente en las industrias de procesamiento de alimentos, los mataderos, las industrias de aceites comestibles, almazaras y las industrias de productos lácteos (Li et al., 2002, Cirne, 2006). En la literatura se puede encontrar la referencia a este tipo residuos con el término de FOG *(Fat, Oil and Grease)* (Mata-Alvarez et al., 2014). El término FOG es también utilizado por otros autores para definir la capa del material rico en lípidos que se forma en el agua residual generada en las industrias de procesamiento de alimentos y cocinas (Suto et al., 2006; Bailey, 2007; Kabouris et al., 2008, 2009a, b; Davidsson et al., 2008; Long et al., 2012). La descarga directa en los sistemas de colección de agua puede ocasionar severos problemas de mantenimiento y altos costes en la EDAR, pues, una vez en las tuberías el FOG se acumula en las paredes formando una capa endurecida que limita la capacidad de conducción (He et al., 2011).

La separación de FOG de las aguas residuales, se realiza a través de unidades denominadas "trampas de grasa" o interceptores. Estos dispositivos de reducción de grasa son generalmente módulos de separación por gravedad de flujo continuo no mecanizados que retienen la grasa y los alimentos sólidos en suspensión, proporcionando tiempo suficiente para la flotación/sedimentación del influente (Figura 2.2).

Figura 2.2 Trampa de grasas EDAR

2.2.2 Residuos agroindustriales de biorrefinería

En el contexto actual, el concepto "biorefineria verde" juega actualmente un papel importante en términos de la utilización de materias primas no alimentarias como pasto y residuos agrícolas para la fabricación de una variedad de productos tales como, biocarburantes, productos químicos, fibras o energía, por medio de procesos flexibles y eficientes. Asociado a este nuevo planteamiento, los países industrializados han comenzado a considerar los residuos agroindustriales como una materia prima idónea para la producción de productos químicos y energía.

Dichos procesos de transformación generan sub-productos y/o residuos que deben ser adecuadamente gestionados para evitar problemas ambientales futuros. Una buena alternativa para el aprovechamiento de estos materiales resultantes es la producción de bioenergía por distintas vías: bien la valorización energética directa para obtención de electricidad y/o calor o la producción de biogás mediante digestión anaerobia.

Dentro de una biorrefinería verde (Figura 2.3) la biomasa fresca se fracciona mecánicamente en una prensa produciendo una torta compuesta principalmente por fibras y un jugo verde cuya composición principal es proteínas y carbohidratos.

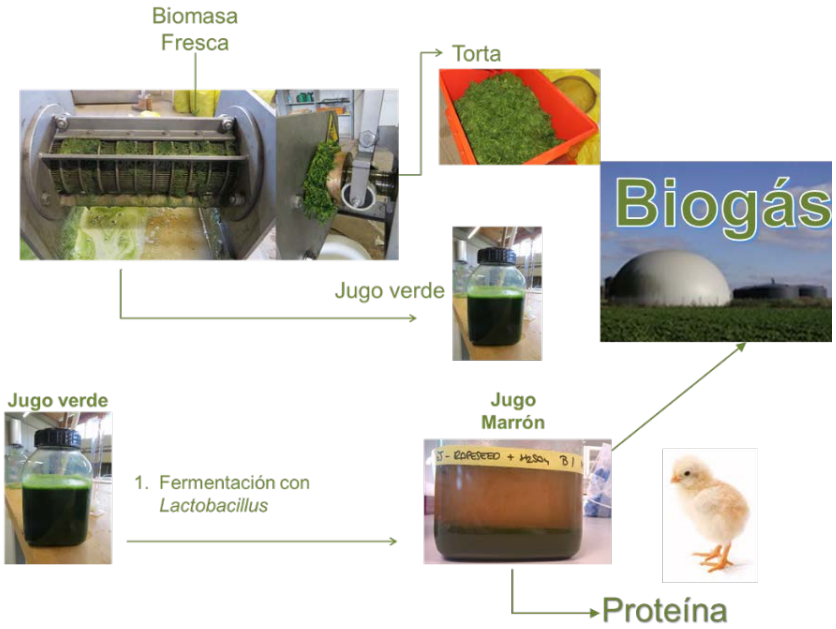

Figura 2.3 Diagrama de refinería verde

El jugo verde es inoculado con una bacteria específica del ácido láctico para llevar a cabo una fermentación y obtener la precipitación de proteínas. La proteína aislada se utiliza esencialmente como alimento para animales. Por otro lado, el residuo obtenido tras la

fermentación del jugo verde es conocido como **jugo marrón,** dicho residuo está compuesto por carbohidratos, proteínas y minerales haciéndolo factible como materia prima para la producción de biogás mediante digestión anaerobia.

2.3 Digestión anaerobia

La digestión anaerobia consiste en la degradación biológica de sustancias orgánicas complejas en ausencia de oxígeno libre y mediante la acción de un grupo de microorganismos específicos, que descomponen la materia orgánica en productos gaseosos (CH_4, CO_2, H_2, H_2S, etc.), y digestato, que es una mezcla de productos minerales (N, P, K, Ca, etc.) y compuestos orgánicos de difícil degradación (Dhamodharan & Kalamdhad, 2014). A pesar de que se trata de un proceso complejo, su uso para el tratamiento de los residuos orgánicos y la estabilización de biorresiduos se ha incrementado notablemente en los últimos años (Hansen et al., 1999; Bolzonella et al., 2003). Actualmente, el interés de estas tecnologías está motivado principalmente por la creciente sensibilización hacia los problemas del medio ambiente, el elevado precio de los combustibles fósiles, el empobrecimiento de los suelos agrícolas en materia orgánica, el encarecimiento de los fertilizantes minerales, etc.

La digestión anaerobia es un proceso complejo en el que intervienen diferentes grupos microbianos de manera coordinada y secuencial. El principal producto obtenido es el biogás, una mezcla gaseosa formada principalmente por metano y dióxido de carbono y pequeñas proporciones de otros gases como H_2S, H_2, NH_3, etc, aunque la riqueza en metano del biogás dependerá del residuo degradado, así como del propio proceso (Appels et al., 2008).

La interacción cooperativa de varios grupos fisiológicos de procariotas permite la formación de metano a partir de sustancias de elevado peso molecular, tales como polisacáridos, proteínas y grasas. Los precursores inmediatos del metano son el hidrógeno y el dióxido de carbono, que se generan por las actividades de los fermentadores anaerobios.

El proceso de la digestión anaerobia se puede representar, de forma química, mediante la reacción:

$$\mathbf{CmHnOp \rightarrow r\ CH_4 + s\ CO_2 + H_2O}$$

Donde r+s = m

Esta simple expresión para la conversión de materia orgánica de un sustrato complejo en metano y dióxido de carbono refleja en realidad un conjunto de etapas, en serie o serie/paralelo, en las que están implicadas un número considerable de microorganismos (Baraza et al., 2003).

Tradicionalmente, la degradación anaerobia ha sido considerada como un proceso en dos etapas, tal y como muestra el esquema representado en la Figura 2.4 **:** 1) Hidrólisis y fermentación de la materia orgánica compleja en ácidos orgánicos simples e hidrógeno, y 2) Conversión de ácidos orgánicos en metano, aceptando la existencia de dos grandes grupos de microorganismos, las bacterias formadoras de ácidos o acidogénicas y los microorganismos responsables de la formación de metano o metanógenos (McCarty, 1981).

Figura 2.4 Esquema del proceso de digestión descrito en dos etapas

Sin embargo, una descripción más detallada del proceso obliga a considerar hasta cuatro etapas sucesivas (Breure, 1986; Romero, 2002), siendo la primera la ***hidrólisis*** donde las bacterias celulolíticas, hidrolíticas y acidógenas excretan exoenzimas, que permiten la ruptura de los polímeros orgánicos hasta subunidades más pequeñas, fácilmente transportadas al interior celular. La siguiente etapa se corresponde con la etapa ***fermentativa o acidogénica***, en la cual las bacterias acidificantes transforman la materia orgánica disuelta, originando como productos principales ácidos grasos volátiles (AGV - acetato, propionato, butirato, sucinato), así como pequeñas cantidades de ácido láctico y etanol, dióxido de carbono e hidrógeno (Stams, 1994). En presencia de ácidos grasos de cadena larga debe tener lugar su oxidación. Estos ácidos grasos libres son introducidos en la célula a través de la pared celular. Este proceso puede ser desarrollado por un gran número de microorganismos. Una vez dentro de la célula el ácido es convertido en el correspondiente tio-ester-CoA, lo que sirve tanto para activar su degradación, como para disminuir su efecto tóxico. La ruta principal de oxidación de los ácidos grasos de cadena larga es la β-oxidación, la cual se desarrolla en espiral de modo que se libera una molécula de Acetil-CoA en cada bucle, produciendo principalmente ácido acético.

En la etapa ***acetogénica*** los componentes más reducidos son oxidados. Así, los AGV con tres o más carbones son oxidados a acético, hidrógeno y dióxido de carbono. Esta conversión es sólo posible si la presión parcial de hidrógeno se mantiene en valores bajos, con presiones parciales menores de 10-3 atm (Zinder, 1984). Esta oxidación es llevada a cabo por bacterias facultativas denominadas "acetógenas" u "organismos protón-reductores obligados" (McCarty, 1981). Se produce también la respiración acetogénica de bicarbonato por bacterias homoacetogénicas las cuales catabolizan mezclas de dióxido de carbono e hidrógeno a compuestos de carbonos múltiples.

La ***metanogénesis*** es la etapa final e implica dos tipos de reacciones, aquellas en las que el dióxido de carbono e hidrógeno se combinan para producir metano y agua, y las que convierten el acetato en metano y dióxido de carbono. En esta etapa, la mayor parte de la energía química contenida en el sustrato es convertida en metano por la actuación de Archaea. Se pueden establecer dos grandes grupos de microorganismos, en función del substrato principal, dividiéndose en los "hidrogenotróficos", que consumen hidrógeno, y los "acetoclásticos", que consumen grupos metilos del acetato y como productos finales se obtiene metano y dióxido de carbono. Esta reacción es llevada a cabo por dos únicos géneros de Archaea: *Methanosarcina* y *Methanotrix*.

Para el correcto funcionamiento del proceso es necesario que las velocidades de transformación metabólica de los diferentes grupos bacterianos estén equilibradas, ya que los productos finales de una etapa son consumidos en la siguiente, dando lugar a una relación simbiótica que estabiliza el proceso. El crecimiento y la actividad de dichos grupos bacterianos están influenciados significativamente por diversos parámetros tanto ambientales como operacionales.

Los parámetros ambientales que hay que controlar hacen referencia a condiciones que deben mantenerse o asegurarse para el desarrollo del proceso, tales como el pH y alcalinidad, nutrientes y componentes tóxicos e inhibidores. Por otro lado, los parámetros operacionales hacen referencia a las condiciones de trabajo de los reactores como la temperatura, agitación y tiempo de retención hidráulica.

El principal problema para la aplicación de la digestión anaerobia en la estabilización y gestión de lodos, radicará en las características y composición del lodo secundario, ya que la hidrólisis de las sustancias complejas de dicho lodo, puede ocasionar rendimientos pobres en el proceso. En los últimos años, muchas investigaciones se han centrado en aumentar la digestibilidad del lodo secundario y eliminar los problemas de espumas asociados a su co-digestión con el lodo primario mediante la aplicación de diferentes pre-

tratamientos (Lu et al., 2008; Tomei et al., 2008; Mottet et al., 2009; Naddeo et al., 2009; Erden & Filibeli, 2010; Montusiewicz et al., 2010). También se ha planteado la co-digestión de lodos con la fracción orgánica de los residuos sólidos urbanos (FORSU) para tratar de valorizar estos dos residuos de difícil aceptación pública (Rintala et al., 1996; Sosnowski et al., 2008; Pahl 2008). A su vez, se han logrado avances importantes en la reducción de los tiempos de residencia de los reactores por medio de la elevación de la temperatura consiguiendo biosólidos con un menor contenido en patógenos (Ponsá et al., 2008). Un breve resumen de las experiencias de digestión anaerobia en los últimos años se presenta en la Tabla 2.2.

La digestión anaerobia de los residuos grasos es una opción atractiva debido al alto potencial de metano que estos residuos ofrecen. Sin embargo, durante la digestión de grasas, se debe tener en cuenta que en el proceso de degradación anaerobia de los lípidos, se presenta inicialmente la hidrólisis de los mismos por lipasas extracelulares para generar ácidos grasos de cadena larga y glicerol. Este último, es un compuesto que presenta una alta solubilidad y que es degradado fácilmente por los microorganismos, convirtiéndolo rápidamente a ácidos grasos de cadena corta y posteriormente a metano. Por otra parte los ácidos grasos de cadena larga generados en la hidrólisis, son primero adsorbidos sobre la superficie de los microorganismos y finalmente degradados mediante β -oxidación. Este último paso conlleva a la generación de acetato e hidrógeno, los cuales son posteriormente transformados en biogás (Battimelli et al., 2010). Estos residuos se suelen caracterizar por presentar un bajo contenido en nutrientes generando a su vez sistemas de digestión con una baja alcalinidad (Angelidaki et al., 1997a; Angelidaki et al., 1997b). Uno de los problemas principales está relacionado con la toxicidad que ejercen los intermediaros de AGCL generados en el mismo proceso (Hanaki et al., 1981; Hwu et al., 1997; Hwu et al., 1998). Otro de los problemas está asociado a la absorción de los lípidos sobre la masa microbiana causando a su vez la flotación de la biomasa (Hwu et al., 1997) y su posterior lavado del reactor.

El grado de inhibición observado por AGCL depende del tipo de microorganismos, la superficie específica de los lodos, la longitud de la cadena de carbono y del grado de saturación de los AGCL (Hwu et al., 1996; Salminen et al., 2000). La acumulación de estos componentes durante el proceso de digestión puede provocar la inhibición de acetógenos y metanógenos (Hanaki et al., 1981; Hwu et al., 1996; Salminen et al., 2002). Sin embargo, en estudios recientes se ha descrito que dicha inhibición podría ser reversible, siendo la aclimatación un factor clave para evitar efectos negativos sobre las comunidades microbianas. Martín-González et al., 2010; Pereira et al., 2004; 2005; Cuetos et al., 2008

reportaron la digestión de residuos de alto contenido en lípidos con éxito tras un período prolongado de aclimatación. Otra alternativa utilizada para evitar la inhibición por AGCL es la co-digestión.

Tabla 2.2 Experiencia de digestión anaerobia de lodos (régimen mesófilo)

Sustrato	Mezcla (%)	Escala	Comentarios	Potencial de Biogás (m^3/kg VS)	Referencia
Lodo secundario	100	Piloto	Pre-tratamiento, TRH 20–30días	0,6	*Nah et al., 2000*
Lodo/residuos frutas	82:18*	Completa	TRH 20días	0,57[a]	*Edelmann et al., 2000*
Lodo secundario	100	Laboratorio	Pre-tratamiento, TRH 8–12días	0,15–0,25[a]	*Lafitte-Forster, 2002*
Lodo depuradora	100	Laboratorio	Discontinuo 1-4g SV/l	0,12[a]	*Kim et al., 2003*
Lodo/residuos de comida	50:50*	Laboratorio	Discontinuo 1-4g SV/l	0,21[a]	*Kim et al., 2003*
Lodo depuradora	100	Piloto	-	0,27[a]	*la Cour Jansen et al., 2004*
Lodo/residuos comida	80:20*	Piloto	-	0,32[a]	*la Cour Jansen et al., 2004*
Lodo secundario/FORSU	84:16*	Completa	Carga orgánica 1,0 a 1,2 kgSV/m^3día	0,17[a]	*Bolzonella et al., 2006*
Lodo depuradora	100	Laboratorio	-	0,32[a]	*Sosnowski et al., 2008*
Lodo secundario/FORSU	75:25*	Laboratorio	-	0,44[a]	*Sosnowski et al., 2008*
Lodo/Glicerina	99:1(v/v)	Laboratorio	TRH 23–25días	0,68	*Fountoulaki et al., 2010*
Lodo/Aceite usado	94:6*	Completa	TRH 57días	0,63	*Pastor et al., 2013*

**Base sobre sólidos volátiles, [a] Expresado en m^3 CH_4/Kg SV*

2.4 Estrategias para mejorar la digestión anaerobia

2.4.1 Co-digestión de residuos

Se denomina co-digestión de residuos a la digestión de al menos dos residuos diferentes. La co-digestión es una opción interesante para aumentar la producción de biogás de algunos residuos mediante el efecto sinérgico que se establece en el medio (Mata-Alvarez, 2003). Además del incremento en la producción de biogás, la co-digestión presenta las siguientes ventajas (Mata-Álvarez et al., 2000):

- Dilución de compuestos tóxicos o inhibidores del proceso.
- Incremento de la carga orgánica en el digestor y mejor aprovechamiento de su volumen.
- Aumento de la estabilización del digerido.
- Mayor reducción de emisiones de gases de efecto invernadero a la atmósfera.
- Ahorro por el aprovechamiento de equipo y costes al tratar varios residuos en el mismo lugar.

La co-digestión anaerobia de los residuos grasos se ha convertido en una opción interesante debido a su alto potencial de metano (0,7 – 1,0 m^3 CH_4 /kg SV). La adición de pequeñas cantidades de grasa incrementan significativamente los rendimientos. Los estudios realizados por diversos autores están resumidos en la Tabla 2.3, obteniéndose resultados prometedores en la co-digestión de lodos con grasas recolectadas en la trampa de grasas de mataderos (Luostarinen et al., 2009), la co-digestión de purines y grasas de cocinas (Lansing et al., 2010), etc.

Tabla 2.3 Experiencias de co-digestión en régimen mesófilico de residuos con alto contenido en lípidos y lodos

Sustrato	Mezcla (%)	Carga orgánica (kg SVm^3/d)	Rendimiento (m^3 CH_4/kg SV)	Referencia
Lodo/FOG	40:60*	3,50	0,49	*Noutsopoulos et al.,2013*
Lodo secundario/FOG	48:52*	1,2	0,55	*Girault et al., 2012*
Lodo/FOG	77:23*	1,6	0,37	*Silvestre et al., 2011*
Lodo /FOG	77:23*	1,58	0,63	*Razaviarani et al., 2013*
Lodo secundario/FOG	64:36*	2,34	0,60	*Wan et al., 2011*
Lodo/FOG	99.8:0.2 (v/v)	0,77	0,30	*Martinez et al., 2012*
Lodo/aceite usado	80.6:19.4*	0,91	0,47	*Pastor et al., 2013*
Lodo secundario/FOG	34.5:65.5*	2,16	0,75	*Wang et al., 2013*
Lodo/residuo matadero	95:5 (p/v)	2,68	0,62	*Pitk et al., 2013*
Lodo/residuo matadero	7:1 (p/v)	2,8	0,43	*Luste & Luostarinen, 2010*

**Base sobre sólidos volátiles, [a] Expresado en m^3 CH_4/Kg SV*

2.4.2 Pre-tratamientos

Como ya se mencionó anteriormente la digestión anaerobia es una tecnología eficiente y comúnmente utilizada para la estabilización y gestión de los lodos. Pero en la digestión anaerobia de lodos activados, la hidrólisis es considerada como el paso limitante. Una mejora en la eficiencia de la misma se estima de mucha importancia para el proceso.

La naturaleza recalcitrante de los lodos activados (secundarios) se debe a los exopolímeros que forman parte del flóculo y a la pared celular de los microorganismos presentes en el lodo. Para aumentar la eficiencia del proceso es necesario propiciar tanto la ruptura de la célula como la hidrólisis de las macromoléculas y otros compuestos celulares. También es necesario promover la hidrólisis de lípidos y otros materiales poliméricos que provocan la formación de espumas.

Con la intención de acelerar la desintegración del floculo y la ruptura de la célula, se han estudiado diferentes pre-tratamientos que permiten aumentar la disponibilidad de los sustratos, romper la pared celular y liberar las proteínas, carbohidratos, lípidos y ácidos nucleicos, los cuales forman la mayor parte del material celular.

Dichos pre-tratamientos se dividen en:

- Mecánicos: Desintegración de las partículas sólidas.
- Químicos: Destrucción de compuestos orgánicos complejos por medio de ácidos o bases fuertes.
- Térmicos: Hidrólisis térmica.
- Enzimáticos y microbianos.
- Estimulación de microorganismos anaerobios: Adición de algunos compuestos que actúan como estimulantes del crecimiento bacteriano.

2.4.2.1 Tratamientos mecánicos: Ultrasonidos

El ultrasonido se propaga por un medio (agua) mediante una sucesión de compresiones y expansiones periódicas con una frecuencia mayor a 20 kHz (Figura 2.5). Dependiendo de la frecuencia alcanzada se distinguen tres regiones: Ultrasonido (20–100 kHz), Ultrasonido de alta frecuencia (100 kHz–1 MHz), y Ultrasonido de diagnóstico (1–500 MHz). Estas tres regiones tienen amplias aplicaciones en diversos campos, como química, física, medicina, etc.

El avance de la tecnología ha permitido, entre otras cosas, recuperar el material intracelular mediante la destrucción de las paredes celulares, obteniendo buenos resultados en su posterior aplicación a gran escala para la desintegración de lodos (Hogan et al., 2004).

Cuando la onda de ultrasonido se propaga genera compresiones y rarefacciones. Los ciclos de compresión ejercen una presión positiva sobre el líquido empujando las moléculas entre ellas y la rarefacción ejerce una presión negativa tirando de las moléculas entre sí. Esta presión negativa, al alcanzar cierto nivel, provoca la formación de microburbujas (burbujas

de cavitación) en las regiones de rarefacción.Estas microburbujas crecen en ciclos sucesivos y llegan a un tamaño inestable que colapsa violentamente (alrededor de 5 000 °C y 500 atm.). Este proceso de formación de burbujas, crecimiento y colapso violento es conocido como cavitación (Figura 2.6) (Flint & Suslick 1991; Suslick, 1991; Onyeche et al., 2002; Gogate & Kabadi 2009).

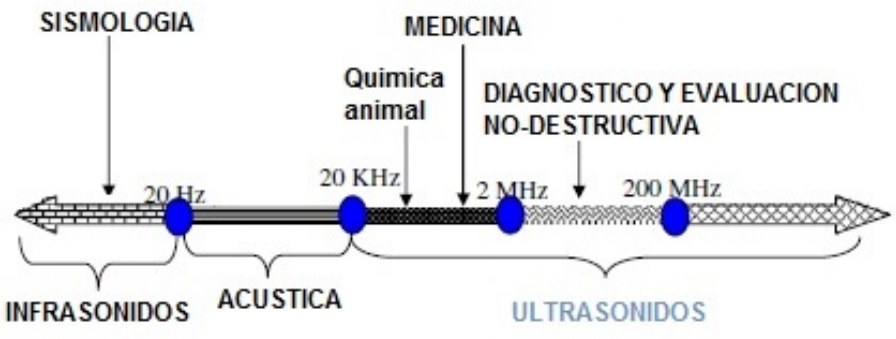

Figura 2.5 Esquema Ultrasonido

Las burbujas de cavitación generadas a rangos de frecuencias bajas (20-100 kHz) dan lugar a esfuerzos cortantes muy potentes. En estas condiciones tiene lugar la máxima desintegración celular, la desinfección, la ruptura de polímeros y la liberación de enzimas al medio.

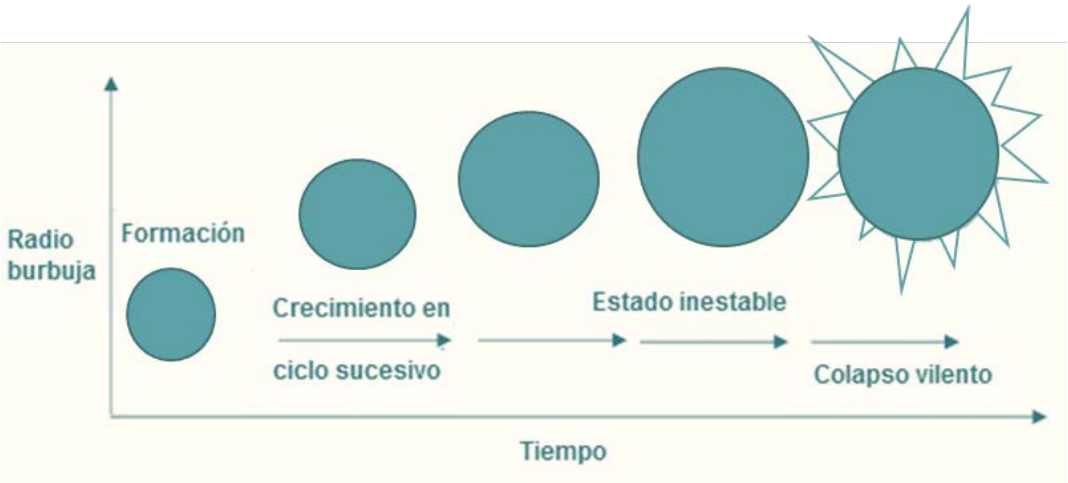

Figura 2.6 Desarrollo y colapso de la burbuja de cavitación

La eficiencia en la desintegración de los lodos por esta técnica se basa en los factores que afectan al fenómeno de cavitación:

- Presión externa: El aumento de la presión externa aumenta la presión de rarefacción, lo que aumenta la intensidad de la cavitación (Shah et al., 1999; Thompson & Doraiswamy, 1999).
- Gas y material particulado: La presencia de gas/aire en el líquido disminuye el umbral cavitacional reduciendo la intensidad de la onda de choque liberada. El material particulado reduce el efecto de cavitación (Mason & Lorimer, 2002).
- Viscosidad del medio: Las fuerzas de cohesión físicas que actúan en el líquido actúan sobre la presión negativa en la expansión o ciclo de rarefacción, por lo que para aumentar el umbral de cavitación las fuerzas cohesivas naturales necesitan ser altas (Capote & de Castro 2007).
- Tensión superficial: La adición de tensoactivos a una solución acuosa facilita la cavitación. El aumento de la viscosidad del disolvente y la tensión superficial, reduce la tasa de formación de microburbujas pero aumenta la intensidad de colapso de la burbuja.
- Presión de vapor: La cavitación resultará difícil si la presión de vapor del líquido es baja (Thompson & Doraiswamy, 1999).
- Frecuencia aplicada: La fase de rarefacción se acorta mediante el aumento de la frecuencia de la irradiación, pero para mantener una cantidad equivalente de energía de cavitación en el sistema la potencia debe aumentarse. De este modo, a una frecuencia más alta se requiere más energía para mantener el mismo efecto cavitacional (Lorimer & Mason 1987; De Visscher & Langenhove, 1998).
- Temperatura: El umbral de cavitación aumenta con la disminución de la temperatura. Con el aumento de la temperatura, el disolvente alcanza el punto de ebullición y produce un mayor número de burbujas, que actúan como barrera para la transmisión de la onda y anulan la efectividad de la energía de ultrasonido.
- Densidad del sonicado: El incremento de la densidad de sonicado aumenta los efectos de cavitación.
- Intensidad acústica: El aumento de la intensidad de ultrasonidos aumenta los efectos de sonicación (Shah et al., 1999).
- Atenuación: La intensidad del ultrasonido se atenúa a medida que progresa a través del medio. La atenuación es inversamente proporcional a la frecuencia de ultrasonidos, es decir, la energía se disipa en forma de calor.

La potencia/energía aplicada para la desintegración de lodos por diversos autores está expresada de acuerdo a las siguientes ecuaciones (Nels et al., 2000; Tiehm et al., 2001; Feng etal., 2009):

Energía Específica (kJ/KgST):

$$E_s = {}^{P \times t}/_{V \times ST}$$

Dosis de Ultrasonidos (J/L):

$$U_{D0} = {}^{P \times t}/_{V}$$

Densidad de Ultrasonidos (W/L):

$$U_D = {}^{P}/_{V}$$

Intensidad de Ultrasonidos (W/cm²):

$$U_I = {}^{P}/_{A}$$

Donde, ***P*** es la potencia (kW); ***T*** es el tiempo de sonicado (s); ***V*** es el volumen (L); ***ST*** son los sólidos totales del medio (kg/L) y ***A*** es el área de la superficie (cm²).

El esfuerzo cortante producido por la onda de alta presión que genera el ultrasonido rompe la pared celular y libera las sustancias intracelulares en fase acuosa. Esto cambia las propiedades físicas y químicas durante el pre-tratamiento de lodos. Por lo tanto, el grado de desintegración debe ser evaluado sobre la base de los cambios físicos (distribución de tamaño de partícula, turbidez, decantación y el examen microscópico), químicos (aumento de material orgánico, concentración de proteína, contenido de polisacáridos, nitrógeno y la liberación de amonio).

La aplicación de ultrasonidos para aumentar la digestibilidad de los lodos ha sido ampliamente estudiada, identificándose como una herramienta rentable para mejorar la producción de biogás durante la digestión anaerobia (Climent et al., 2007; Aldin et al., 2010; Elbeshbishy et al., 2011). Estudios sobre pre-tratamiento de lodos secundarios aplicando energías específicas que van desde 1 000 a 10 000 kJ/kg ST demostraron provocar aumentos en la producción de biogás de hasta un 40% (Khanal et al., 2007).

El pre-tratamiento del lodo con ultrasonidos ha probado tener efectos significativos en la biodegradabilidad durante el proceso de digestión anaeróbia, incrementando la generación

de biogás y mejorando las características inherentes al lodo, por lo que se ha convertido en una tecnología emergente para el tratamiento de lodos a escala industrial.

En el trabajo deTiehm et al. (2001), se evaluó el efecto de la digestión de lodos después del pre-tratamiento con ultrasonidos estudiando el tiempo de sonicado, encontrándose un porcentaje de reducción mayor en los sólidos volátiles cuanto mayor era el tiempo de sonicado, así como valores más altos de producción de gas. Dicho efecto también se evaluó por Wang et al. (1999). El biogás se incrementó en 12, 31, 64 y 69%, correspondiente a un tiempo de sonicado de 10, 20, 30 y 40 min, respectivamente.

Respecto a las propiedades físicas, la aplicación de ultrasonidos tiene también importantes efectos. El tamaño de partícula del lodo disminuye con el incremento de energía aplicado, pero más allá de cierto punto, la aplicación de energías más altas puede ocasionar un nuevo incremento en el tamaño de partícula debido a la re-floculación.

2.4.2.2 Tratamientos térmicos: Microondas

Se denomina microondas a una forma de energía electromagnética situada en el rango de frecuencias (300 -300 000 MHz) correspondiente a una longitud de onda entre 1m-1mm. Al incidir sobre un cuerpo, la radiación de tipo microondas afecta la rotación de las moléculas de la sustancia que lo conforma, mientras que su estructura molecular se mantiene inalterable.

Contrario al proceso de calentamiento convencional en el que el calor es conducido a través de las paredes del recipiente, en el calentamiento por microondas, el transporte de calor no es dependiente de la conductividad térmica del recipiente que contiene el material. Se genera como resultado un super-calentamiento localizado de forma instantánea.

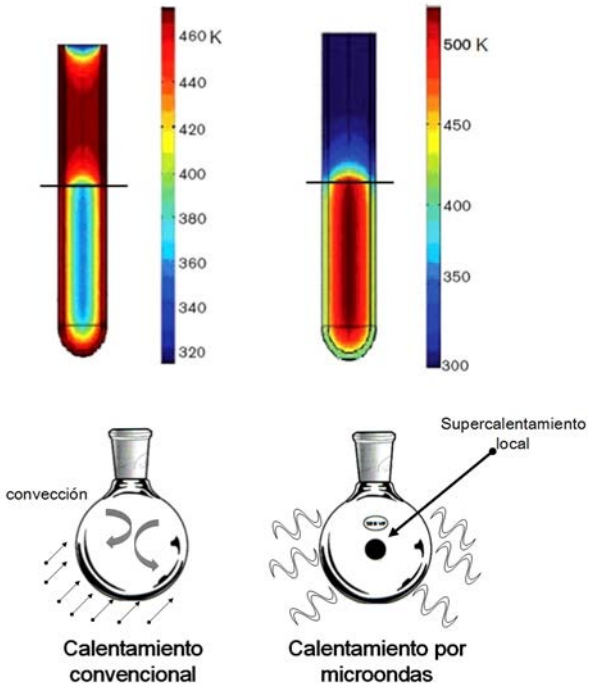

Figura 2.7 Mecanismo de calentamiento

Los dos mecanismos de transferencia de energía por microondas para conseguir el calentamiento de la muestra son la rotación dipolar y la conducción iónica (Figura 2.7).

- -Rotación dipolar: La rotación dipolar es una interacción en la cual las moléculas polares intentan alinearse sobre sí mismas a medida que el campo eléctrico de la radiación de microondas cambia. El movimiento rotacional de la molécula intenta orientarse en la dirección del campo, consiguiendo de esta forma una transferencia de energía.
- -Conducción iónica: La conducción iónica se da cuando hay iones libres presentes en la disolución. El campo eléctrico genera un movimiento iónico mediante el cual las especies intentan orientarse al cambio del campo eléctrico de la radiación de microondas, y de forma análoga a la rotación dipolar se produce un super-calentamiento.

Las microondas no afectan a la energía de activación, pero proporcionan la suficiente energía (casi de forma instantánea) para superar esta barrera y completar la reacción más rápidamente y con un mayor rendimiento respecto a los métodos de calentamiento convencionales.

Las ventajas prácticas de la aplicación industrial de esta técnica se pueden resumir a continuación:

- Tiene lugar un mecanismo de transferencia de energía en lugar de transferencia de calor.
- Se produce un calentamiento selectivo y orientado del material.
- El calentamiento es rápido.
- Los efectos térmicos son reversibles.
- El calentamiento por microondas resulta una eficaz vía alternativa a los métodos convencionales respecto al ahorro energético.

Diversos autores han observado que la irradiación con microondas sobre lodo activado mejora la desintegración de las partículas y la solubilización de la materia orgánica (Park et al., 2004; Eskicioglu et al., 2006; Climent et al., 2007; Eskicioglu et al., 2009; Toreci et al., 2009; Park et al., 2010).

En los estudios de Park et al. (2004) se observaron los efectos de la irradiación por microondas en el lodo secundario utilizando tiempos de entre 0-15 min y una potencia de 700 W. Estos autores obtuvieron un incremento de la demanda química de oxígeno soluble

(DQOs) respecto de la demanda química de oxígeno total (DQOt) con el incremento del tiempo de irradiación con microondas. El aumento de la DQOs indica la ruptura de paredes celulares y la liberación de material citoplasmático del lodo al medio (por la solubilización de sustrato). Por otro lado se observó un aumento de la concentración de Ca^{2+} lo cual indica que las microondas podrían desintegrar agregados de lodo.

En los ensayos de Eskicioglu et al. (2009), se observó una relación lineal entre la temperatura alcanzada con irradiación por microondas y el nivel de hidrólisis del lodo mediante un calentamiento lento y progresivo de este, de 1,2-1,4 °C/min. A una temperatura de 175 °C se obtuvo un incremento en la DQOs del 74,3% y una producción de biogás 31% superior comparado con la muestra sin pretratar. En los estudios de Toreci et al. (2009) se obtuvo un aumento en la producción del biogás del 83% aplicando un tiempo de retención hidráulica (TRH) de 5 días al realizar un tratamiento del lodo mediante microondas con una velocidad de calentamiento de 3,75 °C/min hasta 175 °C, mientras que el sistema control fue operado con un TRH de 20 días.

Climent et al. (2007) llevaron a cabo un estudio del comportamiento de lodo que consistió en el pre-tratamiento con microondas durante 5 min, a 800 W y con una energía específica de 13 000 KJ /KgST. Estos autores obtuvieron un incremento del 311% en el ratio sólidos volátiles disueltos: sólido volátil total (SV d/SV) cuando se compara con la muestra sin pretratar, aunque al ser comparado este resultado con otros tipos de pre-tratamiento (térmico a alta y baja temperatura y ultrasonidos), el tratamiento con microondas obtenía un incremento menor de solubilización que el resto. Por otro lado, no encontraron diferencias en la producción de biogás ni en su velocidad de producción al ser comparados todos los tratamientos con el control.

2.5 Objetivos

2.5.1 Objetivo general

Este trabajo tiene como objetivo general la evaluación del proceso de DA tanto de los lodos de depuradora (primario, secundario y mezcla) como de residuos agroindustriales con alto contenido en lípidos (grasas) y de biorefineria. Mediante la **co-digestión y la utilización de pre-tratamientos** se pretende conseguir un incremento en la producción de biogás, al mismo tiempo que se logra mantener en el reactor las condiciones que permitan un funcionamiento estable y unos efluentes con características óptimas para su disposición final y/o reutilización.

2.5.2 Objetivos específicos

De manera específica, las actividades desarrolladas en este trabajo pretenden dar respuesta a los siguientes objetivos particulares:

- Evaluación del proceso de digestión anaerobia en operación discontinua y semi-continua del lodo de depuradora.
- Evaluación del proceso de digestión anaerobia de lodo de depuradora con residuos de alto contenido en lípidos (grasas) como co-sustratos.
- Aplicación de técnicas espectroscópicas para la evaluación de digeridos.
- Aplicación de pre-tratamientos (ultrasonidos y microondas) evaluación sobre el efecto en el proceso de digestión anaerobia, la evolución del tamaño de partícula y las propiedades de deshidratabilidad de los lodos.
- Evaluación del proceso de digestión anaerobia de un residuo agroindustrial de biorefineria conocido como jugo marrón, en un reactor UASB a escala laboratorio, determinación del potencial de biogás y el efecto de la suplementación de nutrientes.

2.5.3 Esquema de trabajo

El trabajo fue realizado de acuerdo al siguiente esquema (Figura 2.8):

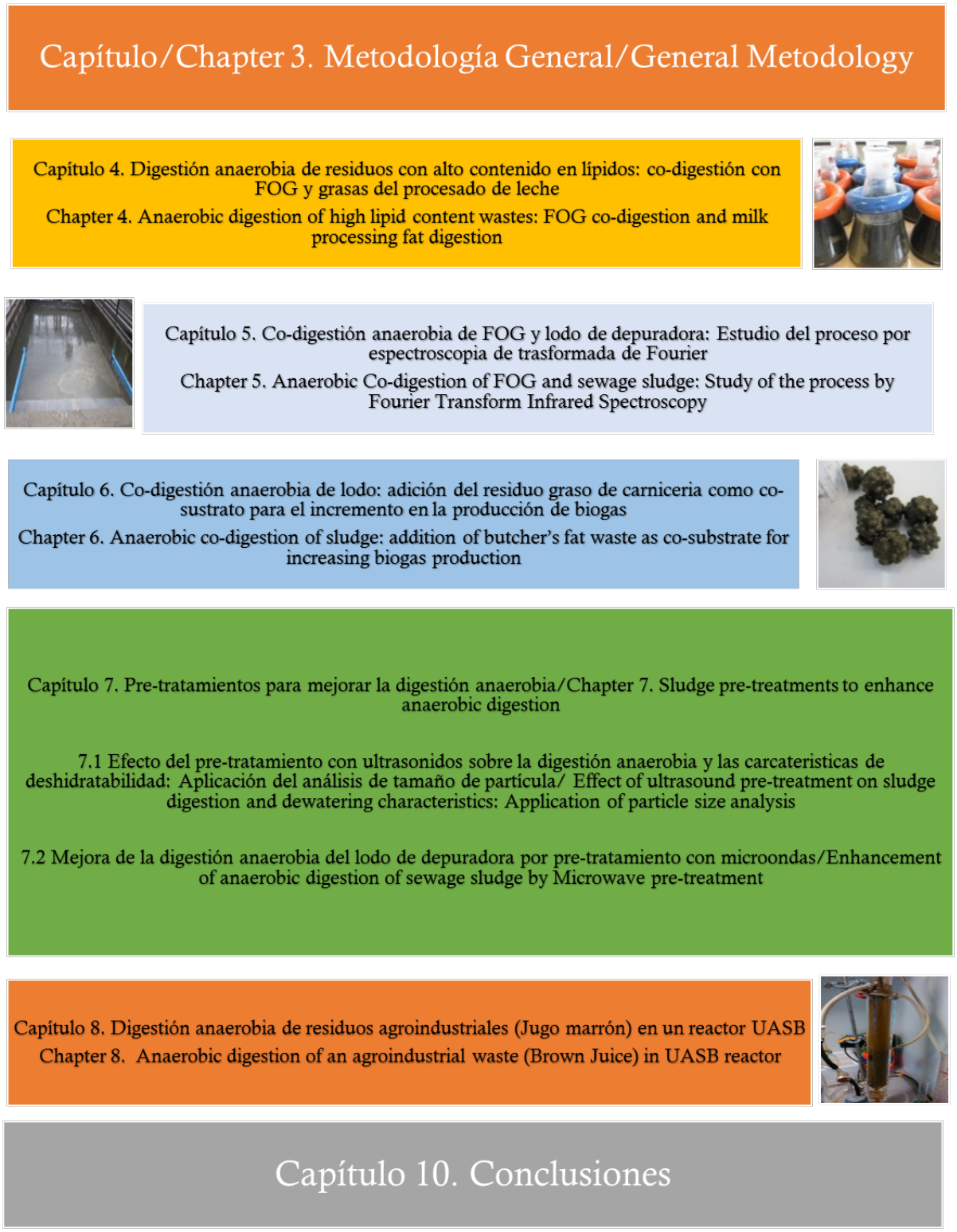

Figura 2.8 Esquema de trabajo

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Capítulo/Chapter 3

3. Material y métodos

3.1 Descripción de los sustratos empleados

3.1.1 Características del inóculo utilizado

El inóculo empleado en todos los ensayos se obtuvo del digestor anaerobio de la Estación Depuradora de Aguas Residuales (EDAR) de León, excepción hecha para los ensayos correspondientes a los capítulos 6 y 8. Esta instalación trata un caudal de aguas residuales de 4 460 m^3/h, dando servicio a los habitantes de León, su alfoz, y el área industrial, depurando en total el agua residual de 350 000 habitantes equivalentes (SALEAL, 2014).

El lodo empleado como inóculo se toma del tanque de espesado de fangos digeridos (Figura 3.1).

El contenido en sólidos promedio oscila entre el 1,5 - 5% de sólidos totales (ST), con un contenido en sólidos volátiles (SV) de aproximadamente el 70% de los ST.

Figura 3.1 Lodo digerido utilizado como inóculo

Como excepción en los capítulos 6 y 8 se utilizó como inóculo lodo digerido de la EDAR de Cáceres. Esta instalación trata un caudal promedio de 1 312 m^3/h de aguas residuales, dando servicio a aproximadamente 95 000 habitantes. (ACCIONA, 2014).

El contenido en sólidos promedio de este digerido oscila entre el 0,9 – 1,2% de ST, y un contenido en SV de aproximadamente el 75% de los ST. El contenido en sólidos del inóculo empleado en el arranque de los reactores puede ser mayor al haber sido espesado por decantación del mismo.

Los resultados de la caracterización química de los inóculos empleados se recogen en la Tabla 3.1.

Tabla 3.1 Características químicas del lodo digerido utilizado como inóculo

Parámetros (*)	Lodo digerido EDAR León	Lodo digerido EDAR Cáceres
Materia orgánica (%)	31,0±3,1	52,0±2,0
Nitrógeno total (%)	4,2±0,5	5,9±0,2
Relación C/N	4,4±0,4	4,7±0,2
Fósforo total (ppm)	440,0±22	30 920±90
Calcio (ppm)	576,0±17	30 470±95
Magnesio (ppm)	108,0±3,2	8 524±89
Potasio (ppm)	232,0± 6,9	6 797±80
Sodio (ppm)	856,0±77	n.r.
Hierro (ppm)	22,0±1,0	n.r.
Manganeso (ppm)	381,0±32	n.r.
Cinc (ppm)	895,0±71	n.r.
Cobre (ppm)	181,0±15	n.r.
Boro (ppm)	75,0±7,0	n.r.
Mercurio (ppm)	1,5±0,1	n.r.
Plomo (ppm)	81,0±7,1	97,0±4,8
Níquel (ppm)	30,0±4,0	31,0±1,7
Cadmio (ppm)	0,9±0,1	2,97±0,1
Cromo (ppm)	91,0±10	22,0±1,0

(*) Valores expresados en base seca, (n.r.) parámetro no reportado.

3.1.2 Características de los sustratos empleados

De forma general los sustratos utilizados en este trabajo fueron lodos (primario, secundario), grasas y jugo marrón. La caracterización del sustrato utilizado en cada ensayo se describe de manera más detallada en el material y métodos del capítulo correspondiente.

El lodo primario es producido durante los procesos de tratamiento primario de las aguas residuales (Figura 3.2). Este fue recogido de la depuradora correspondiente, en la conducción que comunica los sedimentadores primarios con el espesador por gravedad. Estos lodos presentaron una concentración promedio del 3-4% de ST y un porcentaje aproximado de sólidos volátiles SV del 70%.

Figura 3.2 Lodo primario

El lodo secundario utilizado en los ensayos, se obtuvo de los lodos en exceso resultantes del proceso biológico provenientes de la unidad de flotación. (Figura 3.3). Dicho lodo contiene partículas no hidrolizables y biomasa resultado del metabolismo celular. Estos lodos presentaron una concentración promedio del 2-3% de ST y un contenido en SV del 75% aproximadamente.

Figura 3.3 Lodo secundario, detalle de la unidad de flotación de lodos

Independiente a la evaluación individual de los lodos, se realizaron ensayos con la mezcla de lodo primario-lodo secundario a una relación volumétrica de 30:70% (V/V), dicha proporción fue fijada de acuerdo a la producción media de lodo en las depuradoras.

El residuo graso designado como FOG, en los capítulos 4 y 5 se obtuvo de la trampa de grasas de la depuradora de León (Figura 3.4). Las proporciones estudiadas (0.2-1.8% V/V) se fijaron de acuerdo a la producción de dicho residuo en la depuradora. Este residuo mostró un porcentaje de grasa bruta entre 20-63%.

Figura 3.4 Separación del FOG en trampa de grasas

En el capítulo 4 también se evaluó un residuo graso proveniente del procesamiento de la leche (Figura 3.5). Este residuo procedía de una industria láctea ubicada en Hospital de Órbigo, León. El residuo presentó un porcentaje de grasa bruta de entre 10-60%. La evaluación en continuo de este residuo se realizó directamente, previa dilución con agua, fijando una carga orgánica que permitió el arranque del sistema.

Figura 3.5 Residuo graso del procesamiento de lácteos

3.2 Descripción de los sistemas de digestión

3.2.1 Reactores empleados en régimen discontinuo.

Como reactores para los ensayos en discontinuo se emplearon matraces Erlenmeyer de 250 mL de volumen (Figura 3.6). Los ensayos y los análisis realizados fueron evaluados por duplicado. Se utilizó un sistema de digestión como blanco que contenía inóculo libre de sustrato y al que se le adicionó agua hasta completar el volumen equivalente a los sistemas de muestras. La producción de metano obtenida a partir del inóculo (lodo digerido de depuradora) se sustrajo de los resultados obtenidos de los sistemas conteniendo la muestra, de este modo se elimina la contribución del inóculo en el valor total de producción de gas. El ensayo se realizó sin suplemento alguno de nutrientes, utilizando la corrección a pH 7 mediante la adición de solución alcalina cuando resultó necesario.

Las cantidades de sustrato e inóculo a añadir a cada Erlenmeyer, se seleccionaron de modo que la relación inóculo/alimentación (I/A) fuera la adecuada en cada caso (con base en el contenido en sólidos volátiles). Este parámetro muestra los efectos de la concentración del sustrato y de la concentración del inóculo en la degradación anaerobia y producción de metano, y es importante para permitir la comparación entre los resultados obtenidos con las diferentes muestras evaluadas.

Para mantener constante la temperatura de digestión, los matraces Erlenmeyer se situaron en un baño termostatizado modelo Selecta Digiterm 100, el cual cuenta con una placa de

agitación IKA® RO15 en su parte inferior para garantizar la correcta mezcla de sustrato e inóculo.

El volumen de biogás generado se mide mediante el desplazamiento de una solución salina de los recipientes de acumulación de gas que están a su vez conectados a los matraces Erlenmeyer.

Figura 3.6 Ensayos en régimen discontinuo

3.2.2 Reactores de mezcla completa en régimen semi-continuo

La planta semi-piloto utilizada está constituida por cinco reactores de mezcla completa de 3 L de capacidad (Figura 3.7), agitados mecánicamente y termostatizados. Cada reactor está provisto de agitadores de tipo RZR1-Heidolph y constan de un dispositivo para la regulación de la velocidad de agitación. Los reactores son de metacrilato transparente de 5 mm de espesor montados sobre una estructura autoportante de acero inoxidable. Están provistos de un encamisado exterior por el que circula agua de calefacción que permite mantener el sistema a una temperatura controlada. El sistema de calefacción dispone de un depósito en

el que el agua es calentada. La temperatura en el depósito se mide con una sonda Pt-100 y se regula mediante un controlador digital PID. El agua de calefacción es permanentemente bombeada hacia las camisas y desde éstas vuelve al depósito.

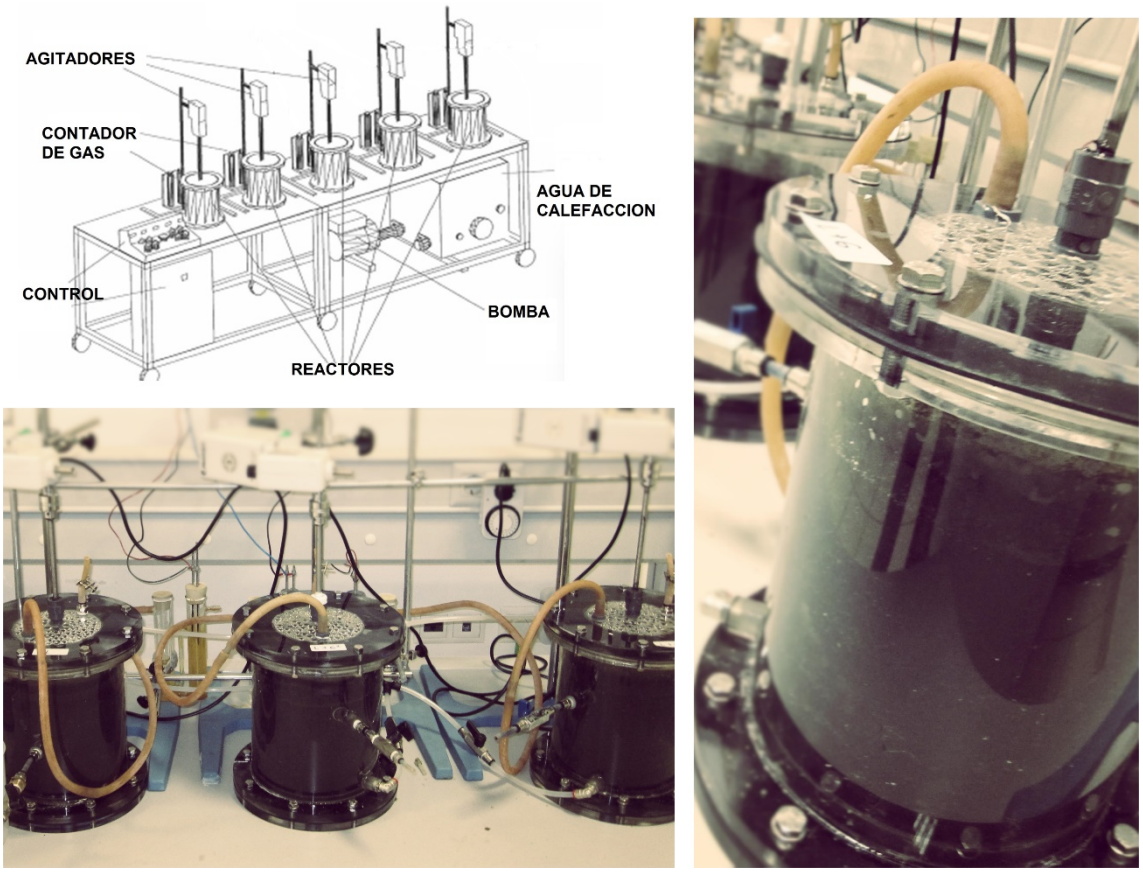

Figura 3.7 Esquema de la planta semi-piloto de reactores de mezcla completa de 3L, detalle de los reactores

Los reactores cuentan con una toma de fondo, otra superior por donde entra la corriente de alimentación, y una toma lateral para la salida del efluente digerido. También en la parte superior cuenta con una abertura para la salida del biogás generado hacia los contadores. Cuando las características de los sustratos lo permiten, alimentación y efluente son bombeadas mediante bombas peristálticas modelo Dosiper C1R equipadas con programadores. El gas producido en cada reactor es conducido desde la salida superior al contador de gas. La medición del volumen de biogás se realiza mediante el desplazamiento de líquido.

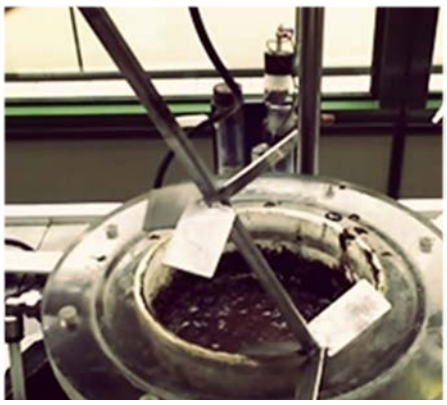

Figura 3.8 Agitador con doble sistema

A fin de impedir la formación de una capa en la parte superior del digestor, o depósitos en el fondo del reactor, se han empleado agitadores con un doble sistema de palas agitadoras (Figura 3.8). Lo que evitó la acumulación de sólidos y posibles obstrucciones para la toma de muestras, además se logró obtener un efluente homogéneo.

3.2.3 Reactor anaerobio de flujo ascendente (UASB)

En el capítulo 8 de este trabajo, se evaluó la digestión anaerobia del residuo agroindustrial conocido como jugo marrón en una configuración de reactor de flujo ascendente a diferencia de los otros experimentos realizados en reactores de mezcla completa (Figura 3.9).

El reactor anaerobio de flujo ascendente conocido por sus siglas en inglés UASB (Upflow Anaerobic Sludge Blanket) es ampliamente utilizado para tratamiento industrial de aguas residuales o residuos líquidos que no cumplen con las regulaciones ambientales para descarga directa por su elevada carga orgánica, bajo pH y presencia de sólidos en suspensión.

Figura 3.9 Reactor anaerobio de flujo ascendente

En este tipo de reactor UASB el influente se alimenta en el fondo del reactor y fluye hacia arriba a través de un lecho de lodo anaeróbico compuesto de comunidades microbianas aglomeradas. A medida que el influente entra en contacto con los microorganismos del lodo anaerobio se produce la degradación de la materia orgánica. El residuo tratado sale por la parte superior del reactor.

El biogás producido causa una turbulencia hidráulica, proporcionando una mezcla adecuada dentro del sistema y eliminando la necesidad de la mezcla mecánica. La retención de la biomasa se ve facilitada por la presencia de un separador de tres fases (sólido-líquido-gas) diseñado especialmente en la parte superior del reactor, donde se separa la fase acuosa de los sólidos y del gas.

3.3 Pre-tratamiento de las muestras

3.3.1 Pre-tratamiento con ultrasonidos

La desintegración por ultrasonidos se llevó a cabo con un procesador de ultrasonido UP200S; Helscher; Germany con una potencia nominal de 300 W y 24 KHz, con un sonotrodo de 7 mm de diámetro (Figura 3.10). La sonicación se realizó sobre 500 mL de lodo secundario, colocado en un vaso de precipitado de vidrio con 1 L de capacidad y a una profundidad conocida (Braguglia et al., 2008).

Figura 3.10 Esquema-Fotografía del Procesador de Ultrasonido: (1) Procesador, (2) asta, (3) sonotrodo

El tiempo de sonicado fue el correspondiente para conseguir la energía específica (Es) de ultrasonidos del ensayo (detallado en el capítulo correspondiente) de acuerdo a la Ecuación 3-1 (Feng et al., 2009).

$$E_s = {}^{P \times t}/_{V \times ST} \qquad \textbf{Ecuación 3-1}$$

Donde, $\boldsymbol{E_s}$ es la energía específica (kJ/KgST), $\boldsymbol{P}$ es la potencia (kW); $\boldsymbol{t}$ es el tiempo de sonicado (s); $\boldsymbol{V}$ es el volumen (L); $\boldsymbol{ST}$ es el contenido en sólidos totales del medio (kg/L).

3.3.2 Pre-tratamiento con microondas

La irradiación con microondas fue realizada en un horno doméstico (Figura 3.11) de la marca comercial SANYO de 900 W de potencia máxima y 2 450 MHz de frecuencia. El pre-tratamiento fue realizado a 200 mL de lodo secundario dispuesto en matraces Erlenmeyer de 500 mL de volumen, los cuales fueron cubiertos para prevenir la evaporación.

Figura 3.11 Microondas utilizado en el pre-trtamiento

Respecto a las condiciones del pre-tratamiento, el tiempo de irradiación y la potencia se fijaron para obtener energías de irradiación de 488, 675, 975, 2 025, 2 700 kJ/L de acuerdo a la propuesta en la Ecuación 3-2 de Uma Rani et al., 2013.

$$E_i = P \times T_i / V \qquad \textbf{Ecuación 3-2}$$

Donde, ***Ei*** es la energía de irradiación (kJ/L), ***P*** es la potencia del Magnetron (kJ/s), ***Ti*** es el tiempo de Irradiación (s) y ***V*** es el volumen de muestra (L).

Las condiciones de operación se resumen en la Tabla 3.2

Tabla 3.2 Condiciones de operación durante el pre-tratamiento con microondas

Energía de irradiación (kJ/L)	***Potencia de Magnetrón (kJ/s)***	**Tiempo de Irradiación (s)**	**Temperatura (°C)**
488	650	150	50
675	900	150	70
975	650	300	90
2 025	900	450	120
2 700	900	600	>100

3.4 Técnicas analíticas

3.4.1 Análisis rutinarios

El seguimiento del proceso de DA en los diferentes ensayos se llevó a cabo mediante una serie de análisis realizados periódicamente tanto sobre el digerido como sobre la alimentación: pH, ST y SV, demanda química de oxígeno (DQO), alcalinidad, amonio, producción y composición del biogás, y concentración de ácidos grasos volátiles (AGV). Todos estos parámetros fueron medidos periódicamente, durante la duración del experimento (Al menos dos veces por semana), a excepción de la producción de biogás, que fue medida diariamente. Las determinaciones de ST, SV, DQO, alcalinidad, y amonio se realizaron de acuerdo con lo indicado en APHA, (2005).

3.4.1.1 pH

Las medidas de pH se realizaron de manera directa empleando un medidor de pH Crison GLP22, previamente calibrado.

3.4.1.2 Contenido en sólidos totales y volátiles

Para determinar los sólidos totales fueron tomados 10 mL de muestra homogeneizada y dispuestos en un crisol tarado previamente, a continuación fueron llevados a una estufa Memmert a 105 °C hasta evaporación total durante 24 h. Para determinar la cantidad sólidos volátiles, los crisoles fueron colocados en una mufla Hobersal 12 PR/300 8B para calcinación de las muestras durante 60 min a 550 °C.

3.4.1.3 DQO

La determinación de la DQO de la muestra se realizó mediante su digestión en presencia de dicromato potásico ($K_2Cr_2O_7$) a 150 °C durante 2 h en un reactor Hanna C9800. Los compuestos orgánicos oxidables se reducen de ion dicromato (naranja) al ion crómico (verde). La titulación se realizó mediante un valorador automático Metrohm 862 compact titrosampler (Figura 3.12).

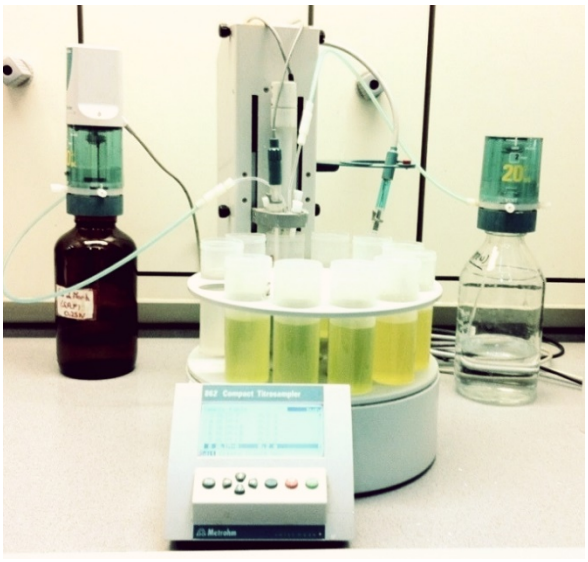

Figura 3.12 Valorador automático

3.4.1.4 Alcalinidad

Se toman 5 mL de la muestra homogeneizada, previa centrifuga-ción durante 5 min a 5 100 r.p.m. con posterior dilución en 50 mL con agua destilada. Se valora la muestra con HCl 0,2 N hasta pH 5,7 y 4,3 para obtener los valores de alcalinidad parcial (AP) y total (AT) respectivamente.

Al igual que la medición de DQO, se valora la muestra mediante el empleo de un valorador automático (862 compact titrosampler Metrohm ion analysis).El ratio de alcalinidad RA se calculó mediante la relación entre la alcalinidad intermedia (AI) y la alcalinidad parcial, determinada a pH 5,7. Se considera que el proceso de digestión es estable cuando el valor RA es inferior a 0,4 (Ecuación 3-3) (Ferrer et al., 2010).

$$\mathrm{RA} = {}^{\mathrm{AT} - \mathrm{AP}}/_{\mathrm{AP}} \qquad \textbf{Ecuación 3-3}$$

Donde, **AT** es alcalinidad total y **AP** alcalinidad parcial.

3.4.1.5 Amonio

El amonio se midió mediante el método del electrodo selectivo, empleando un medidor de pH Methron 781 equipado con un electrodo (No133/1e). Como paso previo a la medición en el equipo, las muestras fueron centrifugadas durante 5 min a 5 100 r.p.m.

La concentración de amoniaco se calcula a partir del equilibrio amonio-amoniaco:

$$\mathrm{NH_3 + H_2O} \xleftrightarrow{\mathrm{K_a}} \mathrm{NH_4^+ + OH^-}$$

En este trabajo se considera como amoniaco total a la suma de ambas especies (NH_4^+ y NH_3), calculando la concentración de amoniaco libre (NH_3) mediante la Ecuación 3-4 y Ecuación 3-5 propuestas por Calli et al., 2005 y Hansen et al., 1998.

$$Amoniaco\ Libre = \frac{Amonio\ Total}{1 + 10^{pK_a - pH}} \qquad \textbf{Ecuación 3-4}$$

$$pK_a = 0{,}09018 + \frac{2\,729{,}92}{T + 273{,}15} \qquad \textbf{Ecuación 3-5}$$

Donde, $\boldsymbol{pK_a}$ es la constante de disociación para el ion amonio y $\boldsymbol{T}$ la temperatura en °C, (pK_a tiene un valor de 8,95 a 35 °C).

3.4.1.6 Composición del biogás

La composición del gas obtenido de los digestores anaerobios se midió mediante cromatografía de gases empleando un cromatógrafo modelo Varian CP-3800 GC, equipado con un detector de conductividad térmica (TCD) (Figura 3.13). Se utilizó para determinar

el contenido en H_2, CH_4, CO_2, N_2 y O_2. El cromatógrafo cuenta con una columna HayeSep Q 80/100 de 4 m de longitud, seguida de una columna de tamiz molecular Molecular Sieve (1,0 m x 1/8''x 2,0 m). El gas portador fue helio y se operó a 331 kPa y a una temperatura de 50 °C.

Figura 3.13 Cromatógrafo de gases utilizado

3.4.1.7 Contenido en ácidos grasos volátiles (AGV)

Los AGVs fueron determinados en un cromatógrafo modelo Bruker-3800 GC equipado con un detector de ionización de llama (FID) y una columna capilar Nukol (30 m x 0,25 mm x 0,25 µm) de Supelco (Figura 3.13). El gas portador empleado fue helio. Las temperaturas del inyector y del detector fueron de 220 y 250 °C respectivamente. La temperatura del horno se mantuvo a 150 °C durante 3 min y después fue incrementada a 180 °C. El límite de detección para el análisis de AGV fue de 5,0 mg/L. El sistema se calibró con una mezcla de ácidos estándar de Supelco (para análisis de los ácidos grasos C2 - C7), empleando fenol 50 mM como patrón interno. Las muestras fueron previamente centrifugadas (10 min, 3 500 x g) y el sobrenadante filtrado con filtros de celulosa de 0,45 µm y 0,20 µm.

3.4.1.8 Contenido en ácidos grasos de cadena larga (AGCL)

Para la determinación de los AGCL las muestras fueron preparadas siguiendo la metodología descrita por Fernández et al. (2005). Se tomaron 5 mL de n-heptano a los cuales se añadió la misma cantidad de muestra homogénea. La mezcla fue primero agitada en un vortex durante 30 min para favorecer la extracción de los AGCL. Posteriormente se centrifugó la muestra, recuperando la fase correspondiente a la extracción de los ácidos grasos, la cual fue filtrada mediante filtros de celulosa de 0,2 µm. A continuación 0,8 mL de la muestra filtrada se añadieron a un vial junto con 0,2 mL de ácido pentadecanoico, empleado como patrón interno.

La determinación de AGCL se realizó en un cromatógrafo Perkin-Elmer AutoSystem XL equipado con un detector de ionización de llama (FID) y una columna de Polyethylene Glycol (15 m × 0,53 mm × 0,5 μm). El gas portador empleado fue helio. Las temperaturas del inyector y detector fueron fijadas en 250 y 275 °C respectivamente. La temperatura inicial del horno, de 100 °C, fue mantenida durante 1 min, y posteriormente fue aumentada hasta los 250 °C a razón de 5 °C/min.

La calibración del cromatógrafo se realizó utilizando una mezcla de AGCL realizada a partir de ácidos individuales con número par de carbonos (C6 - C24) de Sigma. A fin de determinar la eficacia de la extracción se realizaron blancos empleando inóculo y cantidades conocidas de AGCL. El límite de detección para el análisis de AGCL fue de 5 mg/L.

3.4.2 Técnicas analíticas puntuales

A continuación se describen las técnicas analíticas empleadas en la caracterización de los sustratos empleados y técnicas utilizadas puntualmente.

3.4.2.1 Análisis de materia orgánica, carbono orgánico y relación C/N.

El análisis de materia orgánica se determinó mediante el método Walkley-Black (MAPA, 1994), consistente en la oxidación de la materia orgánica presente en la muestra mediante la digestión en presencia de ácido sulfúrico y dicromato potásico 1 N. Posteriormente la muestra se valora empleando una disolución de sulfato ferroso amónico 0,5 N (Sal de Mohr), y difenilamina como indicador, para estimar la cantidad de dicromato en exceso que no interviene en la reacción. El carbono orgánico se determinó por diferencia entre el dicromato original y el valorado con la sal de Mohr. La materia orgánica oxidable se obtiene indirectamente mediante la consideración de que el 58% de la materia orgánica es carbono orgánico.

La relación C/N se obtiene mediante la división del valor obtenido como carbono orgánico y contenido en nitrógeno total.

3.4.2.2 Nitrógeno total

El nitrógeno total se determinó por el método Kjeldahl (MAPA, 1994). El nitrógeno así cuantificado es la suma del nitrógeno orgánico y el nitrógeno amoniacal. En este método la muestra es digerida con ácido sulfúrico concentrado en un digestor modelo DS-20 de Tecator a 400 °C durante 35 min, lo que produce la transformación del nitrógeno en sulfato amónico. Posteriormente, se alcaliniza el medio y se destila el amoniaco en una unidad de destilación modelo UDK 140 de Velp Scientifica, recogiéndose en un vaso con ácido bórico

(4%). El amonio se determina mediante valoración volumétrica con ácido clorhídrico 0,5 N hasta que el pH llegue a 4,8 en un valorador automático modelo 702 SM Titrino de Metrohm.

3.4.2.3 Contenido en fósforo, potasio y metales pesados.

La determinación de estos elementos se realizó mediante espectroscopía de emisión atómica en plasma acoplado por inducción (ICP-AES) y espectrometría de masas con fuente de plasma de acoplamiento inductivo (ICP-MS). En función de la naturaleza del elemento a determinar. Ambas técnicas se basan en la vaporización, disociación, ionización y excitación de los diferentes elementos químicos de una muestra en el interior de un plasma. Previo a su determinación en el equipo las muestras fueron digeridas en ácido nítrico 65% en un digestor a presión atmosférica con reflujo programado con una rampa de calentamiento.

Se dejó enfriar y se aforaron los tubos digestores a 50 mL con agua milliQ. Posteriormente se realizaron las diluciones oportunas y se adicionaron como patrones internos 10 ppb de Sc, Pd, Pt y Rh para los elementos que se midieron en un ICP-MS (Fe, B, Zn, Cu, Mn, Mo, Al, Co y Ni) y 1 ppm de Sc para los que se midieron en un ICP-AES (S, P, Na, Ca, K y Mg).

3.4.2.4 Contenido en lípidos

El contenido en lípidos se llevó a cabo empleando una modificación del método Soxhlet estándar (APHA, 2005). Se empleó la técnica de extracción Randall con un extractor Ser 148/6 Velp. Esta técnica se basa en un proceso de extracción líquido-sólido en continuo empleando como disolvente éter de petróleo en el aparato Soxhlet, con posterior evaporación del disolvente y pesada final del residuo. El rendimiento del proceso es mayor que un sistema Soxhlet convencional ya que la primera parte de la extracción se efectúa mediante la inmersión de la muestra en el disolvente en ebullición, luego sigue un enjuague con el disolvente frío. La rápida solubilización realizada por el disolvente caliente permite reducir notablemente los tiempos de extracción.

3.4.2.5 Carbono Orgánico Total (COT)

La cantidad de carbono total fue analizada en el equipo multi N/C® (Figura 3.14): analizador de TOC de alto rendimiento por combustión de la muestra a 980 °C, mientras que la determinación de carbono inorgánico se realiza por medio de la acidificación de la muestra al 10% de ácido fosfórico (H_3PO_4), lo cual provoca la emisión de CO_2 con la reacción del ácido y los carbonatos.

El carbono orgánico se obtuvo mediante la diferencia de carbono total y carbono inorgánico. Los valores se expresan en porcentaje en peso.

Figura 3.14 Analizador de TOC

3.4.2.6 Parámetros de deshidratabilidad

Tiempo de succión capilar (TSC)

El test de (TSC) es una forma fácil y rápida para evaluar la filtrabilidad de los lodos y observar los efectos de los pre-tratamientos y procesos aplicados en la efectividad para deshidratar lodos. Se basa en la fuerza de gravedad y en la succión capilar de un trozo de papel filtro grueso para retirar agua de una muestra de lodos acondicionados.

Se vertió 5 mL de muestra dentro de un tubo de acero inoxidable con un radio interno de 0,535 cm y colocado en un filtro Whatman Nº 17 de grado cromatografía marcado con 2 círculos de 1 y 3 cm de radio. El TSC se define como la cantidad de tiempo requerido para humedecer el papel de filtro entre los radios de 1,0 y 3,0 cm a lo largo de las direcciones x/y, respectivamente (Chang et al., 2001; Feng et al., 2009).

Resistencia específica a la filtración (REF)

La REF es la resistencia que opone a la filtración una cantidad de lodo depositada en un área de la superficie filtrante. Esta prueba tiene gran utilidad para comparar las características de filtración de distintos lodos provenientes de plantas potabilizadoras y determinar las necesidades de tratamiento para producir una torta que ofrezca mínima resistencia y optimizar el funcionamiento de la deshidratabilidad del lodo.

Se filtró un volumen (V) de muestra dada una presión de vacío hasta que la torta es formada y se impide la filtración. La REF se calculó con la Ecuación 3-6, de acuerdo a la pendiente obtenida en la gráfica volumen frente a la relación tiempo/volumen (Christensen & Dick, 1985; Sorensen et al., 1995).

$$r = P \times A^2 b/_{\mu\, c}$$ **Ecuación 3-6**

Donde, ***r*** es la resistencia específica a la filtración (cm/g), ***P*** es la presión de vacío aplicada (dinas/cm^2), ***A*** es el área del filtro (cm^2), ***b*** es la pendiente (s/cm^6), ***μ*** es la viscosidad del filtrado (poise = g/cm s), ***c*** es la concentración de sólidos totales por volumen de filtrado (g/cm^3).

Agua libre/ligada

La muestra de lodo se centrifugó a 1 000 r.p.m. durante 10 min. La muestra fue secada en una termobalanza con un flujo de aire constante de 300 mL/min a una temperatura de 105 ˚C. La distribución de agua se obtuvo de la curva del tiempo de secado frente al contenido de agua (masa de agua / masa sólido) de la muestra (Kopp & Dichtl, 2000).

3.5 Análisis espectroscópico y termogravimétrico

3.5.1 Espectroscopía infrarroja por Transformada de Fourier (FT-IR)

La espectroscopía infrarroja (IR) es una técnica analítica de gran aplicación para la identificación de compuestos orgánicos. Debido a su sensibilidad para analizar grupos funcionales orgánicos, ha sido ampliamente usada para evaluar procesos de degradación de la materia orgánica tales como compostaje (Meissl et al., 2007) y digestión anaerobia (Gómez et al., 2011; Martínez et al., 2012; Wu et al., 2011).

El espectro IR de un compuesto es una representación gráfica de los valores de número de onda frente a los valores de absorbancia o transmitancia. La absorción de radiación IR de un compuesto a una determinada longitud de onda (o número de onda) origina un descenso en el porcentaje de transmitancia, lo que se pone de manifiesto en el espectro como un pico o una banda de absorción.Al absorber radiación IR, la molécula experimenta un cambio en el momento dipolar como consecuencia de su movimiento de vibración o rotación, solo en estas circunstancias el campo eléctrico alternante de una radiación puede interaccionar con la molécula y causar cambios en la amplitud de algunos de sus movimientos.

Existen dos tipos básicos de vibraciones, de tensión o estiramiento (stretching) que consisten en un cambio en la distancia interatómica a lo largo del eje del enlace entre dos

átomos, y de flexión (bending), en las que cambia el ángulo entre dos enlaces.Como una molécula está compuesta por distintos átomos y enlaces, en el espectro de absorción aparecerán bandas a distintos valores de frecuencia y longitud de onda. La región situada entre 4 000 y 1 400 cm^{-1} es de especial utilidad para identificar grupos funcionales, mientras que la zona del espectro comprendida entre los 1 400 a 600 cm^{-1} es única para cada compuesto, pudiendo compararse con el espectro de compuestos puros para su identificación (Cuetos et al., 2007).

3.5.1.1 Metodología de análisis

El residuo obtenido tras la centrifugación fue secado en estufa Memmert a una temperatura de 105 ± 2 °C durante 24 h y posteriormente molido con un molino de bolas de la marca Retsch MM200.

Las pastillas se prepararon con 2 mg de cada una de las muestras junto con 200 mg de bromuro potásico (KBr) previamente desecado, la mezcla se trituró en un mortero hasta alcanzar una granulometría muy fina y una perfecta homogenización. A continuación la mezcla se introdujo en una prensa hidráulica Graseby de Specac donde fue comprimida durante 10 min, obteniéndose unas pastillas casi transparentes que posteriormente se introdujeron en el espectrómetro. Junto con cada grupo de muestras se preparó y analizó un blanco que consistía en la realización de una pastilla únicamente con 200 mg de KBr (Figura 3.15).

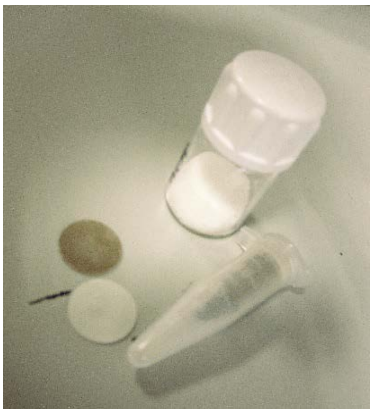

Figura 3.15 Pastillas de Muestra con KBr para la FT-IR

El análisis de las muestras se llevó a cabo mediante espectroscopía FT-IR en un espectrómetro marca Perkin Elmer modelo System 2 000. El espectro FT-IR de cada muestra fue registrado entre los 4 000 cm^{-1} y los 400 cm^{-1} de número de onda en intervalos de 0,5 cm^{-1}, lo que supone un total de 7 201 puntos para cada uno de los espectros a una velocidad de 0,5 cm/s. Se realizaron 100 barridos para cada una de las muestras analizadas.

Los espectros fueron tratados con el software Spectrum GXI v.S.3.1, de modo que se realizó la media de las dos réplicas de cada una de las muestras tras la sustracción del espectro correspondiente al blanco.

Se llevó a cabo el procedimiento de normalización de Meissl et al. (2007). Este procedimiento consiste en realizar el promedio de los valores de absorbancia. Este valor

promedio se resta a cada uno de los valores del espectro, y así hacer pasar el eje de las X por la mitad del espectro. Se calcula entonces la suma del cuadrado de los valores de la Y y el espectro se divide por la raíz cuadrada de esta suma.

3.5.1.2 Análisis estadístico

Dos métodos estadísticos multivariantes, el análisis de conglomerados jerárquico (AC) y el análisis de componentes principales (ACP), se utilizaron para la evaluación de las muestras basadas en sus espectros de FT-IR en la región comprendida entre 4 000 a 800 cm^{-1}. Para dichos análisis se utilizaron los datos de altura y área de señales de las bandas correspondientes a los espectros analizados. Para obtener estos datos se utilizó el programa Spectrum GXI v.S.3.1 eligiendo rangos asignados a grupos funcionales de interés.

El ACP es una técnica estadística descriptiva que tiene como punto de partida una matriz de datos y cuyo propósito fundamental consiste en la reducción de la dimensión de los datos con el fin de simplificar el problema en estudio. En esta técnica, se busca encontrar transformaciones ortogonales de las variables originales para conseguir un nuevo conjunto de variables no correlacionadas, denominadas componentes principales (CP), que se obtienen en orden decreciente de importancia. Los CP son combinaciones lineales de las variables originales y se espera que solo unas pocas recojan la mayor parte de la variabilidad de los datos, obteniéndose una reducción de la dimensión en los mismos (Villardón, 2002).

La obtención de los CP puede realizarse por varios métodos alternativos:

- Buscando aquella combinación lineal de las variables que maximiza la variabilidad.
- Minimizando la discrepancia entre las distancias euclídeas entre los puntos calculadas en el espacio original y en el subespacio de baja dimensión. (Coordenadas principales, Gower).
- Mediante regresiones alternadas (métodos Biplot).
- Buscando el subespacio de mejor ajuste por el método de los mínimos cuadrados. Minimizando la suma de cuadrados de las distancias de cada punto al subespacio (Matriz de Pearson). Este último método fue el utilizado en el presente trabajo.

El análisis de conglomerados jerárquico o análisis cluster (AC) es una técnica multivariante que busca agrupar elementos o variables tratando de lograr la máxima homogeneidad en cada grupo y la mayor diferencia entre los grupos, mediante una estructura jerarquizada que permite decidir el nivel jerárquico apropiado para establecer la clasificación (Baños et

al., 2014). Este análisis permitió observar las similitudes y clasificó los espectros examinados en grupos. La agrupación se llevó a cabo utilizando el algoritmo de Ward. Estos análisis, AC y ACP, se realizaron con el software SPSS.

3.5.2 Análisis termogravimétrico

Los análisis térmicos abarcan una serie de técnicas en las cuales se mide una propiedad física de una sustancia en función de la temperatura. Para ello se somete una muestra a un programa de temperatura determinado y en una atmósfera controlada. Se distinguen varios métodos térmicos, destacando la termogravimetría (TG) y el análisis térmico diferencial (DTA). El análisis termogravimétrico ha sido utilizado para investigar la valoración energética de residuos (Díez, 2003), para estudiar la evolución de la materia orgánica durante el compostaje (Carballo et al., 2008; Dell'Abate et al., 1998; Smidt and Lechner, 2005) y en este trabajo va a servir como herramienta para evaluar los cambios y el grado de estabilización de los digestatos tras el proceso de DA (Cuetos et al., 2009; Gómez et al., 2007a, 2007b, 2005).

La termogravimetría informa de la ganancia o pérdida de masa de la muestra, cuantificando esta variación, mientras que el DTA informa si un proceso es endotérmico o exotérmico. Por otro lado, mediante la calorimetría diferencial de barrido (DSC) se puede medir la variación de energía térmica de una muestra.

La termogravimetría es una técnica en la cual la masa de una muestra se mide continuamente en función de la temperatura mientras es sometida a un programa controlado de calentamiento. El registro continuo de estos datos permite obtener la curva TG. La termogravimetría derivada (DTG) es una forma de representar los resultados de TG, por medio de la primera derivada de la curva, en función de la temperatura o el tiempo.

El análisis térmico bajo atmósfera oxidante se realizó utilizando una termobalanza de TA Instruments, modelo SDT2960, que registra simultáneamente medidas de TG-DTA. En la Figura 3.16 se presenta un esquema del instrumento, que consta de una balanza de precisión, un horno en el que se controla la temperatura y un sistema de registro de la señal.

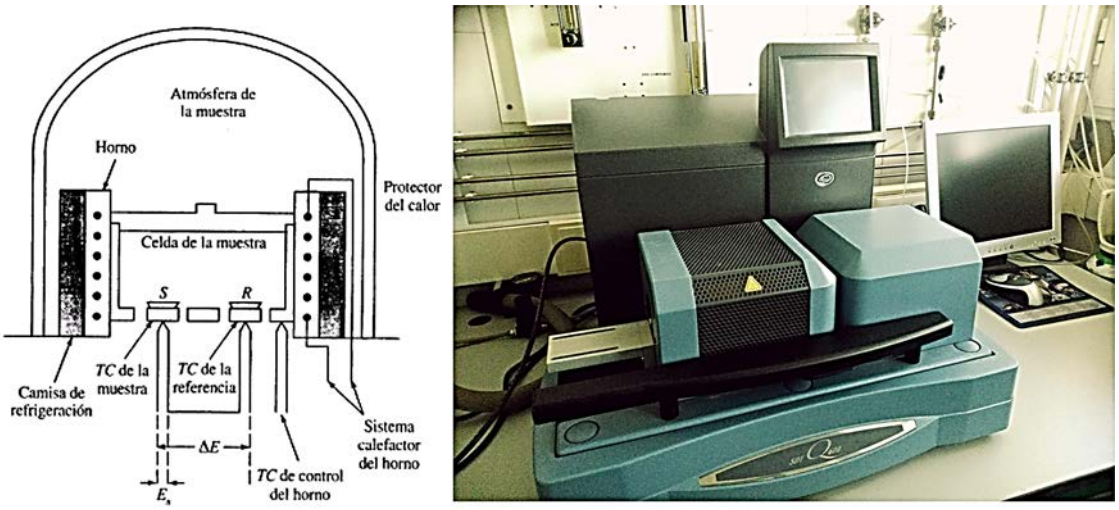

Figura 3.16 Esquema y fotografía y de la termobalanza utilizada

3.6 Análisis de tamaño de partícula

El tamaño de partícula es una propiedad muy importante para caracterizar sistemas de partículas, tiene una influencia directa en las propiedades de las sustancias como la velocidad de reacción o disolución, la estabilidad en suspensión, textura, viscosidad, densidad y porosidad. En un nivel básico podríamos definir a una partícula como una discreta subporcion de una sustancia. Los materiales constituidos por partículas pueden ser polvos, gránulos, pigmentos, suspensiones, lodos, estiércoles, etc.

3.6.1 Distribución de tamaño de partícula

La distribución de tamaño de partícula (DTP) es la manera de representar el tamaño de partículas de una sustancia en forma de una curva de distribución de frecuencias o en una curva de distribución acumulativa. La manera de constituir dichas curvas es mediante la comparación del diámetro de una esfera equivalente que tiene las mismas propiedades tales como volumen, masa o área de superficie específica, debido a ello la DTP puede ser de número, volumen, peso, etc.

La DTP de NÚMERO es una técnica de recuento que dará una distribución ponderada donde cada partícula se le da el mismo peso, independientemente de su tamaño, esta técnica es utilizada cuando el número absoluto de partículas es importante.

La DTP de VOLUMEN es una técnica de dispersión de luz estática como la difracción láser y darán una distribución ponderado en volumen. La contribución de cada partícula en la distribución se refiere al volumen de esa partícula (equivalente a la masa si la densidad es

uniforme), es decir, la contribución relativa será proporcional al tamaño. Esto es a menudo extremadamente útil desde un punto de vista comercial dado que la distribución representa la composición de la muestra en términos de su volumen/masa.

3.6.1.1 Estadísticas de la DTP

Con el fin de simplificar la interpretación de los datos de distribución de tamaño de partícula, una se puede calcular y reportar una serie de parámetros estadísticos. La elección del parámetro estadístico más apropiado para cualquier muestra dada dependerá de cómo se utilizan esos datos y de lo que se comparará. Los parámetros más comunes son:

- Media: tamaño "medio" de una población.
- Mediana: tamaño donde el 50% de la población está por debajo o por encima.
- Moda: tamaño con mayor frecuencia.

Si la forma de la distribución del tamaño de partícula es asimétrica, como es a menudo el caso en muchas muestras, puede que dichos valores no sean exactamente equivalentes, como se ilustra en la Figura 3.17.

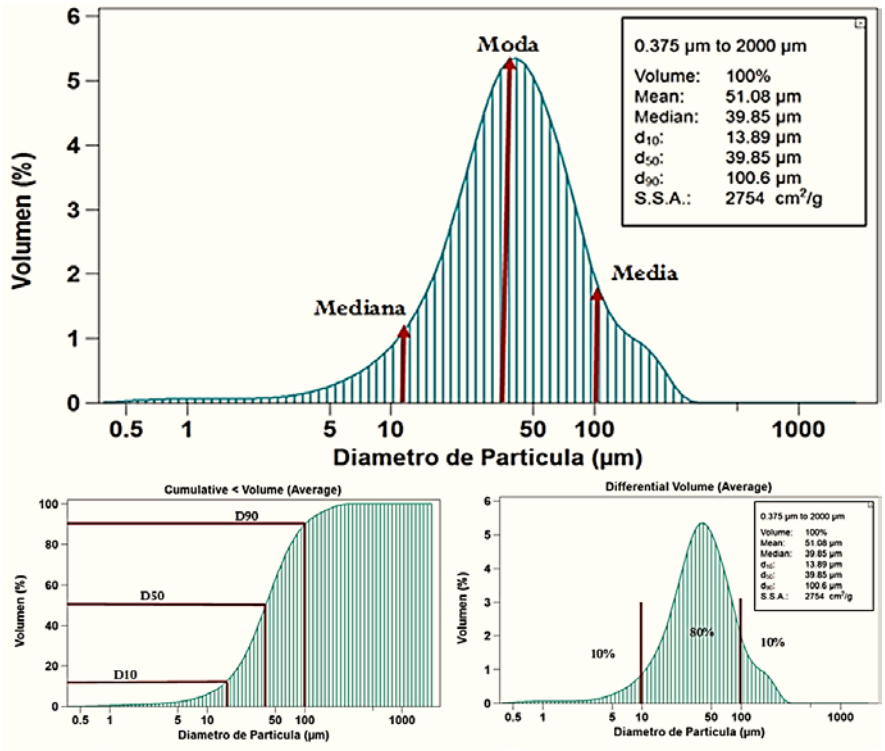

Figura 3.17 Parámetros estadísticos en la DTP, Percentiles de volumen en términos de graficas acumulativas y de frecuencia

Los percentiles más comunes son 10%, 50% y 90% representados en la distribución de frecuencias y la distribución acumulada en la Figura 3.17.

Por último, el área de superficie específica o superficie específica (ASE) es uno de los parámetros más relevantes cuando lo que se observa en la sustancia es la biodisponibilidad, la reactividad y la disolución. Este parámetro es más sensible a la presencia de partículas finas en la distribución.

3.6.1.2 Metodología del análisis de tamaño de partícula

El análisis de tamaño de partícula se llevó a cabo en el analizador de tamaño de partícula por difracción de rayos laser LS 13 320 de Beckmann Coulter (Figura 3.18), con un rango de 0,35 a 2000 µm. (sin ultrasonido) Para el análisis cada muestra fue diluida en agua del grifo 50:1 antes de ser analizada 10 veces.

Durante el análisis el equipo utilizó dos fuentes de luz: la fuente de luz principal que cuenta con Diodo de láser de 5 mW con una longitud de onda de 750 nm y fuente de luz secundaria que cuenta con una lámpara de tungsteno-halógeno (PIDS) con longitud de onda de 450, 600 y 900 nm. En el análisis de las muestras se utilizó el módulo de análisis Universal de líquidos (ULM).

Figura 3.18 Analizador de tamaño de partículas

3.7 Metodología de superficie de respuesta

La metodología de superficie de respuesta (RSM) es un conjunto de técnicas matemáticas utilizadas en el tratamiento de problemas en los que una respuesta de interés está influida por varios factores de carácter cuantitativo. El propósito inicial de estas técnicas es diseñar un experimento que proporcione valores razonables de la variable respuesta y, a continuación, determinar el modelo matemático que mejor se ajusta a los datos obtenidos. El objetivo final es establecer los valores de los factores que optimizan el valor de la variable respuesta. Para ayudar a visualizar la forma de una superficie de respuesta, con frecuencia se utiliza la gráfica de contornos de la superficie de respuesta.

Generalmente se desconoce la relación entre la respuesta y las variables independientes, por ello requerimos un modelo que aproxime la relación funcional entre la respuesta (Y) y las variables independientes (X). Si la respuesta se describe adecuadamente por una función lineal de las variables independientes se utiliza el modelo de primer orden, si la respuesta no es lineal entonces debe realizarse la aproximación con un polinomio de orden más alto.

Una vez que el polinomio de segundo orden ha sido ajustado a la respuesta, es necesario determinar los máximos, mínimos o puntos de silla que puedan encontrarse en la región experimental seleccionada. Esto se logra mediante el cálculo de los valores propios de la matriz formada por el coeficiente de segundo orden del modelo cuadrático. La magnitud de los valores propios ayuda al usuario comprender mejor la respuesta del sistema.

Si todos los valores propios son positivos, entonces se ha encontrado un punto mínimo. En contraste, cuando todos los valores propios son negativos, se encuentra un punto máximo y el procedimiento de optimización de la respuesta seleccionada depende de la determinación de este punto estacionario. El máximo se encuentra resolviendo el sistema de ecuaciones obtenido a partir de la derivada parcial de la respuesta con respecto a cada variable independiente.

La ecuación para el modelo de segundo orden para un diseño de tres factores se puede escribir como (**Ecuación 3-7**):

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2$$

Ecuación 3-7

Esta aproximación de segundo orden puede ser escrita en forma matricial como:

$$\hat{Y} = \boldsymbol{\beta_0} + \boldsymbol{x'b} + \boldsymbol{x'Bx} \qquad \textbf{Ecuación 3-8}$$

Donde el vector $\boldsymbol{b}$ está formado por los coeficientes de la parte lineal (efectos principales) y la matriz $\boldsymbol{B}$ está formado por los coeficientes correspondientes a las interacciones y términos cuadráticos puros.

El máximo es calculado resolviendo el sistema dado por:

$$\frac{\boldsymbol{d}\hat{Y}}{\boldsymbol{dx}} = \boldsymbol{b} + \boldsymbol{2Bx} = \boldsymbol{0} \qquad \textbf{Ecuación 3-9}$$

El punto estacionario está dado por:

$$\boldsymbol{x_s} = -\left(\frac{\boldsymbol{1}}{\boldsymbol{2}}\right) \boldsymbol{B^{-1}b} \qquad \textbf{Ecuación 3-10}$$

3.8 Referencias

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Capítulo/Chapter 4

4. Anaerobic digestion of high lipid content wastes: FOG co-digestion and milk processing fat digestion

Abstract

The digestion of two different residues with high lipid content was investigated in the present research. The first part of the experimental work focussed on an assessment of the co-digestion of sewage sludge and fat, oil and grease (FOG) residues separated from the grease trap in the biological pre-treatments of wastewater treatment plants, as proposed in the present study. The second part of the experimental work studied the individual digestion of fat recovered from the grease trap of a milk processing plant. The digestion process was performed under batch and semi-continuous operation at mesophilic temperatures. Successful digestion of wastes was attained, with no inhibitory consequences due to the accumulation of long-chain fatty acids. An increase in biogas production was observed under batch digestion of sewage sludge when FOG was added as a co-substrate. However, the small increase reported was in accordance with the limited volume of this residue added. With regard to the digestion of the milk processing fat, although the addition of nutrients may be necessary for maintaining long-term operation, with the long hydraulic retention time tested no foam accumulation was observed and successful operation was achieved.

Keywords: Anaerobic digestion, FOG, sewage sludge, lipid wastes.

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4.1 Introduction

There are numerous sources of high lipid content wastes, which include waste water treatment plants (WWTPs), animal slaughterhouses, and food processing installations [1]. Co-digestion using these kinds of wastes has become an area of interest to researchers, owing to the multiple advantages that the process offers. Co-digestion is the term used to describe the combined treatment of several wastes with complementary characteristics, this being one of the main advantages of anaerobic technology. [2]. Anaerobic digestion is a complex process which requires strict anaerobic conditions to transform organic matter into biogas, while also allowing the biochemical stabilization of sludge and reducing the amount of solids finally remaining for disposal [3]. Therefore, it is one of the most promising alternatives intended for the treatment of residues and for the production of energy by taking advantage of biogas. The process is considered an environmental friendly option [4] for the treatment of biowastes, since it not only allows stabilization of organic matter, reducing its potential to putrefy, but also contributes to the reduction of greenhouse gas emissions. Biogas is currently seen as an important future contributor to European energy supplies [3]. The volume of biogas produced is related, among other things, to the content and quality of organic matter fed into the digester. In this way, increases in the concentration of solids in the incoming substrate may lead directly to greater biogas yields.

Lipid-rich waste which can be collected in the grease trap of wastewater treatment plants is also called fat, oil and grease (FOG) waste. This residue presents a great potential for increasing methane yield as noted by several studies [5-8]. Published reports record the benefits obtained from the co-digestion of this waste under mesophilic and thermophilic conditions can result in a doubling, or even higher increase, in methane production. Lipids are one of the major types of organic matter found in food wastes and some industrial wastewaters, such as those coming from slaughterhouses, dairy plants or fat refineries [9]. The addition of FOG, greases, or residues from slaughterhouses with high lipid contents has been evaluated in various different co-digestion processes [7, 10-12]. The addition of the co-substrate has been tested with substrates presenting volatile solid (VS) concentrations as high as 46% and successful results have been achieved, with no foam accumulation or inhibition due to long-chain fatty acids (LCFAs) [7-10]. The break-down of LCFAs takes place through the β-oxidation pathway, which has been reported as the rate-limiting step of the whole anaerobic digestion process [13]. The accumulation of these components during the digestion process may cause inhibition, because of their known toxicity affecting acetogens and methanogens [13-15]. However, recent studies have stated that inhibition

caused by LCFAs could be reversible, with acclimatization being a key factor in avoiding negative effects on microbial communities [8, 16-18]. Cuetos et al. [12] reported successful digestion of high lipid content wastes after a long acclimatization period, whilst recording inhibition problems and foam accumulation when the same residue was treated directly without first submitting micro-organisms to an adaptation period.

Another waste product of similar characteristics is the fat obtained from milk processing installations. The digestion of this type of waste has been studied previously [19-20]. These studies report the hydrolysis phase of the process as the limiting factor. Under continuous operation, most reactors treating organic wastes with high loads are usually reported to work with long hydraulic retention times (HRTs). In this way, these systems may be particularly suitable for overcoming problems associated with the low hydrolysis rate of fatty milk wastes. With this in mind, the present study aimed to assess anaerobic digestion of FOG and fat obtained from a milk-processing plant. In the first part of the experimental work, the FOG obtained from the grease trap of the WWTP was co-digested with sewage sludge in volumes proportional to the production of these wastes in the plant. In the second experimental phase, the individual digestion of fat obtained from a milk-processing factory was evaluated.

4.2 Material and methods

The sewage and digested sludge was obtained from the WWTP of the city of Leon. Primary and secondary sludge (waste-activated sludge) (PS and WAS) were used as the substrate for the experiments when digesting sewage sludge under batch and semi-continuous conditions. The grease used as co-substrate was obtained from the grease trap. FOG was added in a proportion of 0.2% V/V, on the basis of data from the WWTP regarding the production of wastes. Later experiments under semi-continuous operation were performed with higher volumetric proportions of the co-substrate (0.8% and 1.8% V/V). Digested sludge from the WWTP digester was used as inoculum. This digester treated a mixture of PS and waste-activated .The temperature of the digestion process was 32 °C and the average HRT was 26 days.

The fat used for the second phase of experiments was obtained from a local milk-processing factory. The digestion systems were inoculated with the same digested sludge used in the previous set of experiments. The digestion of this substrate was also evaluated under batch and semi-continuous conditions.

4.2.1 Batch digestion

Batch experiments were performed to determine the biochemical gas potential of the substrates used in this study. Experiments were carried out until the cessation of gas production was observed. The batch reactors (Erlenmeyer flasks of 250 mL) were filled with inoculum and the corresponding amount of substrate in order to attain the desired proportion of VS between substrate and inoculum. Two reactors were used for measuring gas production and composition. A batch reactor containing only inoculum was used as blank. The biogas produced by this reactor was subtracted from the corresponding tests. The temperature of digestion was 34 °C. Agitation was provided by means of magnetic stirrers. The gas volumes were measured using bottle gasometers and corrected to standard temperature and pressure (STP), 0 °C and 760 mmHg, respectively.

The PS used in the batch experiment presented a total solid (TS) concentration of 37.6 g/L with 72% of volatile solids (VS). WAS presented a TS content of 24.4 g/L with 75% of VS. The Erlenmeyer flasks were inoculated with 150 mL of digested sludge presenting a TS content of 20 g/L and a VS concentration of 12 g/L. Table 4.1 shows the characteristics of the grease collected at two different points in the grease trap (denoted FOG_1 and FOG_2). The proportion of VS between the inoculum and substrate for this experiment was 1:1. Digestion systems were denoted, according to the substrate used, either PS or SS, followed by the label for the grease used, in the case of co-digestion systems.

Table 4.1 Characteristics of the high lipid content wastes used in the study for batch and semi-continuous digestion

	FOG_1	FOG_2	GM1	GM2
Organic Matter (%)	79.8	83.7	92.5	85.5
Kjeldahl Nitrogen (%)	3.1	5.8	0.83	4.8
Grease (%)	17.6	63.5	58.5	10.8
COD (g/L)	149	92	240	117
TS (g/L)	133	55	246	109
VS (g/L)	107	48	232	91

Percentages are expressed on dry basis

The characteristics of the grease obtained from the milk processing factory (GM1) are also presented in Table 4.1. Batch digestion for this substrate was performed with several different proportions of VS between the inoculum and substrate, as follows: 0.4, 0.8, 1.0 and 1.5. The Erlenmeyer flasks were inoculated with 200 mL of digested sludge.

4.2.2 Semi-continuous digestion

The digestion process was carried out in completely mixed reactors provided with mechanical stirrers. The reactor had a working volume of 3 L. The systems were kept thermostatically at a temperature of 34± 1 °C. Reactors were provided with an outer jacket for circulating heating water that kept the system at a controlled temperature. Each reactor was provided with liquid and gas sampling ports. The reactors worked on a semi-continuous basis, being fed manually once every day. Before the reactor was fed, an equivalent volume was withdrawn. All the samples taken from the reactors were obtained after complete homogenization and previous feeding of the systems. Daily gas production was measured using a reversible device with liquid displacement and a wet-tip counter.

Reactors were inoculated with digested sludge. The PS used in this experiment presented a concentration of 33.5 g/L TS with 72% of VS. The WAS sludge presented a TS concentration of 29 g/L with 78% of VS. In this second stage of experimentation, co-digestion with sewage sludge was evaluated using FOG_1 as co-substrate. The co-digestion of sewage sludge and FOG_1 was performed only with a mixture at 0.2% (V/V) of FOG (following the proportions tested in batch experiments). The HRT was set at 30 days. The sewage sludge in this case was composed of a mixture of PS and WAS with a volumetric proportion of 30:70 based on the proportions of volumes of sewage sludge produced in the WWTP. A second reactor was used for treating a mixture with a higher volumetric proportion of FOG. The reactor was started up by applying an adaptation period where the feeding substrate had a proportion of FOG of 0.2%. This ratio was gradually increased to 1.8%. This reactor was evaluated at an HRT of 30 days during 120 days. Reactors were denoted in accordance with the substrate being digested in each case.

The digestion process for the fat from the milk-processing factory was performed using the same experimental apparatus and conditions as described above. In this phase of experimentation the fat obtained from the industry was denoted GM2. The reactor used was denoted R_GM2 and the HRT applied was 40 days.

4.2.3 Analytical techniques

Nitrogen concentrations were determined by the Kjeldahl method. Organic matter was analyzed in accordance with the Walkely-Black method (Walkley and Black, 1934). Grease content was determined by Soxhlet extraction using Velp Scientifica SER 148/3 in accordance with APHA Standard Methods [21]. Chemical oxygen demand (COD), total solids (TS), volatile solids (VS), ammonium and pH were monitored during the digestion process.

These parameters were determined in accordance with the APHA Standard Methods [21]. COD was determined using a Metrohm 862 Compact Titrosampler. The homogenized sample was digested in the presence of dichromate at 150 °C for 2 h in a Hanna C9800 reactor. The composition of the biogas was analysed using a gas chromatograph (Varian CP3800 GC) equipped with a thermal conductivity detector. A packed column (HayeSep Q 80/100; 4m) followed by a molecular sieve column (1m) was used to separate CH_4, CO_2, N_2, H_2 and O_2. The carrier gas was helium and the columns were operated at a pressure of 331 kPa and a temperature of 50 °C. Volatile fatty acids (VFAs) were determined on the same gas chromatograph, using a flame ionization detector (FID) equipped with a Nukol capillary column (30m × 0.25mm × 0.25μm) from Supelco. The carrier gas was helium. Injector and detector temperatures were 220 and 250 °C, respectively. The oven temperature was set at 150 °C for 3 min. and thereafter increased to 180 °C. The detection limit for VFA analysis was 5.0 mg/L. The system was calibrated with a mixture of standard volatile acids from Supelco (for the analysis of fatty acids C2 to C7). Samples were previously centrifuged (10 min., 3500 × g) and the supernatant filtered through 0.45 μm cellulose filters. Gas chromatography was used for the analysis of the long chain fatty acids (LCFAs). Samples for LCFA analysis were extracted as described by Fernández et al. [22]. Samples were mixed with n-heptane, the solution was then centrifuged for 30 min. at 3500 × g and filtered through a 0.2 μm Millipore Millex-FGS filter. The sample was injected into a Perkin-Elmer AutoSystem XL chromatograph equipped with a FID detector and a PEG (100% Polyethylene Glycol) column (15 m × 0.53 mm × 0.5 μm). The carrier gas was helium. The initial oven temperature of 100°C was maintained for 1 minute, and then increased to 250 °C, with a ramp of 5 °C per minute, this temperature being maintained for 5 min. Injector and detector temperatures were 250 °C and 275 °C, respectively. The system was calibrated using a mixture of LCFAs from individual acids with concentrations in the range of 0 to 100 mg/L. The detection limit for LCFA analysis was 5.0 mg/L. The acids analyzed were C6:C24 (with even numbers of carbon atoms) all from Sigma.

4.3 Results

4.3.1 Sewage Sludge and FOG digestion

The results obtained from batch digestion assays are presented in Figure 4.1 for PS and WAS systems. Since the addition of co-substrate was limited to the volumetric proportions of production in the WWTP, the benefits of this addition were scarcely noticeable in the minor increase in cumulative methane production in the PS systems. The specific methane production obtained for the individual digestion of PS was 462 mL/g VS, whilst this value

increased to 542 mL/g VS (average values for both co-digestion systems). The small addition of complex wastes resulted in a decrease in the biogas production rate, as may be observed from the sigmoid behaviour of the cumulative methane graph. With regard to the WAS system, no improvement was observed, all systems presenting an average specific methane production of 358 mL/g VS. Contrary to what was the case for the previous system evaluated, the addition of high lipid content waste did not affect in any significant way the methane production of the co-digestion system. Although an increase in the total volume of biogas produced was obtained in the first case, modification of the rate of biogas production was also observed. This delay may be rationalized as an adaptation of micro-organisms to the presence of the complex substrate, resulting in sigmoid curves of cumulative methane production.

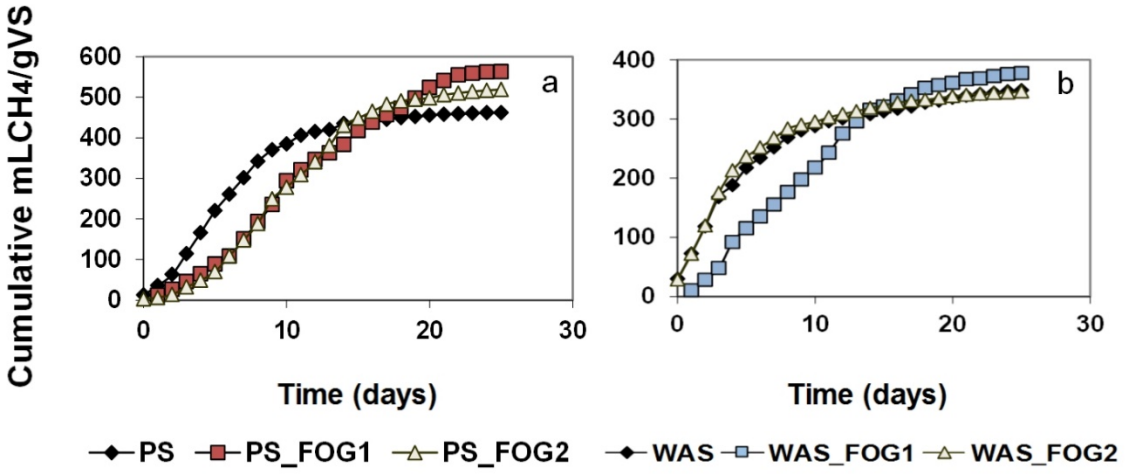

Figure 4.1 Gas Production from batch digestion test of Sludge and FOG for (a) PS Systems and (b) WAS Systems

Under semi-continuous operation FOG_1 was selected for the assessment of the digestion process, because to its higher solid contents. Additionally, the residue denominated FOG_2 presented particles of greater size which might render normal operation difficult. Daily biogas production is presented in Figure 4.2a. As may be observed, the gas evolution was practically constant with similar values for both reactors evaluated. The addition of the co-substrate in the proportions applied did not translate into an increase in the biogas rate. As it might be expected from batch experiments, the low addition of volatile solids did not represent any significant increase in the organic load supplied to the co-digestion reactor. VS measured during experimentation showed stable behaviour with no significant modifications.

The effect of increasing the volumetric proportions in the mixture may be seen in Figure 4.2b. The gradual increase in FOG addition resulted in poor performance from the reactor. Table 4.2 shows the values obtained for the parameters monitored during digestion. The specific methane production calculated for this second reactor treating the mixture of FOG at 1.8% was lower than that obtained for the previous reactor. This value was calculated for the period between 75 and 120 days of operation. Owing to the small amounts of the co-substrate added to the first system (0.2% FOG) no observable effects were measured. Results obtained for this reactor presented low values for VFA concentrations. Additionally, the LCFAs detected showed low concentrations of octanoic (C8), decanoic (C10), and myristic (C14) acids, with values below 50 mg/L. Increasing the FOG content of the mixture did not result in higher VFA values. This may be rationalized by the mechanisms of inhibition of LCFAs. Accumulation of LCFAs may inhibit anaerobic digestion because of direct toxicity to acetogens and methanogens, the two main groups involved in LCFA breakdown [13]. Another inhibiting mechanism is the result of the adsorption of surface active acids onto the cell wall [23], thus affecting the processes of transportation and protection.

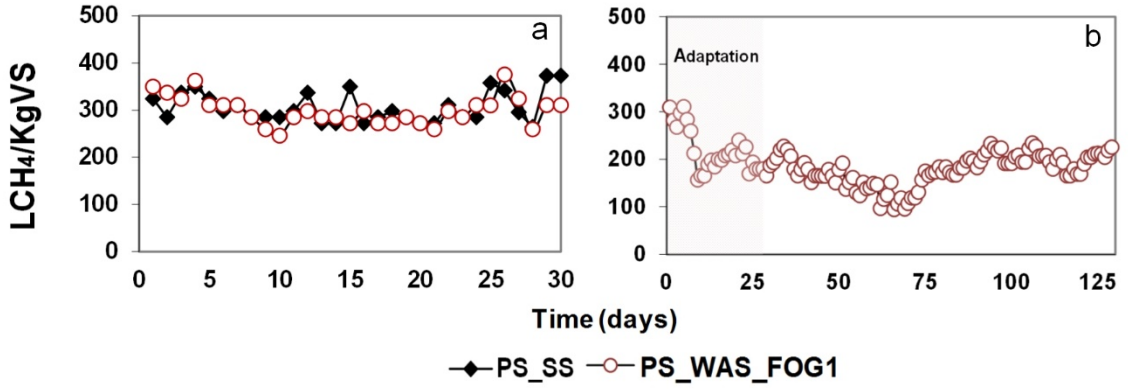

Figure 4.2 Specific methane production of reactor treating the mixture of primary sludge and secondary sludge (PS_WAS) and the co-digestion mixture (PS_WAS_FOG1) at 0.2 % (b) Daily gas production of reactor treating the mixture at 1.8%

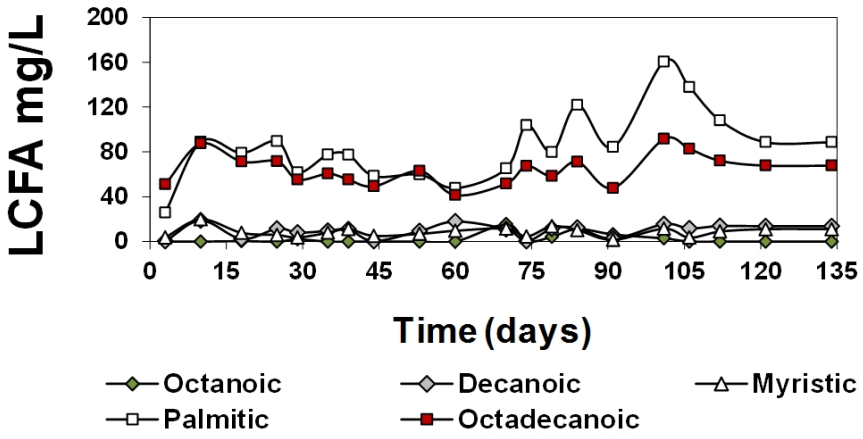

Figure 4.3 LCFA concentration measured from the reactor treating the co-digestion mixture (PS_WAS_FOG1) with increasing proportion of FOG

Figure 4.3 shows the LCFA concentrations measured in the reactor treating the FOG mixture at 1.8%. Values obtained here were lower than those reported in the literature as causing inhibition [24]. Thus, the lower gas yield of the reactor was probably due to adsorption of the FOG components onto cell walls.

Table 4.2 Parameters of Reactors for Semi-continuous digestion

Parameters	PS_WAS	PS_WAS_FOG1 0.2%	PS_WAS_FOG1 1.8%	R_GM2
Organic loading rate (g VS/m³/day)	771	776	790	652
Specific CH_4 production mL/g VS/day	304	298	200	440
Average daily gas production (L/day)	1.08	1.06	0.70	1.9
% CH_4 in biogas	65	65	67	63
VS (g/L)	14.4	12.6	12.9	9.0
TS (g/L)	21.8	19.4	18.5	14.4
COD (g/L)	13.8	12.2	12.5	13.8
T NH_4^+ (mg/L)	1103	1114	824	509.2
VFA concentration:				
Acetic (mg/L)	126	84		135
Propionic (mg/L)	13	--	--	23
Butyric (mg/L)	--	--	--	5

The addition of fat residues to digesters has been recommended by several authors who have evaluated the co-digestion of greases by applying either continuous supplementation or pulsed addition of waste [11, 25-27]. The addition of high lipid content wastes seems a plausible option for increasing biogas production in already existing digestion systems, as has been demonstrated by the practical implantation of this option in WWTPs [28].

However, in the present study an increase in the FOG concentration resulted in inhibition of the digestion process, highlighting the relevance of testing modifications under pilot scale conditions prior to undertaking operational changes in industrial plants. Another relevant aspect deals with operating considerations which should also be carefully evaluated, to avoid clogging of process piping when delivering this co-substrate [29].

4.3.2 Milk processing waste digestion

Figure 4.4 shows the results for the gas production obtained under batch digestion of milk-processing waste. The increase in substrate concentration results in an inhibitory effect, as may be observed from the lower production of gas obtained as the Inoculum to Substrate ratio (I/S) increases. Inhibition associated with the concentration of LCFAs has been reported under continuous operation and digestion assays [30-32]. From the results here obtained, it may be rationalized that increasing amounts of the substrate resulted in higher concentrations of LCFAs, which in turn decreased the biogas yield.

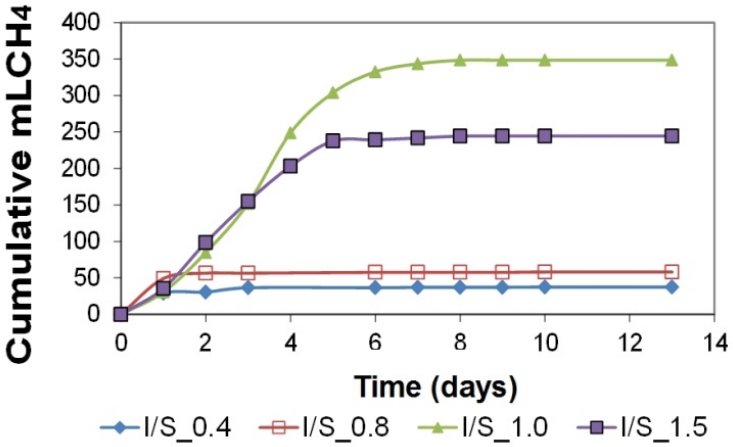

Figure 4.4 Cumulative methane production of batch reactors treating the milk processing waste at different ratios of VS of inoculum/substrate (I/S)

Sage et al. [20] studied the degradation of milk fat, reporting a lag phase of several days prior to degradation of fat by anaerobic micro-organisms, with this lag phase before biogas production being mainly due to unsaturated free fatty acids (FFA). Conversion to biogas occurred at a lower rate for saturated than for unsaturated FFA.

Taking into consideration the low methane yield obtained with the increase in substrate concentration, semi-continuous digestion was evaluated applying a low organic loading rate. Figure 4.5a shows the daily biogas production of the reactor working under semi-continuous operation (R_GM2). The reactor was daily fed with an organic loading rate of

0.65 g VS/L day with an HRT of 40 days. Under these conditions, steady production of gas was observed, presenting an average methane content of 63%. Data relating to the performance of this reactor are also presented in Table 4.2.

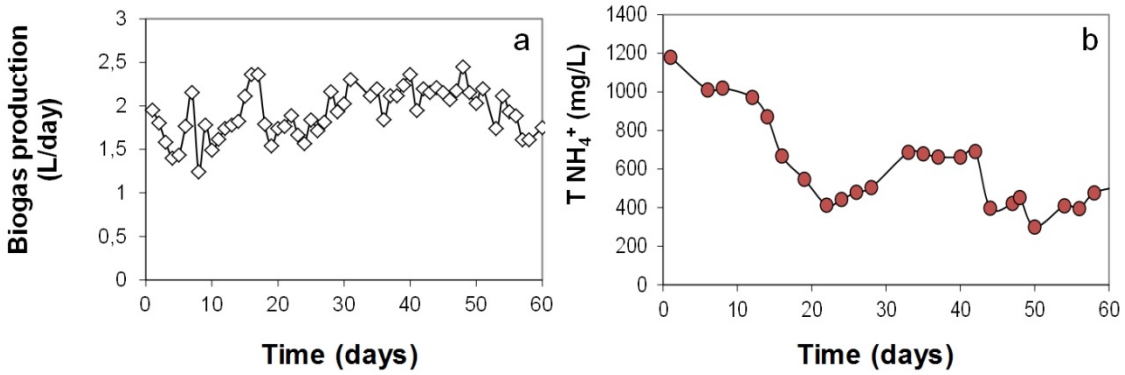

Figure 4.5 (a) Daily gas production of reactor treating the waste obtained from the processing milk factory (R_GM2), (b) Total ammonium concentration inside the reactor

The specific methane production obtained was higher than the value reported for the sludge digestion systems, corroborating the high methane potential of this waste. Although inhibition was probably the cause of the limited production of methane under batch digestion in some of the experiments carried out, this situation may be circumvented under semi-continuous operation by the application of a low organic loading rate to the reactor. In this way, the concentration of LCFAs was low during the period of experimentation, with the main acids detected being Palmitic, Stearic and Arachidic, their average concentrations being 89, 80 and 50 mg/L respectively. Although steady gas production was attained, one of the main problems when considering the digestion of food processing wastes is the concentration of nutrients needed to maintain stable operation of biological treatment processes during long-term performance. In the present study, the concentration of ammonium in the reactor was initially that of the inoculum used for starting-up the digestion process. However, as the time of experimentation elapsed, a significant reduction was observed, as may be seen in Figure 4.5b.

4.4 Conclusion

The Digestion of high lipid content waste was successfully attained for both substrates evaluated. The studies undertaken using sewage sludge as the main substrate resulted in no observable modifications to specific methane production with a FOG content of 0.2% (V/V). However, an increase in the addition of FOG to 1.8% resulted in significant detriment of the

performance of the process. On the other hand, when digesting fat obtained from a milk-processing factory, the results showed successful operation of the semi-continuous reactor operating with an HRT of 40 days, even though the high inhibition was reported from batch tests performed.

4.5 Acknowledgements

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Capítulo/Chapter 5

5. Anaerobic Co-digestion of FOG and sewage sludge: Study of the process by Fourier Transform Infrared Spectroscopy

Abstract

The digestion of residues with high lipid content was investigated. The experimental work focussed on the assessment of co-digestion of sewage sludge and fat, oil and grease (FOG) residues separated from the grease trap of the pre-treatment system of a wastewater treatment plant. A 0.2% volumetric ratio was set, based on the volumetric production of these streams in the plant. The digestion was carried out under semi-continuous operation at mesophilic temperatures. The operation of reactors at a hydraulic retention time (HRT) of 30 days resulted in no observable modifications of the specific methane production when compared to results obtained from sludge digestion and co-digestion systems. However, a decrease in HRT resulted in the detriment of the co-digesting reactor, which was attributed to an inhibitory process associated with the adsorption of FOG onto active sludge.

Keywords: Anaerobic Co-digestion, FOG addition, FTIR, sludge, wastes, adsorption.

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5.1 Introduction

The biological treatment of wastewater has greatly contributed to reducing the impact of human activities on the environment. Nevertheless, some technical aspects still need to be resolved in the near future, particularly those related to the monitoring of pharmaceuticals, as suggested by Kochkodan et al. (2010), and concerns related to the environmental impacts of organic waste disposal procedures, which require a careful evaluation (Sablayrolles et al., 2010). Municipal wastewater treatment plants (WWTPs) generate sludge as a product of the physical, chemical and biological processes used during treatment (Appels et al., 2008). Typical sewage sludge is composed of primary sludge separated from wastewater during pre-setting and biological excess sludge from an activated sludge system (Luostarinen et al., 2009). The anaerobic digestion of sewage sludge has been applied at WWTPs for decades; most often, sewage sludge is digested alone, though co-digestion with other substrates could be beneficial with respect to increase biogas production (Luostarinen et al., 2009). Anaerobic digestion technology plays an important role in transforming organic matter into biogas and in reducing the amount of final sludge solids and pathogens present in the sludge (Appels et al., 2008).

The co-digestion of sewage sludge and other organic wastes could enhance biogas production and organic matter degradation due to benefits such as diluted inhibitory compounds and a more balanced carbon-to-nitrogen ratio (Luostarinen et al., 2009). This may be the main reason why there is increasing interest in this line of research. Although the co-digestion process offers multiple advantages, the main one is the improvement obtained in methane yield with the addition of a co-substrate. In some cases, this factor may be considered relevant to increase the economic feasibility of treatment plants. In this regard, lipid-rich waste has become an interesting co-substrate because it is produced in large quantities by several industries. Lipid-rich waste, which can be collected in the grease trap of wastewater treatment plants, is also called fat, oil and grease (FOG) waste; this material must be separated from wastewater prior to entering a sewage system primarily because of its propensity to block municipal sewer lines and disrupt the effective operation of downstream treatment processes (NCDENR, 2002).

The high theoretical methane potential of lipid wastes with respect to that of other substances makes these wastes a desirable substrate to be treated in anaerobic digestion processes (Angelidaki and Sanders, 2004). However, organic wastes with high lipid content

present several problems such as adsorption of lipids onto biomass, which can cause sludge flotation and washout (Hwu et al., 1998; Pereira et al., 2003). In addition, the accumulation of these components during the digestion process may cause inhibition. This inhibition is mainly due to the toxicity of a given number of fatty acids on anaerobic microorganisms. Long-chain fatty acids (LCFAs) inhibit H_2-producing bacteria responsible for β-oxidation (Hanaki et al., 1981; Sage et al., 2008). Nevertheless, recent studies have suggested that the inhibition caused by these acids could be reversible, with acclimatisation being a key factor in avoiding negative effects on microbial communities (Pereira et al., 2004; Pereira et al., 2005; Cuetos et al., 2008; Cavaleiro et al., 2009; Martín-González et al., 2010); thus, they report the successful digestion of high lipid content wastes after a long acclimatisation period but record inhibition problems and foam accumulation when the same residue is treated directly without first submitting microorganisms to an adaptation period.

Fourier transform infrared spectroscopy (FTIR) is a technique applied for the rapid analysis of complex biological samples and characterisation of heterogeneous organic matter. This technique is a valuable tool that provides information regarding the chemical characteristics of samples (Carballo et al., 2008). Recently, it has been widely applied in monitoring the anaerobic digestion of biowastes like slaughterhouse wastes, organic fraction of municipal solid wastes and pig slurry (Gómez et al., 2005; Gómez et al., 2007; Marcato et al., 2008; Cuetos et al., 2010) with successful results. FTIR is a technique sensitive to changes in organic functional groups, and it is well suited for the study of organic transformations during biological processes. Thus, anaerobic co-digestion of FOG waste collected from the grease trap of a waste water treatment plant (WWTP) was conducted in this study. The process was studied by the aid of FTIR analysis of digested samples with the aim to assess the effect of FOG addition over the organic matter characteristics of the digested material and to study the stability of the final effluents.

5.2 Material and methods

5.2.1 Substrates and digestion characteristics

Sewage and digested sludge were obtained from the WWTP of the city of León. The plant was managed by the companies Acciona Agua and Acciona Infraestructuras. Reactors were inoculated with digested sludge with a total solid (TS) content of 20 g/l and a volatile solid (VS) concentration of 12 g/l. The primary sludge (PS) used in this experiment presented a concentration of 33.5 g/l TS with 72% VS. The waste-activated sludge (WAS) presented a

TS concentration of 29 g/l with 78% VS. The sewage sludge (SS) was composed of a mixture with a volumetric proportion of 30:70 (PS:WAS) based on proportions of sludge produced in the WWTP. The sewage sludge was co-digested with FOG at different hydraulic retention times (HRTs). FOG was added at a 0.2% volumetric ratio (based on the volumetric production of these streams in the plant), the feeds were stored at 4 °C until they were needed. The HRTs evaluated were 30, 26 and 21 days. The chemical characteristics of FOG collected from the grease trap are shown in Table 5.1.

Table 5.1 Characteristics of the FOG used in the study for semi-continuous digestion

Chemical parameters	FOG
Organic Matter (%)	79.8±0.2
Kjeldahl Nitrogen (%)	3.1±0.1
Grease (%)	17.6±0.3
COD (g/l)	149±20
TS (g/l)	133±15
VS (g/l)	107±10
% of total LCFAs	
Palmitate	62.6±0.6
Oleate	n.d
Stereate	25.0±0.6
Myristate	3.7±0.5
Araquidic	3.7±0.2
Capric	5.0±0.1
Lauric	0.5±0.1

Percentages are expressed on dry basis, n.d -not detected

The digestion process was carried out in completely stirred reactors, which were provided with mechanical stirrers and sampling ports for the extraction of liquid and gas samples. The reactors had a working volume of 3 l and were also provided with an outer jacket to circulate heating water, which kept the systems at a controlled temperature of 34 ± 1°C. The reactors were run on a semi-continuous basis and manually fed once a day. Samples were taken from reactors three times a week and were obtained after complete homogenisation and previous to feeding the systems. Daily gas production was measured using a reversible device with liquid displacement and a wet-tip counter. Reactors were denoted as SS and SS_FOG in accordance with the substrates being treated (sewage sludge and sewage sludge co-digested with FOG, respectively). Another co-digesting reactor with 1.8% (volumetric

ratio) FOG was also studied by Martinez et al. (2011) to evaluate the effect of an increment in lipid waste addition over biogas production at an HRT of 30 days. Data from this reactor were used for comparison.

5.2.2 Analytical techniques

Nitrogen concentration was determined by the Kjeldahl method (MAPA 1994). Organic matter was analysed in accordance with the Walkey-Black method (Walkey and Black, 1934). Grease content was determined by Soxhlet extraction using Velp Scientifica SER 148/3 in accordance with the APHA Standard Methods (1989). Chemical oxygen demand (COD), TS, VS, ammonium and pH were determined in accordance with the APHA Standard Methods (1989) and were regularly monitored during the digestion process. COD was determined using a Metrohm 862 Compact Titrosampler. The homogenised sample was digested in the presence of dichromate at 150 °C for 2 h in a Hanna C9800 reactor.

The composition of biogas was analysed using a gas chromatograph (Varian CP3800 GC) equipped with a thermal conductivity detector. A packed column (HayeSep Q 80/100; 4 m) followed by a molecular sieve column (1 m) was used to separate CH_4, CO_2, N_2, H_2 and O_2. The carrier gas was helium, and the columns were operated at a pressure of 331 kPa and a temperature of 50 °C. H_2S was detected using a pulse flame photometric detector (PFPD) with an FA-II capillary column (60 m × 0.32 mm × 0.25 μm) from Varian. The carrier gas was helium. The injector and detector temperatures were 220 °C and 300 °C, respectively. The oven temperature was set to 150 °C for 12 min and the detection limit for H_2S analysis was 2000 ppm.

Volatile fatty acids (VFAs) were determined using the same gas chromatograph and a flame ionisation detector (FID) equipped with a Nukol capillary column (30 m × 0.25 mm × 0.25 μm) from Supelco. The carrier gas was helium. The injector and detector temperatures were 220 °C and 250 °C, respectively. The oven temperature was set to 150 °C for 3 min and increased to 180 °C with a ramp of 10 °C/min. The detection limit for VFA analysis was 5.0 mg/l. The system was calibrated with a mixture of standard volatile acids from Supelco (for the analysis of fatty acids C2 to C7). Samples were previously centrifuged (10 min, 3500 × g) and the supernatant filtered through 0.45 μm cellulose filters, both methods: biogas composition and VFAs determination are described by Cuetos et al. (2008).

Gas chromatography was used for the analysis of long chain fatty acids (LCFAs), samples were extracted with n-heptane (99% purity, Panreac, Spain), the n-heptane was added to 5ml of sample and mixed for 30 min and the suspension was centrifuged (30 min, 3500 × g), the resulting supernatant filtrated through a Millipore Millex-FGS filter (0.2 μm) (Fernández et al., 2005).The sample was injected into a Perkin-Elmer AutoSystem XL chromatograph equipped with an FID detector and a PEG (100% polyethylene glycol) column (15 m × 0.53 mm × 0.5 μm). The carrier gas was helium. The initial oven temperature of 100 °C was maintained for 1 min. and then increased to 250 °C with a ramp of 5 °C/min; this temperature was maintained for 5 min. The injector and detector temperatures were 250 °C and 275 °C, respectively. The system was calibrated using a mixture of LCFAs from individual acids with concentrations in the range of 0 to 100 mg/l. The detection limit for LCFA analysis was 5.0 mg/l. The acids analysed were C6:C24 (with even numbers of carbon atoms) and all obtained from Sigma.

5.2.3 FTIR analysis

The substrate and final effluents obtained from the reactors herein described and the effluent from the reactor described in Martinez et al. (2011) were studied. Samples were dried at 105 °C in a furnace for 48 h and then ground in a laboratory ball mill (Retsch mill model MM200) before analysis. Two milligrams of separate dried milled samples were ground with 200 mg KBr (FTIR grade) and homogenised in an agate mortar. KBr pellets were compressed under vacuum in a standard device under pressure of 6.000 kg/cm for 10 min. Infrared spectra were recorded using an FTIR Perkin-Elmer 2000 spectrophotometer over the 4000–400 cm^{-1} range at a rate of 0.5 cm/s. Fifty scans were collected, averaged for each spectrum and corrected against ambient air as background as described by Cuetos et al. (2009). FOG samples and inoculum were analysed, and the mean values of three replicates were estimated for each sample. Digestate samples were obtained at the end of experiments prior to dismantling the reactors. Commercial yeast and humic acid sodium salt CAS-1415-93-6 from Sigma Aldrich were also analysed to facilitate the interpretation of the spectra. Moreover, spectra were vector normalised for comparison following the procedure proposed by Meissl et al. (2007).

Data set collected were statistically analysed by one way analysis of variance (ANOVA) using Origin 6.0 software.

5.3 Results and discussion

5.3.1 Anaerobic digestion

Daily biogas production is presented in Figure 5.1. Reactor SS showed an increase in biogas production with the decrease in HRT (increasing organic loading rate). This behaviour may be rationalised by the low complex nature of the substrate, which allowed for its rapid conversion. Although the reactor was not studied during an extended period at each HRT, a trend of increasing biogas production was observed with increasing organic loading. Thus, the decrease in the residence time of the waste inside the reactor did not exert any negative effect on the performance of the digestion process. On the contrary, when the mixture of SS and FOG were treated in the reactor, no major variations were observed in biogas production at an HRT of 30 days. This may be explained by the fact that the additional organic load provided by the lipid-rich waste was too low (due to the volumetric ratio used (0.2%)) to exert any observable effect on the performance of the reactor. However, a subsequent decrease in the HRT did not lead to significant increases in biogas production, which contradicts the results previously obtained from the SS reactor. The progressive decrease in specific methane production obtained for this co-digesting reactor at HRTs of 26 and 21 days may be associated with an inhibitory effect. With regard to the biogas production, the mean values obtained for each HRT differed significantly when ANOVA analysis was applied at a confidence level of 5%.

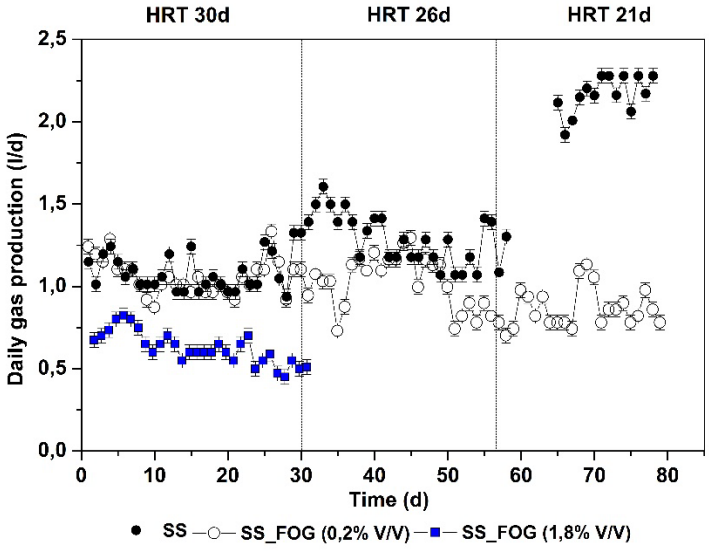

Figure 5.1 Daily gas production of the reactor treating the mixture of primary sludge and secondary sludge (SS) and the co-digestion mixture (SS_FOG)

The study of FOG co-digestion with SS at a higher volumetric proportion (1.8%) was reported by Martinez et al. (2011). It was found that treating a mixture with a higher content of lipid-rich waste resulted in a decrease in specific methane production (SMP), although an adaptation period was applied to the reactor. Table 5.2 shows the parameters obtained during digestion in this reactor (denoted as SS_FOG_1.8%) evaluated at an HRT of 30 days for 3 consecutive HRT periods. These results are indicative of an inhibitory effect exerted by the addition of the co-substrate.

Table 5.2 Parameters of reactors for semi-continuous digestion

PARAMETER	SS	SS_FOG 0.2%	SS_FOG 1.8%[(d)]	SS	SS_FOG 0.2%	SS	SS_FOG 0.2%
HRT (d)	30	30	30	26	26	21	21
OLR [(a)] (gVS/l*d)	0.77±0.07	0.77±0.07	0.79±0.08	0.88±0.07	0.90±0.08	1.10±0.09	1.11±0.08
SMP [(b)] (ml/gVS*d)	304±45	298±54	200±63	288±55	223±51	428±46	144±31
Gas Prod.[(c)] (l/d)	1.08±0.12	1.06±0.16	0.70±0.21	1.29±0.22	1.02±0.21	1.82±0.18	0.80±0.16
% CH_4	65±2	65±2	67±3	59±2	59±3	60±2	60±3
H_2S (ppm)	120±67	180±50	160±65	144±50	118±46	129±26	112±65
VS (g/l)	14.4±0.8	12.6±0.8	12.9±0.9	11.6±1.6	11.8±1.7	9.9±1.40	11.1±0.7
TS (g/l)	21.8±1.1	19.4±1.3	18.5±1.3	16.8±2.1	17.7±2.7	14.6±1.3	17.0±1.2
COD (g/l)	26±5	23±3	12.5±4.0	17±5	15±3	16±4	16±4
pH	7.50±0.03	7.50±0.03	7.40±0.02	7.40±0.10	7.50±0.10	7.40±0.08	7.30±0.05
NH_4^+ (mg/l)	881±68	730±67	824±66	757±96	807±52	800±370	815±81
Acetic (mg/l)	126±57	84±21	---	45±21	33±15	17±7	9±3
Propionic (mg/l)	13.0±1.2	---	---	5.0±0.3	---	---	---

(a)OLR: Organic loading rate; (b) SMP: Specific methane production; (c) Gas Prod: Average daily gas production, (d) Parameters of FOG co-digestion with SS at higher volumetric proportion (1.8%) was reported by Martinez et al. (2011).

Table 5.2 also shows the measured values of biogas composition and parameters measured from the reactors studied (SS and SS_FOG). The methane content in the biogas was similar for both reactors, with this value presenting a decrease with the reduction in the HRT of

each system. Similar values of H_2S content were also measured for both reactors at the different HRTs tested. With regard to the concentration of acetic acid in reactors, it was also observed that lower values of this acid were obtained for the co-digesting reactor at each HRT. These results may indicate that the co-substrate may impede the degradation of the readily degradable components of SS.

With regard to the behaviour of the solid concentration and COD values during the period studied, a decreasing trend is observed when comparing consecutive HRT periods for individual reactors. However, both reactors presented similar values when the HRT periods were equal. On the other hand, pH and NH_4^+ concentration followed a constant behaviour during the experiments. The NH_4^+ concentration in digestion systems is associated with protein degradation. Although high values of these parameters may inhibit the digestion process, the values reported in Table 5.2 are far from being inhibitory, as established by Hansen et al. (1998) and Hashimoto (1986). In this sense, the possible inhibition by NH_4^+ was disregarded.

Carbohydrates, lipids and proteins may be considered to be the main components of sludge. In studying primary sludge digestion, Miron et al. (2000) reported that the hydrolysis of lipids and carbohydrates increased with increasing solid retention time (SRT), whereas protein hydrolysis only occurred under methanogenic conditions. These authors also reported a SRT greater than 8 days as needed to achieve methanogenic conditions. In the case of proteins, these compounds are hydrolysed to amino acids and further degraded to VFA either through anaerobic oxidation linked to hydrogen production or via fermentation according to the Stickland reaction (McInerney, 1988). The former is dependent on the presence of hydrogen scavengers, while the latter is independent of the methanogenic activity in the reactor (Nagase and Matsuo, 1982). In the present study, the HRT was set to values much higher than those considered by Miron et al. (2000) to attain methanogenic conditions. The similar values of NH_4^+ and pH measured regardless of the conditions applied may thus be interpreted as the hydrolysis of protein being completely attained at each HRT period for both reactors. Thus, FOG addition did not affect protein hydrolysis.

The results obtained in the present study do not agree with those obtained by Kabouris et al. (2009), who studied the semi-continuous digestion of FOG and SS under mesophilic and thermophilic conditions. These authors reported a significant increase in methane-specific production: 2.95 times more in the case of the mesophilic system and 2.6 times more for the thermophilic digester when they applied a high load of FOG as a co-substrate (48% of the

total VS load). The differences in the results may be explained by the different origins of the FOG fraction. The FOG used by these authors was obtained from grease traps associated with, among others, restaurants, hospitals, schools, assisted living facilities and catering services, while the FOG used in the present study was obtained from the grease trap of the WWTP and was of a grey colour. Several studies have also reported significant increments in biogas production with the addition of greases collected from grease traps. These results are characterised by the absence of inhibitory effects (Davidsson et al. 2008; Luostarinen et al. 2009; Martín-González et al. 2010; Silvestre et al. 2011; Wan et al. 2011).

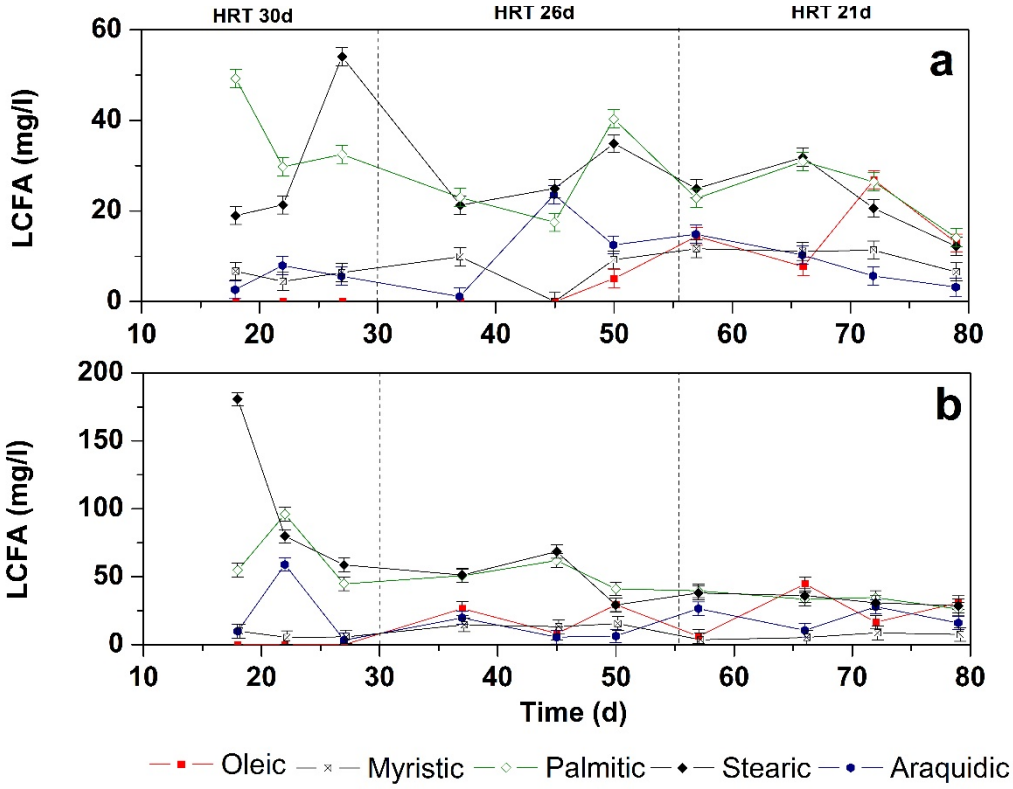

Figure 5.2 LCFA concentration of (a) reactor SS treating the mixture of primary sludge and secondary sludge and (b) the co-digestion mixture SS_FOG

It is well known that the accumulation of LCFAs may inhibit anaerobic digestion because of their direct toxicity toward acetogens and methanogens, the two main groups involved in LCFA breakdown (Hanaki et al., 1981). Another inhibiting mechanism is the adsorption of surface active acids onto the cell wall (Alves et al., 2001), which affects the processes of transportation and protection. Figure 5.2 shows the LCFA concentrations measured in the SS and SS_FOG reactors. The values obtained were lower than those reported in the literature as causing inhibition (Koster et al., 1987). Hence, the most probable reason why

a lower gas yield was obtained from the SS_FOG reactor is the adsorption of FOG components onto sludge, which then would have precluded degradation by microorganisms.

5.3.2 FTIR analysis

The hypothesis of inhibition of the co-digesting reactor due to the adsorption of FOG components was assessed by FTIR spectroscopy; Figure 5.3a and b show the FTIR spectra of FOG, humic acid, yeast and digestate samples. The bands selected as being characteristic of humic acid were those observed at 2914 and 2849 cm^{-1}, as shown in Figure 5.3a. These bands were used to identify biomolecules with high aliphatic content and components of the substrate presenting an important contribution of C-H bonds. The spectrum obtained from the yeast sample was used to characterise biological material, with the main band being identified at 1644 cm^{-1}. It is not possible to unequivocally assign this band due to the overlapping of aromatic C=C vibrations, functional group vibrations such as C=O (carboxylates and amides), C=C (alkenes) and OH from water, which all contribute to this strong band (Meissl et al., 2007). FOG showed a high aliphatic degree with a strong absorption band at around 2800 and 3000 cm^{-1}, which is related to C–H stretching (Reveillé et al., 2003). Specifically, it presented a band of strong intensity at around 2960 cm^{-1}, which was ascribed to the C–H stretching of aliphatic methylene (Hafidi et al., 2005), and the additional bands at 2905 cm^{-1} and 2851 cm^{-1} were assigned to the stretching of aliphatic C–H bonds (Castaldi et al., 2005; Hafidi et al., 2005). In this case, these aliphatic bands were assigned to fats and lipids (Reveillé et al., 2003).

Another region with intense signals was the one observed at 1800 -1485 cm^{-1}. The FOG sample presented similarities with that obtained from commercial yeast in this region, with the latter ascribed to proteins (Naumann 2000; Wilson et al. 2000).The range below 1500 cm^{-1} is significant for deformation, bending and ring vibrations. This range is frequently referred to as the “fingerprint region” of a spectrum (Schmitt and Flemming, 1998), with the bands at 1185 - 900 cm^{-1} being ascribed to polysaccharide components (Naumann et al., 2010).

Figure 5.3b presents the spectra obtained from feedstocks and digestate samples (inoculum and digestates taken at the end of digestion experiments). Bands associated with aliphatic components (2920–2930 and at 2850 cm^{-1}) were registered for all samples but with higher relative intensity being observed in those samples containing FOG as a co-substrate. With

regard to the region ascribed to proteins and polysaccharides, samples taken from digestion experiments presented higher intensities in both regions when compared to the inoculum sample. The comparison with fresh residue bands associated with aliphatic components shows a decreasing trend observed for digestates, with a similar tendency being also observed for the band at 1400 cm^{-1}, and 1730 cm^{-1} (associated with carbohydrates).

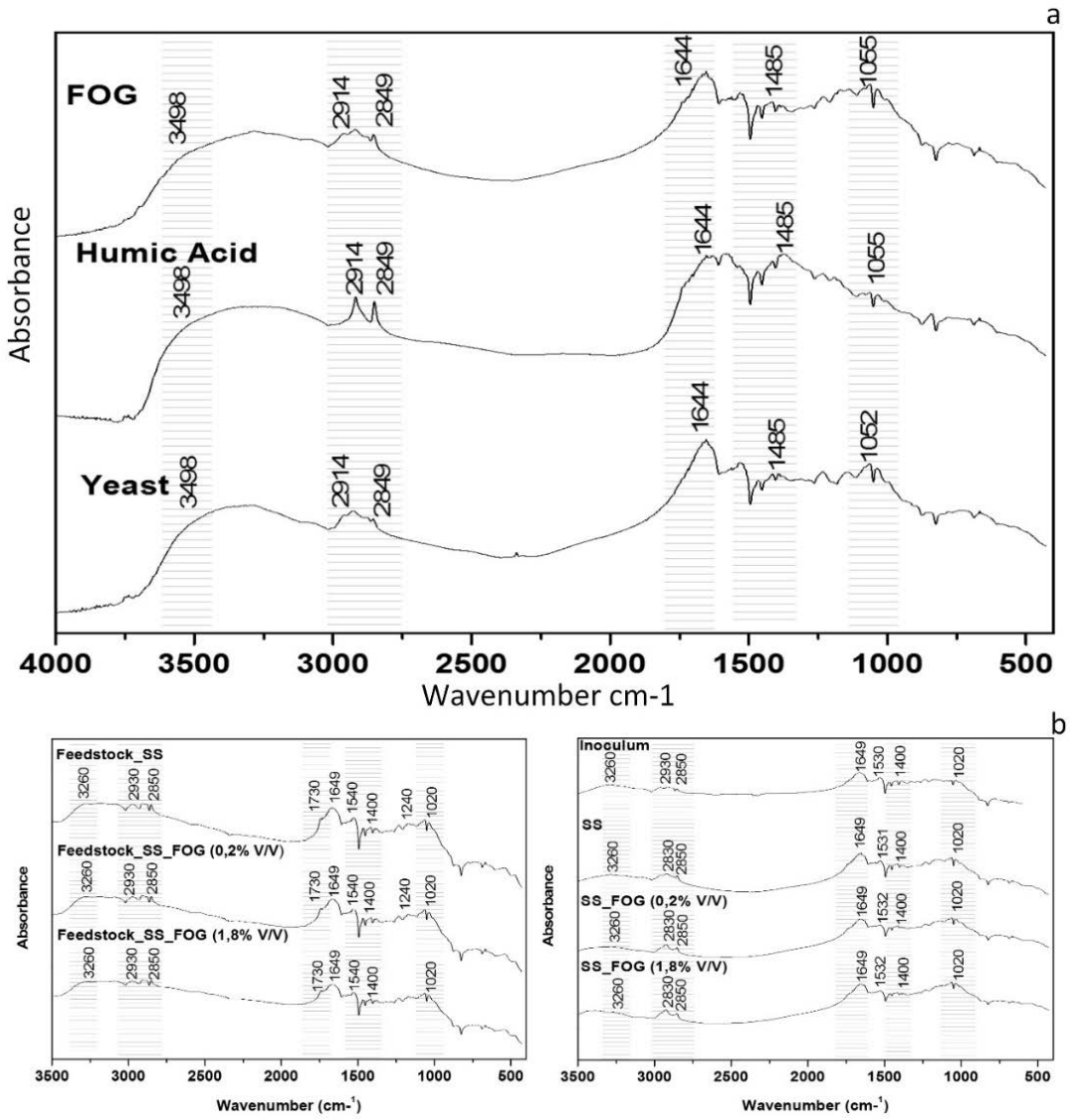

Figure 5.3 (a) FTIR spectra obtained from fresh samples; (b) FTIR spectra obtained from feedstocks and digestate samples

However, with the addition of the co-substrate, the intensity of the polysaccharide region increased. The higher intensities registered for these bands as well as from those obtained from the aliphatic region suggest that FOG components may have been adsorbed onto the

active sludge, and as a consequence the degradation of organic material was not completely achieved during the anaerobic treatment.

5.4 Conclusion

Enhancing biogas production from sewage sludge (SS) by addition of a co-substrate was the goal of the present study. The fat, oil and grease (FOG) mixture used was a lipid-rich waste obtained from the grease trap of the wastewater treatment plant (WWTP) of the city of León. Digestion under semi-continuous operation resulted in no detriment to the specific methane production with FOG addition of 0.2% (V/V) at a hydraulic retention (HRT) of 30 days. However, a continuous decrease in the HRT resulted in the detriment of the performance of the reactor. The FTIR spectra obtained from the co-digestating samples of the studied reactors showed high-intensity bands in the aliphatic region, which where ascribed to fat and lipids, resembling the spectra obtained from the FOG substrate. The results suggest the possible adsorption of lipid components of FOG, which may have impeded the degradation of the substrate during the anaerobic treatment.

5.5 Acknowledgements

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Capítulo/Chapter 6

6. Anaerobic co-digestion of sludge: addition of butcher's fat waste as co-substrate for increasing biogas production

Abstract

Fat waste discarded from butcheries was used as co-substrate in the anaerobic co-digestion of sewage sludge (SS). The process was evaluated under mesophilic and thermophilic conditions. The co-digestion was successfully attained despite some inhibitory stages initially present which had their origin in the accumulation of volatile fatty acids (VFA) and adsorption of long chain fatty acids (LCFA). The addition of a fat waste improved digestion stability and increased biogas yields thanks to the higher organic loading rate (OLR) applied to the reactors. However, thermophilic digestion was characterised by an effluent of poor quality and high VFA content.

Results from spectroscopic analysis suggested the adsorption of lipid components onto anaerobic biomass, disturbing therefore the complete degradation of substrate during the treatment. The formation of fatty aggregates in the thermophilic reactor prevented process failure by avoiding the exposure of biomass to the toxic effect of high LCFA concentration.

Keywords: co-digestion, sewage sludge, thermophilic, mesophilic, butcher's fat waste.

Edition of this manuscript was in accordance with the scientific journal

6.1 Introduction

Environmental concerns and stringent regulations regarding sludge management and disposal entail to seek alternatives and feasible solutions for the treatment of such waste. On the other hand, food-processing industries generate wastewaters and solid fatty by-products, which are difficult to treat and can cause environmental problems when improperly disposed. The main treatment option is incineration but this also results in increased management costs. Lipid rich materials, abundant in food processing industries are known to have high biogas potential. This characteristic makes them an interesting co-substrate to be treated in anaerobic reactors (Cavaleiro et al., 2008; Kim et al., 2004). In the case of butcheries, the amount of animal fat suitable for human consumption being discarded as a waste is increasing due to the changes in customer perception of a healthy diet. The excessive consumption of fats has been associated with higher rates of obesity and growing chronic disease risks (Drewnowski et al., 1997). In addition, the meat industry also needs to deal with the negative perception caused by the high carbon footprint of the business. Therefore, the valorisation of butcher's animal fat for biogas production will aid in increasing green credentials of the meat industry by means of producing a renewable fuel and allowing the recycling of nutrients.

Anaerobic digestion is a well-established process for converting organic matter into biogas and digestate, with this latter being a valuable fertiliser and soil conditioner. Co-digestion offers several benefits over digestion of separate materials. Co-digestion of sewage sludge with other organic wastes improves biogas production and organic matter degradation (Luostarinen et al., 2009), as well as it involves the dilution of inhibitory compounds and favours the degradation of lipids (Mata-Alvarez et al., 2000)

Despite the reported benefits of co-digestion, the accumulation of lipid components during the digestion process may cause inhibition associated with the toxicity of a given number of fatty acids on anaerobic microorganisms. The main LCFAs found at food processing industries are oleic acid (C18: 1), which is mainly found in olive, pecan and teased oils, and stearic acid (C18: 0), which is present in cocoa, tallow and lards (Lalman & Bagley 2001). Under anaerobic conditions these LCFAs are produced when fats and oils are hydrolysed. Several studies have suggested inhibition caused by these acids could be reversible, with acclimatisation being a key factor in avoiding negative effects on microbial communities (Cavaleiro et al. 2008; Pereira et al 2004; Pereira et al. 2005).

Long-chain fatty acids (LCFAs) inhibit H_2-producing bacteria responsible for β-oxidation (Hanaki et al. 1981; Sage et al. 2008) and may disrupt the process in different ways. Adsorption of LCFAs onto the sludge can affect the transport and protective functions of the bacteria wall and form a hydrophobic layer of LCFAs around biomass aggregates reducing the mass transfer between the media and bacteria (Pereira et al., 2004). Entrapment of LCFAs in biomass aggregates can lead to biomass flotation inside the reactor and, as a consequence, to biomass washout (Hwu et al., 1998; Pereira et al., 2003). Precipitation of LCFAs with divalent ions such as Ca_2^+ or Mg_2^+ makes them inaccessible to anaerobic biomass and hence reduces their biodegradability (Pereira et al., 2005) but may also aid in avoiding biomass toxicity.

Many experimental approaches have been used to study interactions of LCFAs in anaerobic digestion of lipid rich materials, potential toxicity, microbial communities, mathematical modelling, and microscopic and macroscopic analyses (Borowski and Kubacki, 2015; Zonta et al., 2013). In this line, Fourier transform infrared spectroscopy (FTIR) is a spectroscopic tool which provides valuable information regarding the chemical characteristics of samples. This technique is sensitive to changes in organic functional groups and it has demonstrated to be well suited for the study of organic transformations during biological processes (Carballo et al., 2008; Gómez et al. 2007; Provenzano et al., 2014).

Another important parameter is the temperature of the digestion process. Mesophilic conditions are commonly studied at 36–40 °C, while thermophilic conditions are usually tested at around 55 °C. The increase up to the thermophilic regimen would allow for an increase in the treatment capacity of the digester due to the capability of thermophilic microorganisms to work at higher organic loading rates (OLRs) and lower hydraulic retention times (HRTs). The aim of the present research was to assess the effect of animal fat obtained from butcher's shops when added as co-substrate on the anaerobic digestion of sewage sludge. Anaerobic co-digestion with animal fat was conducted at mesophilic and thermophilic regimens with the purpose of evaluating the combined effect of temperature and high accumulation of LCFAs. The characteristics of the digested material were evaluated by the use of FTIR spectroscopy.

6.2 Material and methods

6.2.1 Characteristics of substrate and inoculum

Sewage sludge (SS) and inoculum were obtained from the WWTP of Cáceres (Spain). The plant has an anaerobic digester for the treatment of a mixture of primary and secondary sludge and operates under mesophilic regimen. Animal fat (F) was collected from a local butcher shop (León, Spain). The material comprised animal fat discarded by clients due to excessive fat content of veal meat. The chemical characteristics of the materials used in this study are presented in Table 6.1.

Table 6.1 Characterisation of wastes

Parameter	Inoculum	SS	F
TS (g L^{-1})	12.3±0.6	19.0±0.9	98.3±4.9
VS (g L^{-1})	8.0±0.4	14.9±0.7	98.2±4.9
Alkalinity (mg L^{-1})	1316±65	512±26	-
NH_4^+ (mg L^{-1})	276±14	137±7.0	-
Organic Matter (%)	0.87±0.04	1.17±0.05	98.1±5.0
Kjeldahl Nitrogen (%)	0.1	0.1	0.04
C/N	4.7	6.6	1438.7
Lipids (%)	n.d	n.d	0.27±0.01
LCFAs (%)			
Myristic C:18	n.d	n.d	4.43±0.22
Palmitic C:16	n.d	n.d	21.35±1.25
Stearic C:18	n.d	n.d	73.53±4.53

(n.d) not detected
TS: Total solids; VS: Volatile solids.

6.2.2 Experimental set up

6.2.2.1 Batch Digestion

Batch experiments were performed to determine the biochemical methane potential (BMP) of substrates. Experiments were carried out in Erlenmeyer flasks of 250 ml. The temperature was controlled by a water bath and set at 35 ± 1 °C for mesophilic and 55 ± 1 °C for thermophilic tests. Agitation was provided by means of magnetic stirrers. Bottle gasometers were used to measure the volume of gas. Measurements were standardised to temperature and pressure (STP), 0 °C and 760 mmHg, respectively. Erlenmeyer flasks were

inoculated using a ratio of VS inoculum/substrate (I:S) in the range of 1:1 - 2:1. This ratio was selected to avoid the addition of alkali solution for pH control. Batch digestion systems were denoted as MS and TS when digesting sewage sludge under mesophilic and thermophilic conditions, respectively. In a similar way, co-digestion systems were denoted as MS:F and TS:F. These systems were evaluated by adding fat at percentages of 2.5 - 5% w/v.

6.2.2.2 Semi-continuous Digestion

Semi-continuous digestion was carried out in completely stirred reactors. The working volume of the mesophilic system was 3 L and 5 L for the thermophilic reactor. Reactors were equipped with mechanical agitators and outer jackets to circulate heating water. Temperature was controlled at 35 ± 1 °C and 55 ± 1 °C in accordance with digestion regimen. Reactors also had sampling ports for the withdrawal of liquid samples and gas collection. Feeding was manually performed once a day. Samples were taken after complete homogenisation and prior to feeding. Reactors were evaluated at an HRT of 30 days, except for the reactor digesting sewage sludge at mesophilic conditions which was tested at an HRT of 40 days since the application of lower times resulted in acidic conditions. Semi-continuous reactors were denoted as RMS and RTS when digesting sewage sludge under mesophilic and thermophilic conditions, while semi-continuous co-digestion reactors were denoted as RMS:F and RTS:F.

6.2.3 Analytical techniques

Total and volatile solids, pH, ammonia and alkalinity were determined in accordance with APHA Standard Methods (APHA, 1999). Nitrogen concentration was determined by the Kjeldahl method. Organic matter was analysed in accordance with the Walkley-Black method (Walkey & Black, 1934). Lipid content was determined by Soxhlet extraction using Velp Scientifica SER 148/3 in accordance with APHA Standard Methods (APHA, 1999).

Biogas composition was analysed as described in Martínez et al. (2012), using a gas chromatograph (Varian CP3800 GC) equipped with a thermal conductivity detector. A packed column (HayeSep Q 80/100; 4 m) followed by a molecular sieve column (1 m) was used to separate CH_4, CO_2, N_2, H_2 and O_2. The carrier gas was helium, and the columns were operated at a pressure of 331 kPa and a temperature of 50 °C.

Volatile fatty acids (VFAs) were determined as described by Cuetos et al. (2009) using a gas chromatograph and a flame ionisation detector (FID) equipped with a Nukol capillary column (30 m × 0.25 mm × 0.25 μm) from Supelco. The carrier gas was helium. The injector and detector temperatures were 220 °C and 250 °C, respectively. The oven temperature was set to 150 °C for 3 min and increased to 180 °C with a ramp of 10 °C min^{-1}. The detection limit for VFA analysis was 5.0 mg L^{-1}. The system was calibrated with a mixture of standard volatile acids from Supelco (for the analysis of fatty acids C2 to C7). Samples were previously centrifuged (10 min, 3500 × g) and the supernatant filtered through 0.45 μm cellulose filters.

Gas chromatography was used for the analysis of long chain fatty acids (LCFAs). Samples were extracted with n-heptane. The solution was then centrifuged for 30 min at 3500 × g and filtered through a 0.2-μm Millipore Millex-FGS filter. The sample was injected into a Perkin-Elmer AutoSystem XL chromatograph equipped with an FID detector and a PEG (100% polyethylene glycol) column (15 m × 0.53 mm × 0.5 μm). The carrier gas was helium. The initial oven temperature of 100 °C was maintained for 1 min and then increased to 250 °C with a ramp of 5 °C min^{-1}; this temperature was maintained for 5 min. The injector and detector temperatures were 250 °C and 275 °C, respectively. The system was calibrated using a mixture of LCFAs from individual acids with concentrations in the range of 0 to 100 mg/L. The detection limit for LCFA analysis was 5.0 mg L^{-1}. The acids analysed were C8:C18 (with even numbers of carbon atoms) and all obtained from Sigma.

6.2.4 FTIR analysis

Samples were dried at 105 °C in a furnace for 48 h and then ground in a laboratory ball mill (Retsch mill model MM200) before analysis. Two milligrams of separate dried milled samples were ground with 200 mg KBr (FTIR grade) and homogenised. KBr pellets were compressed under vacuum in a standard device under pressure of 6000 kg cm^{-1} for 10 min. Infrared spectra were recorded using an FTIR Perkin-Elmer 2000 spectrophotometer over the 4000 – 400 cm^{-1} range at a rate of 0.5 cm s^{-1}. Fifty scans were collected, averaged for each spectrum and corrected against ambient air as background as described by Cuetos et al. (2009). Digestate samples from semi-continuous experiments and SS feedstock were analysed. Spectra are the representation of mean values of three replicates for each sample. Digestate samples were obtained at the end of experiments prior to dismantling the semi-continuous reactors. Sewage sludge and commercial LCFAs (palmitic and stearic acids)

were also analysed to facilitate the interpretation of the spectra. Moreover, spectra were vector normalised for comparison following the procedure proposed by Meissl et al. (2007).

6.2.4.1 Statistical analysis of FTIR spectra

Two multivariate statistical methods, Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA), were used for the evaluation of the samples based on their FTIR spectra at the region between 4000 – 800 cm^{-1}. The initial data values were standardised, mean centred and autoscaled to variance 1 prior to analysis to avoid any effects of scale of units with which they were measured.

HCA was used to classify the studied samples into different groups, based on the values of the set of data extracted from the FTIR spectra. The clusters were formed by grouping samples according to their similarity and the results were presented in the form of dendrograms. Grouping in clusters was carried out using Ward's algorithm.

PCA was used in order to reduce the initial data from linear combinations of the original variables. The aim of this technique is the reduction of an original set of variables into a smaller set of non-correlated components, which represent most of the information in the original variables and that are called principal components (PCs). Usually, only the first few PCs in a descending order explain the maximum of the total variance of all original variables. The score plot of the first PCs was used to investigate the interrelationships among the samples, as it allowed the observation of clusters of samples. Both HCA and PCA analyses were performed using the SPSS v. 19.0 software.

6.3 Results and discussion

6.3.1 Batch digestion

Results obtained from batch digestion experiments of sludge and co-digestion with animal fat at mesophilic and thermophilic conditions are presented in Figure 6.1a-b. The digestion of sludge was initially performed under mesophilic condition at an I:S ratio of 1. However, the rapid acidification of sludge prevented further digestion of the substrate (data not reported). A second experiment was then performed with an I:S ratio of 2. Results are shown in Figure 6.1a where an initial accumulation of VFAs is observed with their subsequent degradation.

On the contrary, the thermophilic digestion was carried out using an I:S ratio of 1. Once the lag phase has been overcome, the conversion of the organic material was achieved in a short time. The final value obtained for the specific methane production was however much lower than that at mesophilic conditions. The thermophilic experiment was performed using mesophilic inoculum; thereby an extended lag phase was expected in this experiment associated with the acclimation to higher temperatures. The poor results may probably be explained by the prolonged inhibitory state experienced by this reactor and by the fact that the experiment was performed using a non-adapted inoculum to thermophilic conditions. Furthermore, the thermophilic system did not show high VFA accumulation. This behaviour could be explained by the lower capacity of the non-adapted anaerobic microflora to degrade the organic material. VFA produced during the acidogenesis phase accumulated in the system and probably caused an inhibitory stage of acetoclastic methanogen. This unbalanced reaction rate between acidogens and methanogens implies that solubilised compounds (i.e. VFAs) at the thermophilic temperature were not efficiently converted to CH_4 (Kim et al., 2002).

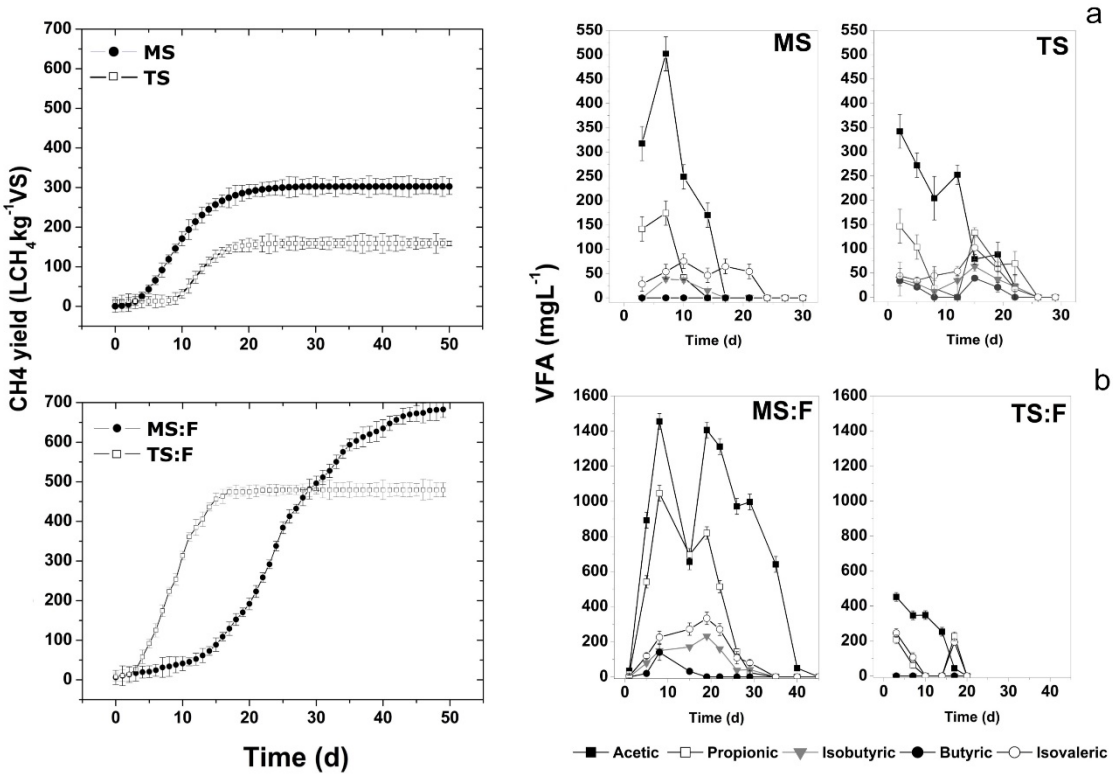

Figure 6.1 Batch digestion experiments of sludge: a) methane yield and VFA results of mesophilic (MS) and thermophilic (TS) and b) co-digestion experiments of sludge with fat: methane yield and VFA results of mesophilic (MS:F) and thermophilic conditions (TS:F)

Results from batch co-digestion tests are presented in Figure 6.1b. There was a significant increase in biogas production at mesophilic regimen when fat was used as co-substrate. The benefits of co-digestion when adding this co-substrate are due to the increase attained in OLR and thus in the effective use of the reactor volume, rather than to an increase in the specific methane production. During the initial stage of digestion, the accumulation of VFAs along with a lag phase in cumulative gas curves was observed. Under thermophilic conditions, the system was capable of rapidly degrading complex lipid materials added with the co-substrate. In this line, VFA build-up was not a cause of inhibition. The gas yield obtained in this experiment was lower than that of its homologous mesophilic reactor, even though an extended adaptation period to thermophilic conditions was applied to the inoculum.

6.3.2 Semi-continuous digestion

Methane yield obtained from RMS and RTS reactors are presented in Figure 6.2a. Low gas production was observed over the entire test with periods of nil gas production at mesophilic conditions. Digestion of this particular sludge proved to be difficult with the need of repetitive inoculation steps being required to increase the pH of this system. Low alkalinity of the reactor resulted in recurrent pH variations being observed during the experiment. It was finally possible to achieve a decrease in VFA content (average value of 529 mg L^{-1}) during the start-up phase to the low values reported in Table 6.2 but at the cost of operating at low OLRs (0.25 g VS L^{-1} d^{-1} equivalent to an HTR of 40 d).

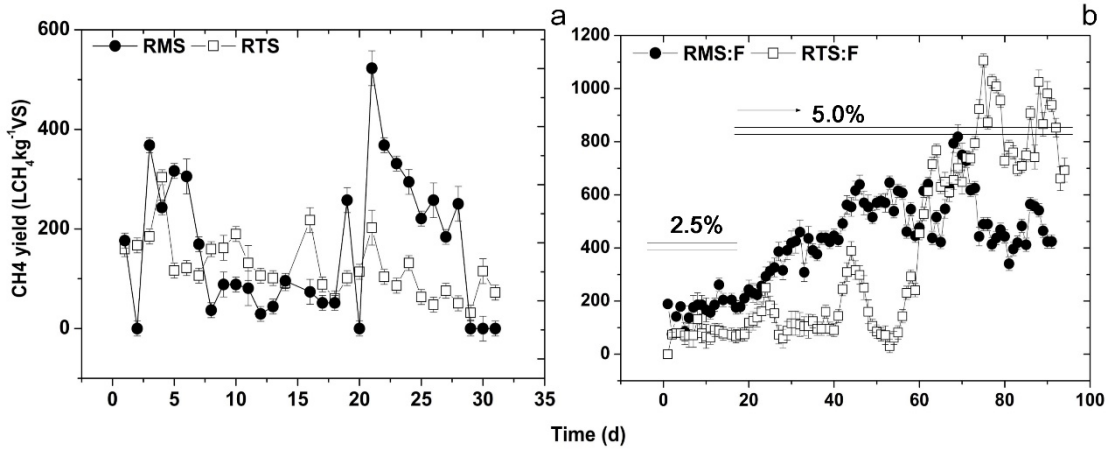

Figure 6.2 Methane yield obtained from digestion of: (a) sludge under mesophilic (RMS) and thermophilic (RTS) conditions and (b) co-digestion with fat under mesophilic (RMS:F) and thermophilic (RTS:F) regimens.

The start-up of reactor RTS was carried out after a 40 day adaptation period to thermophilic conditions (data not shown). Although steady production of biogas was attained during the whole experimental period, results were not satisfactory (Figure 6.2a). OLR applied was just 0.33 g VS L^{-1} d^{-1} to prevent VFA accumulation.

Figure 6.2b shows methane yields obtained from co-digestion systems. Parameters of reactor performance are also reported in Table 6.2. The co-digestion process initiated with a 2.5% addition of co-substrate and this value was increased up to a 5% addition. The RTS system proved to be superior. Although instabilities associated with high VFA concentration when digesting sludge are inferred from Table 6.2, the addition of co-substrate alleviated the inhibitory state. The co-digestion under thermophilic conditions (RTS:F) during the first 30 days showed lower gas yields and higher VFA accumulation, which may be explained by the adaptation needed for the system to degrade complex lipids. Angelidaki and Ahring (1992) also reported the need of an adaptation period to attain the degradation of lipids when adding a fatty co-substrate to the digestion of cattle wastes. Oleate and stearate affected growth by increasing the lag phase, which implies a shock load of fat can make a biogas reactor inactive for long periods.

The concentration of VFA from the different thermophilic systems was higher when compared to mesophilic reactors, but this is not a characteristic associated with a detrimental impact on performance. Thermophilic reactors present higher content of VFA during normal operation as reported by several authors (Öztürk, 1991; Kim et al., 2002; Bolzonella et al., 2012). The addition of a co-substrate resulted in higher values of propionic acid but the content in acetic acid was reduced. The accumulation of these acids may be explained by the increase in OLR when the co-substrate is added. Ammonium values in thermophilic reactors were far from reaching inhibitory levels, so this fact should be discarded as possible explanation for poor reactor performance.

The better performance experienced when fat was added, might indicate a significant effect of C/N ratio on thermophilic performance. Systems co-digesting fat presented an increase in biogas production with the increase in co-substrate addition. Methane yields were 520 L CH_4 kg^{-1} VS for the mesophilic system and 516 L CH_4 kg^{-1} VS, for the thermophilic system. These results were significantly higher than those obtained from the reactor digesting sludge where great difficulties were encountered. The addition of animal fat not only allowed higher gas production but also increased stability of the digestion process.

Results were in accordance with several studies of sewage sludge co-digestion with agro-industrial waste (Davidsson et al.,2008; Loustarinen et al., 2009; Bayr et al., 2012; Iacovidou et al., 2012; Martínez et al., 2012; Fierro et al., 2014), showing a remarkable enhancement. However, there should be borne in mind there is always a limit to which addition of wastes can cause a detriment in process performance based on the complex characteristics of the co-substrate. In the present research the addition of the co-substrate reported a significant increase in OLR which resulted in higher biogas production, but the main effect obtained was the increase in the stability of the digestion process.

The formation of flocculent aggregates (FW) of fat was observed during thermophilic digestion. Their presence might be attributed to the high content of LCFAs inside this reactor. Pereira et al. (2004) also reported floc structures accumulating lipid material when operating with suspended anaerobic biomass in the presence of high LCFA content. Lipid components can be entrapped by this type of sludge with a greater tendency than that presented in granular sludge. Pereira et al. (2005) also observed the accumulation of LCFAs, adsorption onto sludge and entrapment in flocculent aggregates when palmitic and oleic acid were present in a concentration range of 100 - 900 mg L^{-1}.

Table 6.2 Parameters of semi-continuous digestion at mesophilic and thermophilic conditions

Parameter	MS	TS	MS:F			TS:F		
% Co-substrate	0	0	2.5	5	5	2.5	5	5
Days of experiment	32	32	12	17	58	12	18	60
OLR (gVS L^{-1} d^{-1})	0.25±0.01	0.33±0.01	0.90±0.02	1.33±0.02	1.21±0.02	0.87±0.02	1.30±0.02	1.19±0.02
Biogas (mL d^{-1})	284±30	500±50	444±42	1167±60	1894±80	669±60	1460±80	4560±200
CH_4 (%)	55±5.0	50±5.0	61±5.0	62±5.0	61±5.0	55±5.0	51±5.0	66±6.0
CH_4 yield (L Kg^{-1}VS)	163±16	121±12	164±16	293±30	520±50	80±8.0	114±10	516±50
NH_4^+ (mg L^{-1})	730±37	717±36	847±42	901±45	800±40	1014±50	997±50	878±44
Alkalinity (mg L^{-1})	1680±84	1670±83	2470±120	1980±99	2112±106	2650±133	1980±99	2112±106
Total Solid (mg L^{-1})	11.50±0.6	13.80±0.7	14.03±0.7	16.18±0.8	20.20±1.0	12.71±0.6	13.78±0.7	22.08±1.0
Volatile Solid (mg L^{-1})	7.40±0.4	9.10±0.5	9.14±0.5	11.36±0.6	11.38±0.6	8.25±0.4	8.80±0.4	12.74±0.6
VFA (mg L^{-1})								
Acetic	201.9±10	766.9±38	22.8±1.1	29.3±1.2	25.7±1.3	239.5±12	268.6±13.5	369.4±18
Propionic	21.1±1.0	363.9±18	12.2±1.0	n.d	2.8±0.1	621.8±31	912.5±46	778.3±39
Isobutyric	n.d	117.4±6.0	n.d	n.d	n.d	104.3±5.0	170.5±8.5	96.2±2.0
Butyric	n.d	91.8±5.0	n.d	n.d	n.d	129.5±6.5	88.6±4.4	24.4±1.2
Isovaleric	n.d	210.0±11	n.d	n.d	n.d	251.0±13	354.8±18	140.8±7.0
Valeric	n.d	9.3±0.5	n.d	n.d	n.d	58.8±3.0	104.2±5.2	32.7±1.6

Table 6.3 shows the results of LCFA and fat content of digested samples and the fatty aggregate. It can be observed a higher concentration of stearic and palmitic acid in RTS:F digestate and FW aggregate. No inhibitory effects were observed in thermophilic reactor during the last 60 days of operation. This could be due to the entrapment of LCFA into FW aggregates reducing the availability of this toxic compound to anaerobic microflora.

Table 6.3 Long chain fatty acids at digested samples

LCFA (mg L^{-1})	TS	RMS:F	RTS:F	FW
Caprylic C:8	20.9±1.0	3.5±0.2	n.d	34.2±1.7
Capric C:10	n.d	2.5±0.1	n.d	21.7±1.0
Lauric C:12	3.4±0.2	8.5±0.4	n.d	25.9±1.3
Myristic C:14	19.1±0.9	60.3±3.0	87.44±4.4	434.1±22
Palmitic C:16	223.0±11	536.0±27	663.70±33	3042.7±150
Stearic C:18	405.3±20	684.0±34	1952.03±98	7608.9±380
Fat (%)	3.2±0.2	3.2±0.2	5.2±0.3	17.7±0.9

6.3.3 FTIR analysis

FTIR-Spectroscopy was used to obtain information on functional groups of organic matter contained in digestates and fatty aggregates, to correlate principal functional groups of fat and sludge and to assess the hypothesis of inhibition due to fat adsorption.

Figure 6.3a and 6.3b show the FTIR spectra of commercial LCFAs and digested samples illustrating the dominant spectral features associated. Table 6.4 presents band assignation.

Spectra obtained from LCFAs samples (Figure 6.3a) were used to characterise fatty materials. Palmitic and Stearic samples showed a high aliphatic degree with a strong absorption band at around 2800 and 3000 cm^{-1}, which is related to C–H stretching. In particular, a band of strong intensity at around 2917 cm^{-1} is observed, which was ascribed to the C–H stretching of aliphatic methylene, and the additional band at 2851 cm^{-1} was assigned to the stretching of aliphatic C–H bonds. The main band identified at 1704 cm^{-1} was ascribed to its carboxylic nature.

Table 6.4 FTIR bands assignment for functional groups in digestates and LCFA samples

Nominal frecuency of bands (cm^{-1})	Assigment[a]
3600	O-H stretching of water
3400	H-bonded OH groups of alcohols, phenols and organic acids, as well as H-bonded N-H groups
2971	Methyl (-CH_3) asymmetric stretching of lipids
2917	Methylene (-CH_2) asymmetric stretching of lipids
2850	Methylene (-CH_2) symmetric stretching of lipids
1700-1750	C=O stretching vibrations of carboxylic groups involved in an ester linkage
1660-1628	C=O vibrations of primary amides at sludge
1548	C=O vibrations of primary amides
1450-1410	CH_2 scissor deformation vibrations
1230	Phospholipids (PO2) asymmetric stretching , protein amide III band (C-H and N-H)
1070-1048	–C–O–C of carbohydrates, Si–O–C groups
888-738	Scissoring deformation of CH_2

[a]Assigment according to Guillen and Cabo (1997), Tandon et al. (2000), Socrates (2001), Amir et al. (2010), Francioso et al. (2010),; Martínez et al. (2012), De Oliveira (2012) and Hernández et al. (2013).

Figure 6.3b shows spectra obtained from SS feedstock and digestate samples which were taken at the end of semi-continuous digestion experiments. The region ascribed to aliphatic components (2800 to 3000 cm^{-1}) is observed in these samples. Digestate samples containing fat as co-substrate present a band with higher intensity. The higher intensities registered for these bands obtained from the TS:F and FW samples suggest that fat components have been adsorbed onto the active sludge, and as a consequence the degradation of organic material was not completely achieved during the anaerobic treatment. In spite of this fact, no inhibitory conditions were observed during the operation of the thermophilic reactor, indicating that the formation of the fatty aggregates served as protector of the microbial system.

Digestate samples presented two high intensity bands at around 1560 and 1500 – 1400 cm^{-1}. The first band is ascribed to the presence of proteins in samples (primary amines) and the second one is ascribed to the adsorption of lipids onto the biomass surface. This peak is also

easily discerned in spectrum from the FW sample. The range below 1500 cm^{-1} is significant for deformation, bending and ring vibrations. This range is frequently referred to as the "fingerprint region" of a spectrum (Schmitt and Flemming, 1998). Digested samples and SS feedstock presented a band with an important contribution to the region at around 1185 - 900 cm^{-1}, being these signals ascribed to polysaccharide components (Naumann et al., 2010).

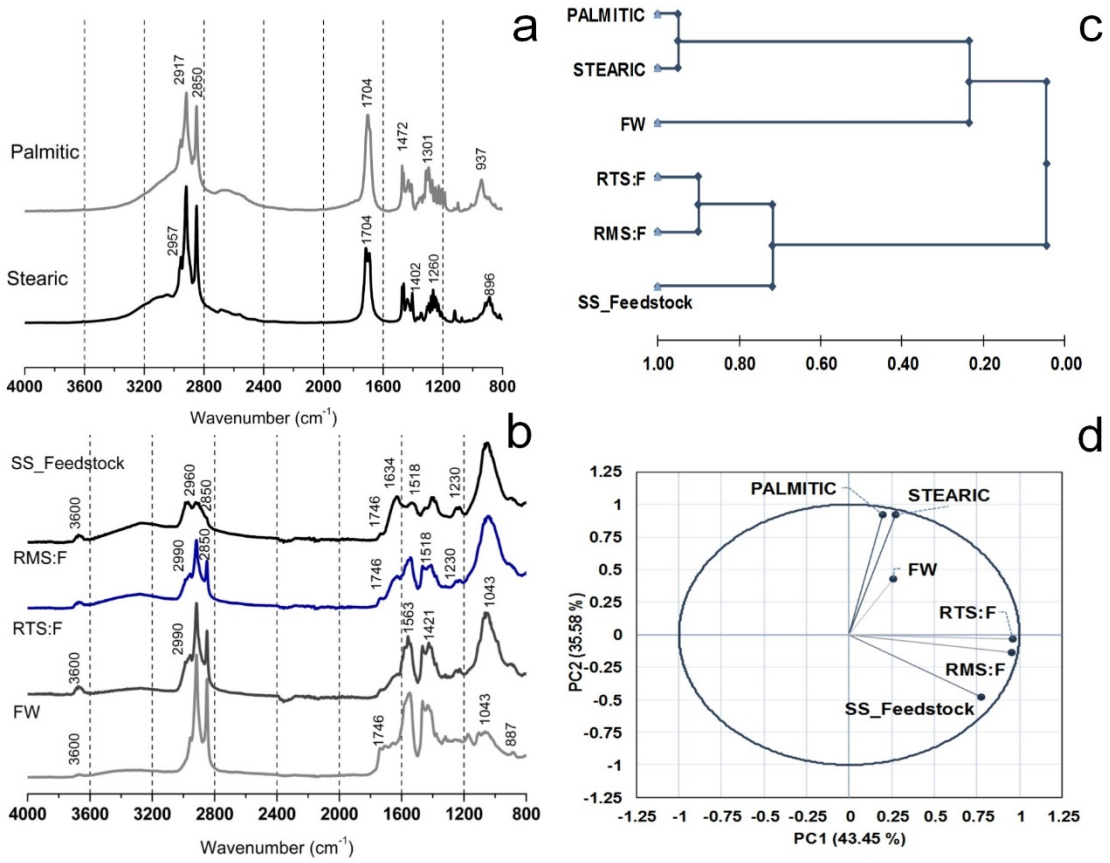

Figure 6.3 FTIR spectra of commercial LCFAs (Palmitic and Stearic acids) (a) and sludge samples (SS_feedstock, RMS:F, RTS:Fand FW) (b) together with statistical analysis of FTIR spectraby HCA (c) and PCA (d)

Statistical analysis of FTIR spectra allowed the evaluation of the samples based on their FTIR results in order to study the relationship between the RMS:F, RTS:F and FW samples with the main functional groups of fat (palmitic and stearic acids) and SS_feedstock. Pearson's correlation matrix in Table 6.5 reported a high correlation between both digestates and SS_feedstock. In addition, Table 6.5 shows a significant correlation between

FW and RTS:F samples, which is in accordance with the high formation of fatty aggregates found in the thermophilic reactor.

Figure 6.3c shows the dendrogram resulting from HCA, while Figure 6.3d shows the PCs plot resulting from PCA analysis of the FTIR spectra. From both figures it can be concluded that RMS:F and RTS:F samples are very close and form a group with SS_feedstock due to the similarity of their FTIR spectra. Likewise, although the distance is slightly higher, samples of palmitic and stearic acids form a group with FW sample according to the spectra results.

Table 6.5 Correlation matrix (Pearson (n)):

	STEARIC	PALMITIC	SS_feedstock	RMS:F	RTS:F	FW
STEARIC	**1**					
PALMITIC	**0,950**	**1**				
SS_Feedstock	-0,155	-0,218	**1**			
RMS:F	0,133	0,075	**0,755**	**1**		
RTS:F	0,184	0,106	**0,682**	**0,900**	**1**	
FW	0,268	0,201	-0,172	0,114	**0,339**	**1**

Values in bold are different from 0 with a significance level alpha=0,05

6.4 Conclusion

The co-digestion of sewage sludge (SS) with fat (F) was successfully performed under batch and semi-continuous conditions. The SS used in the experiments proved to be of difficult degradation with a high tendency for VFA build-up. The addition of the co-substrate improved the digestion stability and biogas yield by the increase in the organic loading rate (OLR). However, the thermophilic digestion was characterised by an effluent of poor quality associated with high VFA content in the effluent.

Results from spectroscopic analysis suggested the adsorption of lipid components onto sludge biomass, which prevented the complete degradation of substrates during the anaerobic treatment. The formation of fatty aggregates in the thermophilic reactor avoided the process failure derived from the toxicity of high concentration of LCFAs.

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Capítulo/Chapter 7

7. Sludge pre-treatments to enhance anaerobic digestion

7.1 Effect of ultrasound pre-treatment on sludge digestion and dewatering characteristics: Application of particle size analysis

7.2 Enhancement of anaerobic digestion of sewage sludge by Microwave pre-treatment

7.1 Effect of ultrasound pre-treatment on sludge digestion and dewatering characteristics: Application of particle size analysis

Abstract

The aim of this work was to study the effect of ultrasonic pre-treatment on sludge digestibility, particle size distribution (PSD) and dewaterability of digestates (measured by capillary suction time (CST) and specific resistance to filtration (SRF). Sludge was pretreated with ultrasounds (24 kHz) at an energy dosage of 4 300 kJ/Kg TS. Digestibility of sludge was increased resulting in higher specific methane production (SMP). Digestate of pre-treated waste activated sludge (WAS) obtained under batch conditions presented better dewatering performance.

Digestion under semi-continuous conditions was evaluated using sewage sludge (mixture of primary sludge and WAS). In this case, digestates presented a much higher mean particle size for both cases evaluated (pre-treated and no pre-treated) than that obtained under batch conditions. A wide PSD was the characteristic of these digestate samples. Flow dynamics inside the reactor resulted in the presence of high diameter flocs, therefore significantly affecting the mean particle size and specific surface area (SSA) values.

Keywords: Anaerobic digestion, dewatering, particle size distribution, ultrasounds.

Edition of this manuscript was in accordance with the scientific journal

7.1.1 Introduction

The management of sewage sludge in wastewater treatment plant (WWTPs) is currently becoming an issue of great concern. The treatment and disposal of sludge may represent up to 50% of plant operating costs. Anaerobic digestion (AD) is a well-known technique used for valorisation of sludge. This process is usually the preferred stabilisation method due to low operating costs and biogas production. In addition, sludge dewatering, which is highly affected by the treatment option of sludge, is also an important issue for decreasing sludge volume and consequently, reducing operating cost in WWTPs. Dewatering is a difficult and costly process in waste water treatment. The process is mainly carried out by physical separation and presents a variable efficiency based on the sludge nature. Even though anaerobic digestion has many advantages, it may adversely affect sludge dewaterability (Dewil et al., 2006).

Anaerobic digesters in WWTPs generally treat a mixture of primary and secondary (waste activated) sludge. However, waste activated sludge (WAS) is known to be more difficult to digest than primary sludge (PS) (Lafitte-Trouqué and Forster, 2002). It has been reported that hydrolysis is the rate-limiting step in anaerobic digestion (Eastman and Ferguson, 1981; Shimizu et al., 1993). Extracellular polymeric substances (EPS) and microbial cells are recalcitrant to direct hydrolysis, therefore pre-treatment of sludge is required to attain the rupture of cell walls, facilitate the release of intracellular material into the aqueous phase and accelerate the low rate of biodegradation and enhance anaerobic digestion (Khanal et al., 2007).

Different pre-treatment methods have been studied to improve anaerobic degradability of sewage sludge: thermal hydrolysis, ozone oxidation, alkaline hydrolysis and ultrasounds (Hogan et al., 2004; Kim and Lee, 2012; Ruiz-Hernando et al., 2014; Zhen et al., 2014). Among these, ultrasonication is a well-known method to break-up microbial cells and extract intracellular material (Tiehm et al., 2001). Several authors have reported an increase in biogas production when applying this type of pre-treatment (Khanal et al., 2007; Braguglia et al., 2009; Kim and Lee, 2012). The impact of ultrasound waves on a liquid causes the periodical compression and rarefaction of the medium (Tiehm et al., 2001). The result of the mechanical phenomenon on sludge depends on the duration and power applied during the pre-treatment, equating to specific energy (Chu et al., 2002). Other effects of ultrasonic energy application are: acoustic streaming, local heating, interface instabilities, agitation and cavitation. These combined effects facilitate the migration of moisture through

natural channels or through those created by wave propagation. This effect could be useful in dewatering suspensions with high concentration of fine particles like slurries and sludge (Riera-Franco et al., 2000). Research work reported by Feng and co-workers (2009) has associated low frequency ultrasound with the ability of aggregating sludge particles and thereby making dewatering easier.

On the other hand, the size of particles comprising sludge has a great influence on its degradability and dewaterability. In addition, rheological properties of sludge may keep a close relationship with the treatment process it has undergone. In this sense, the course of digestion may affect strongly the physical properties of sludge and in turn the distribution of particle size may be used to predict the state of a digestion process. This study was conducted to investigate the effect of ultrasound pre-treatment over the digestion of sewage sludge, methane production and dewatering parameters. Particle size analysis was used to evaluate changes experienced in particle size distribution (PSD) at different stages of digestion.

7.1.2 Material and methods

7.1.2.1 Substrates and digestion tests

WAS, PS and digested sludge were obtained from the WWTP of the city of Cáceres. Digested sludge was used as inoculum. This sludge had a total solid (TS) content of 9.4 g/l and a volatile solid (VS) concentration of 7.4 g/l. WAS and sewage sludge (SS- Mixture of PS and WAS at 30:70 volumetric proportion) were used as feed. SS was concentrated to a solid content of 50 g TS/l by centrifugation.

Biochemical methane potential (BMP) of substrates was determined using batch reactors (Erlenmeyer flasks of 250 ml volume). The temperature was 35 ± 1 °C and agitation was provided by means of magnetic stirrers. Reactors were filled with inoculum and substrate at a VS ratio (inoculum/substrate) of 1.5:1. A reactor containing inoculum were used as blank. Three replicates were used for each BMP tests and blank. Digestion systems were denoted in accordance with substrates evaluated (either PS, WAS or SS). BMP tests were also carried out for ultrasound pretreated samples of WAS and SS. These tests were denoted as U_WAS and U_SS. The evolution of PSD was also evaluated in batch tests for WAS and U_WAS digestion systems.

A semi-continuous reactor was operated using completely stirred reactors (working volume of 3 l at 35 ± 1 °C). This reactor was evaluated using SS as substrate at hydraulic retention time (HRT) of 21 days for 40 days (organic loading rate (OLR) of 1.8 g VS/l d). Subsequently, pretreated SS was used as feed and reactor performance was evaluated at the same OLR during a 45 day period. Reactors were denoted R_SS and RU_SS.

7.1.2.2 Ultrasound pre-treatment

An ultrasonic processor UP400S (Dr. Hielscher, Germany) operating at a nominal power of 300 W and 24 kHz was used. The sonotrode had a diameter of 7.0 mm. 500 ml of sample were prepared in Erlenmeyer flasks of 1 000 mL and mixed previous to sonication. The applied ultrasonication energy (Es) is given by:

$$E_s = {P \times t}/{V \times TS^{\circ}} \qquad \textbf{Equation 7-1}$$

Where P is the ultrasonic power, t is the application time, V is the sample volume and TS is the initial concentration of total solids. Energy input was 4 300 kJ/kg TS based on previous results of Martinez et al., 2013 and Feng et al., 2009.

7.1.2.3 Analytical techniques

Total solid (TS), volatile solid (VS), and pH were determined in accordance with APHA Standard Methods (1989) (APHA, 1989). The composition of biogas was regularly monitored during the digestion process and was analysed using a gas chromatograph (Varian CP3800 GC) as described by Martinez et al., 2012.

Capillary suction time (CST) was measured using 5 ml sample of sludge poured into a stainless steel tube (1.0 cm inner diameter) in contact with Whatman nº17 chromatography grade paper. CST was defined as the time required when the wetting front passed the first radius located at 1.0 cm of the cylindrical reservoir and reached the second radius placed at 3.0 cm. Seven replicates were used.

Specific resistance to filtration (SRF) was measured using a 9 cm standard Buchner funnel (fitted to a constant vacuum pressure) were sludge sample was poured. Filtrate volume and filtration time were recorded. SRF was calculated as the slope of the linear plot of volume vs. time/volume (Lo et al., 2001). The water content of the sludge cake trapped by the filter

paper was measured in accordance with standard methods (APHA, 1989). Seven replicates were used.

Free and bound water in sludge was measured using a thickened sludge sample centrifuged at 1 000 rpm for 10 min. A subsample was collected for drying at a constant airflow of 300 ml/min at 105 °C (Kopp and Dichtl, 2000) using TA Instruments thermobalance. The water distribution was derived from the curve of drying time vs. water content (mass-water/mass-solids) of the sample. Three replicates were used for obtaining the curve.

Particle size analysis was carried out using a Laser Diffraction particle Size Analyser LS 13 320 Beckmann Coulter. Samples were previously diluted in tap water for analysis. 10 measurements were performed for each sample. Inoculum, substrates and digestates were analysed. PSD measurements on samples prior and after digestion were denoted by adding the term initial or digestate to the abbreviation indicating the substrate. The evolution of PSD in batch tests was studied during batch digestion of WAS and U-WAS. Samples were taken from the digestion system at the beginning of the experiments (WAS_D0, U_WAS_D0), after 14 days (WAS_D14, U_WAS_D14) and at the end of the batch test (WAS_digested, U_WAS_digested).

7.1.3 Results and discussion

7.1.3.1 Effect of pre-treatment over batch digestion and particle size of the sludge

Results obtained from BMP tests are presented in Figure 7.1(a-b) for substrates (PS, WAS and SS) and pretreated samples (U_WAS and U_SS). The benefits of ultrasound pre-treatment are noticeable in the increase of cumulative methane production and biogas production rate during the initial stage of the digestion experiments. The values of specific methane production (SMP) obtained were quite similar for substrates tested, although it should be expected a higher value for the PS sample. In addition, this sample presented an initial low rate of gas production during digestion. This behaviour is also observable when digesting the mixture (SS sample). This initial trend may be associated to the presence of complex organic material in PS probably due to the proximity of a slaughterhouse to the WWTP.

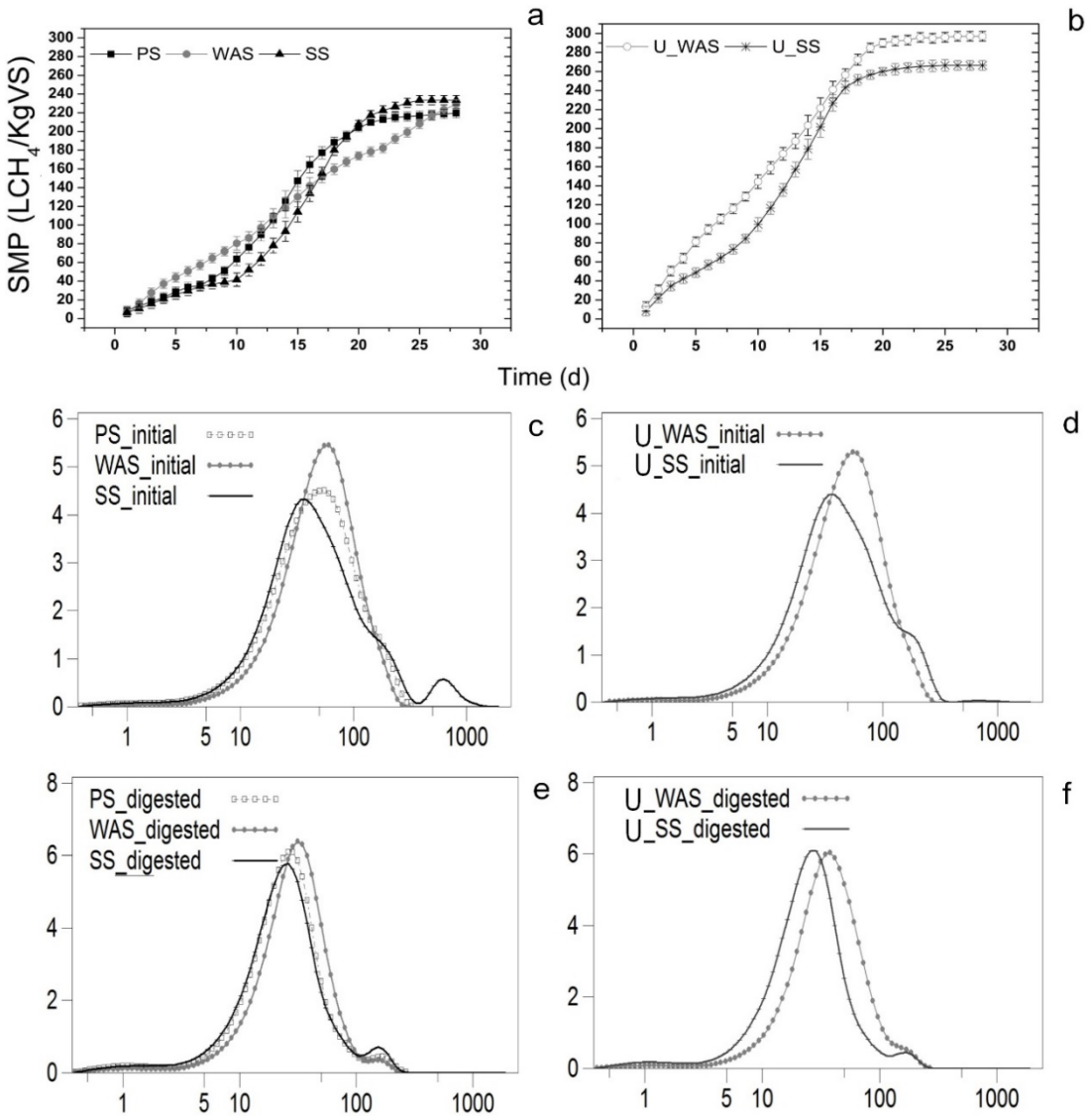

Figure 7.1 (a-b) Specific methane production (SMP) obtained from batch tests for samples: Primary sludge (PS), Waste activated sludge (WAS), Sewage sludge (SS) and ultrasound pretreated samples U_WAS, U_SS. (c-f) Particle size distribution (PSD) of samples taken prior (initial) and after (digested) batch digestion tests

The values of SMP for SS and WAS samples were 233 ± 35 and 229 ± 23 ml CH_4/g VS respectively. This value was increased by 14% when evaluating U_SS sample, while the increment for U_WAS reached 30%. Tiehm et al. (2001) investigated the improvement on anaerobic digestion when WAS was pretreated at an ultrasound frequency of 41–3 217 kHz. These authors reported that sludge disintegration was most significant at low frequencies. Low-frequency ultrasound created large cavitation bubbles, and resulted in sludge floc deagglomeration without the destruction of bacteria cells. Longer sonication brought about the break-up of cell walls, the sludge solids were disintegrated and then dissolved organic

compounds were released. The increase in digestion efficiency obtained in the present study should be explained by deagglomeration of sludge since ultrasound frequency applied was in the low range (24 kHz-4 300 kJ/Kg TS of energy input).

Results from particle size analysis are presented in Figure 7.1(c-d). Feedstocks (denoted by adding the term initial) presented a main peak at around 5-300 μm. The SS sample also showed a pronounced peak for particles of greater size (400-1 000 μm) which may be due to the agglomeration of flocs. On the other hand, the pretreated samples presented a similar distribution but in this case, the graph corresponding to U_WAS shows a curve thinning, indicating that the range of sizes is narrowing thanks to the disintegration of sludge flocs. Table 7.1 shows mean particle size and specific surface area (SSA) for all samples studied. It was observed a decrease in particle size of pretreated samples (U_WAS, U_SS), with a 15% and 8% reduction respectively, when compared with WAS and SS samples. This decrease keeps relation with the increase experienced by the SSA parameter (Table 7.1) and methane production in batch tests. With regard to digested samples, PSD graphics presented in Figure 7.1(e-f), show a higher content of smaller particles (in the range 5-100 μm) and less disperse profile for all samples, obtaining a mean particle size value of less than 40 μm as shown in Table 7.1.

Table 7.1 Particle size analysis of substrate samples before and after ultrasound pre-treatment

Substrate	Initial		Digested	
	Mean μm	SSA cm^2/ml	Mean μm	SSA cm^2/ml
PS	59.9±2.90	2594±129	30.8±1.52	4904±245
WAS	61.0±3.01	2672±133	31.9±1.50	4993±249
SS	65.9±3.25	2031±101	34.8±1.75	3664±183
U_WAS	53.9±2.62	2941±147	31.3±1.50	4695±234
U_SS	60.9±3.03	2203±110	33.8±1.74	3664±183

7.1.3.2 PSD and dewatering parameters at digestion process

Figure 7.2 shows the evolution of PSD of WAS and U_WAS samples during batch digestion. The first samples of these experiments were composed by the mixture of substrate and inoculum; this explains the lower range of particle sizes shown in these graphs. The previous observation of lower mean particle size due to the pre-treatment is still discernible in samples taken at day 0. The pretreated system presents a 9% decrease in particle size

when compared with the WAS_D0 sample. Even though the pre-treatment caused the destruction of sludge flocs and release of biopolymers, floc agglomeration was still observed due to the addition of inoculum (samples taken at day 0 showed particles with size in the range of 500-1 000 μm in PSD graphs). However, these flocs was no longer present in posterior samples. The digestion process finally results in a biosolid with small size particles presenting both systems similar PSD profiles.

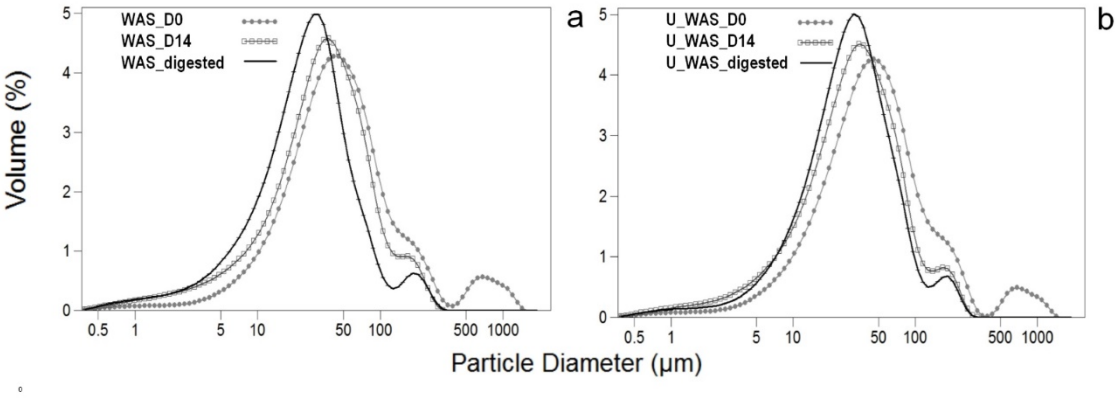

Figure 7.2 Particle size distribution (PSD) obtained from samples taken from batch digestion tests of waste activated sludge (WAS) (a) and ultrasound pretreated WAS (U_ WAS) (b)

It has been suggested that the release of biopolymers and inorganic substances caused by the pre-treatment and posterior digestion might have a detrimental effect over sludge dewaterability (Na et al., 2007; Xu et al., 2011). However, Feng et al. (2009) reported an improvement in this parameter (measured by CST and SRF) when applying low ultrasound dosages (< 2 200 kJ/kgTS), while energy dosages beyond 4 400 kJ/kgTS caused a detriment in these parameters. Shao et al. (2009) found that particle size of sludge flocs had an important effect over dewaterability, reporting a significant correlation between mean particle size and CST when evaluating WAS. In the present research, the dewatering behaviour of WAS was examined along with the effect of ultrasound pre-treatment and digestion on dewaterability. CST and SRF values obtained are shown in Table 7.2.

Pre-treatment of WAS resulted in an increase in CST and SRF parameters, therefore showing an adverse effect over dewaterability. Nevertheless, the pretreated system presented a slight improvement on day 14th. At the final stage of digestion, these values were reduced; reaching values far below those obtained for the original WAS sample. On the other hand, the digestion of WAS resulted in a gradual deterioration of sludge dewatering parameters

during the initial stage of digestion, but at the end of the process the detriment in dewaterability is striking, reaching a CST value higher than 2 000 s. Even though mean particle size of WAS and U_WAS digestates presented similar values, dewatering behaviour of digestates varied significantly as it is observed from Table 7.2.

Table 7.2 Dewatering parameters for WAS at digestion process

Substrate	Sample	CST (s)	SRF (cm/g)
WAS			
	WAS_D0	456±27	9.8127E+13±4.3931E+12
	WAS_D14	550±38	2.2662E+14±9.0648E+12
	WAS_digested	>2 000	3.7959E+14±1.8979E+13
U_WAS			
	U_WAS_D0	608±30	5.5235E+14±2.2094E+13
	U_WAS_D14	535±32	1.3203E+14±6.6015E+12
	U_WAS_digested	267±13	8.0418E+13±4.0209E+12

Drying curves and water distribution as interpreted by Kopp and Dichtl (2001) are shown in Figure 7.3. Three zones are described: free water which is associated with solid particles, interstitial water (trapped inside interstitial spaces of flocs and microorganisms) and chemical bound water. The graph shows results obtained for WAS and U_WAS digestion batch tests.

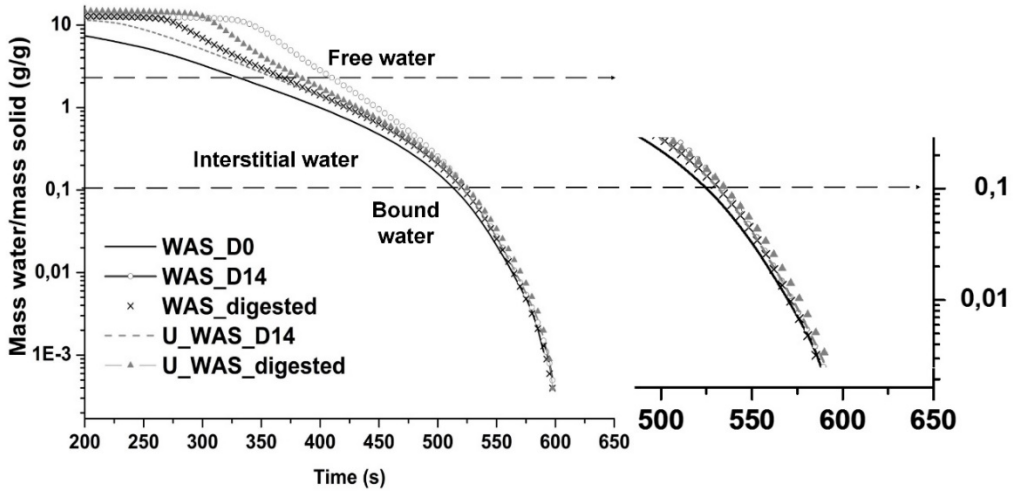

Figure 7.3 Drying curve of sludge samples during the digestion process

The increase in SSA experienced by the samples after applying ultrasound pre-treatment and more over after digestion may be influencing adhesion of water to particles. This would explain the greater amount of water retained which was in consonance with the suggestion of Lawler et al. (1986) about the relevance of SSA over sludge dewaterability, with this being

established as one of the most relevant parameters. In addition, the slope of the curve in the interstitial region was higher for digested samples, which can be rationalised as a positive effect of digestion on the drying process.

The time needed for eliminating interstitial water was much higher for WAS sample. The application of the pre-treatment favoured its removal and a similar effect was also observed for digested samples. With regard to the last region of the curve, the difficulty in eliminating bound water from digested samples was similar disregard of the pre-treatment and digestion stage. The shape of the curve and total drying time are indicating that digested samples needed a similar time for completing the drying process although starting from an initial point of much higher water content.

7.1.3.3 Digestion under semi-continuous operation

Parameters of reactor performance are shown in Table 7.3. Digestion of SS at an HRT of 21 d resulted in an SMP of 120 ml/g VS, while the use of U_SS enhanced methane production. With regard to results obtained from particle size analysis, samples taken from this reactor when digesting U_SS presented a 15% decrease in mean particle size when compared with results obtained when digesting SS. These results were in accordance with those reported by Mahmoud et al. (2006), who also described a transformation of bigger flocs into smaller particles during anaerobic digestion of sludge. Digested sludge is usually characterised by particles of much lower size than those conforming the original substrate, and this was also the case in the present research when batch conditions were tested, but this was not the case when tests were performed under semi-continuous conditions.

The mean particle size of digestates was in the range of 30 - 35 μm under batch conditions; while this value increased to 76 – 87 μm approximately under semi-continuous conditions (see Table 7.3). Operating conditions favoured the aggregation of particles (Figure 7.4) in a similar way to that observed at the initial stage of batch digestion tests when substrate and inoculum were mixed. This effect was more pronounced in R_SS sample which was characterised by a wide PSD with a mode value of 28.5 μm, while its mean particle size was 87.3 μm.

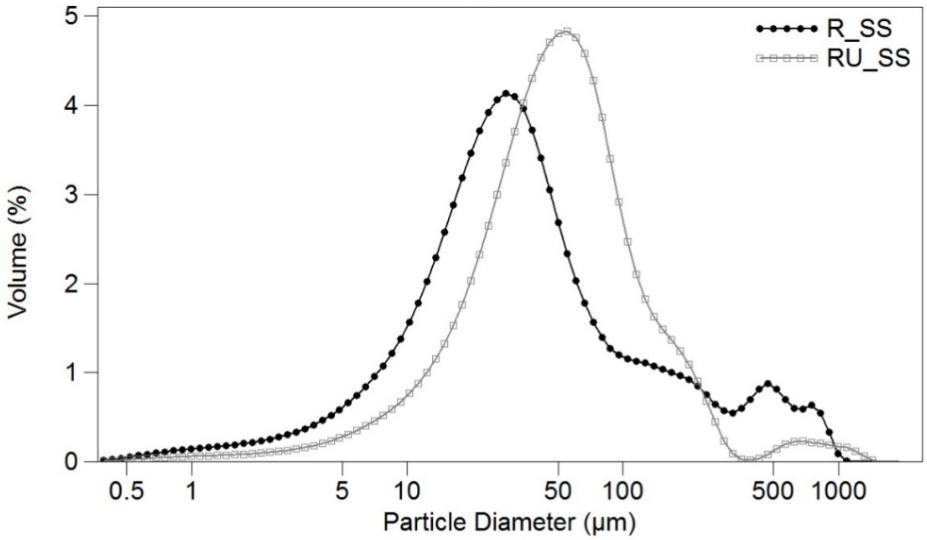

Figure 7.4 Particle size distribution (PSD) obtained from samples taken from semi-continuous digestion of sewage sludge (R_SS) and ultrasound pretreated sewage sludge (RU_ SS)

Table 7.3 Parameters of reactor performance operating under semi-continuous conditions

Performance parameter	R_SS	RU_SS
OLR (a) (g VS/l d)	1.80 ± 0.05	1.80 ± 0.09
SMP [(b)] (ml/g VS)	120 ± 24	224 ± 20
Gas Prod.[(c)] (l/d)	0.92 ± 0.12	1.92 ± 0.12
Mean particle size (µm)	87.30 ± 4.36	76.10 ± 3.80
Mode (µm)	28.5±1.14	55.7±3.32
Dp90 (µm)	230±11.0	144±8.60
SSA(cm^2/ml)	4191 ± 250	2487 ± 149
CST(s)	1765±88	1737±69
SRF(cm/g)	1.9552E+13±7.8208E+11	6.6430E+12±3.3215E+11

[(a)]OLR: Organic loading rate; [(b)] SMP: Specific methane production; [(c)] Gas Prod.: Average daily gas production.

7.1.4 Conclusion

Digestion of sewage sludge was significantly enhanced by ultrasound pre-treatment. Specific methane production (SMP) was improved with the application of ultrasounds thanks to floc deagglomeration and the increase in specific surface area (SSA). Sonication

decreased the mean particle size of sludge. This effect favoured biogas production but also affected sludge dewaterability.

Ultrasound pre-treatment initially caused a detriment in sludge dewaterability. However, at the end of digestion, the pretreated digestate presented better dewatering performance than its counterpart. Ultrasound improved water removal from digested sludge. The use of particle size analysis proved to be a suitable technique for characterising the course of digestion and obtaining an insight in process performance. Further work would be needed to extend these results to evaluate the course of different digestion systems.

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7.2 Enhancement of anaerobic digestion of sewage sludge by Microwave pre-treatment

Abstract

This study assessed the effect of microwave pre-treatment (MwP) over the anaerobic digestion of sewage sludge. Response surface methodology (RSM) was applied to determine the effect of microwave irradiation on organic matter solubilisation at low input energy (<1000 kJ/L). The pre-treatment process was subsequently studied at energies of 488 – 2 700 kJ/L to evaluate the improvement in biogas production under mesophilic conditions. Results showed an increase in organic matter solubilisation with the increase in the energy applied. Modifications in the specific surface area of the organic matter due to the MwP resulted in increments in methane yields. However, an accumulation of complex compounds was observed in thermal profiles at the maximum energy input (2700 kJ/L). Semi-continuous digestion experiments reported a significant increase in methane yield (43%) when evaluating the process at hydraulic retention times (HRTs) of 25 – 10 d.

Keywords: Sewage sludge, anaerobic digestion, microwave pre-treatment, biogas improvement.

Edition of this manuscript was in accordance with the scientific journal

7.2.1 Introduction

Sewage sludge is produced in wastewater treatment plants (WWTP) in large amount as an inevitable by-product of the biological process. The treatment and final disposal of biosolids has a great influence in the energy efficiency of the WWTP. Anaerobic Digestion (AD) has been widely applied and it is usually the sludge management preferred option, since it allows the simultaneous valorisation of biogas. However, waste activated sludge (WAS) has proven to be a material of difficult degradation. Hydrolysis of extra polymeric substances (EPS), complex compounds from cell secretions and lysis, may become a limiting factor of the AD process (Carrère et al., 2010).

Several pre-treatments, such as chemical, thermochemical, shear stress, sonication and oxidation, have been proposed to disrupt cell walls and increase digestibility of EPS (Battimelli et al., 2003; Braguglia et al., 2008; Brougrier et al., 2005; Brougrier et al., 2007; Erden and Filibeli, 2010; Kepp et al., 2000; Valo et al., 2004). These pre-treatments are intended to minimise the time required for digestion by increasing the solubilisation of the sludge and therefore maximise biogas production. However, their success depends on sludge characteristics, age, solid content and conditions of the digestion process.

Microwave (Mw) irradiation has extensively been studied as a thermal pre-treatment method because it allows the destruction of pathogens and increases the solubilisation of complex material thereby reducing the lag-phase during digestion (Beszédes et al., 2011; Guo et al., 2008; Eskicioglu et al., 2009). Mw irradiation is electromagnetic radiation with a wavelength between 1 mm and 1 m and corresponds to a frequency range of 300 MHz to 300 GHz. Domestic microwave ovens generally operate at 2.54 GHz causing thermal and non-thermal effects which are also dependant on substrate interactions. The Mw irradiation acts on pathogen destruction, biodegradability and AD efficiency by changing the dipole orientation of the molecules leading to polarised side-chains of macromolecules and the breakage of hydrogen-bonds (Beszédes et al., 2011; Hong et al., 2004; Toreci et al., 2009). This later effect is known as a non-thermal effect caused by the high frequency electromagnetic field. The modification of complex material usually increases with the increase in Mw power and irradiation time. In this line, the relationship between these two parameters can be used as a measure of the efficiency of the pre-treatment. In addition, the effect of Mw pre-treatment over the organic matter may condition the final behaviour of the AD process.

During biological stabilisation of organic matter, the readily biodegradable materials are converted into structurally complex substances (e.g. humus or humic substances) and the energy available for the metabolism of micro-organisms is reduced. Thermogravimetric analysis (TG) techniques have been proposed to study the mineralisation and conversion of organic compounds during biological stabilisation. These techniques are capable of evaluating in a fast and reliable way the increase in the quality of the organic matter along with the increase in the degree of mineralisation when submitting the sample to a controlled thermal process (Dell'Abate et al., 1998; Otero el al., 2002; Pietro and Paola, 2004; Gómez et al., 2007).

The objective of the present work was to evaluate the effect of Mw pre-treatment over the anaerobic digestion of sewage sludge under batch and semi-continuous conditions. The modifications experienced by the organic material were also studied as an attempt to better understand the effects caused by the pre-treatment.

7.2.2 Material and methods

7.2.2.1 Sludge samples

The sewage and digested sludge (used as inoculum) was obtained from the WWTP of the city of León, Spain. Primary sludge (PS) was collected from the sedimentation tank and WAS was collected from the dissolved air flotation unit. Sludge samples were posteriorly stored at 4 °C. Their main characteristics are shown in Table 7.4.

Table 7.4 Characteristics of substrates used in the present work: primary sludge (PS) and waste activated sludge (WAS)

Parameter	*Unit*	*PS*	*WAS*
TS	g/L	37.6 ± 1.9	25.6 ± 1.3
VS	g/L	27.1 ± 1.4	21.8 ± 1.4
pH	-	7.6 ± 0.1	6.4 ± 0.1
COD	g O_2/L	39.1 ± 1.9	31.8 ± 1.3
NH_4^+	mg/L	874 ± 42	241 ± 16
C/N	-	6.4 ± 0.2	3.2 ± 0.2

7.2.2.2 Pre-treatment procedure

The Mw pre-treatment was performed using a domestic microwave oven (2 450 MHz frequency). A 500 mL covered Erlenmeyer flask was used to avoid water evaporation. Sludge samples were irradiated with a power output of 650 – 900 W. The energy applied was calculated in accordance with Equation 7-2, as suggested by Uma Rani et al., 2013.

$$\mathbf{I_e}\left(\frac{\mathbf{kJ}}{\mathbf{L}}\right) = \mathbf{Magnetron\ power}\left(\frac{\mathbf{kJ}}{\mathbf{s}}\right) \times \mathbf{Irradiation\ time\ (s)\ /Sample\ volume\ (L)}$$

Equation 7-2

7.2.2.3 Response surface methodology

A factorial design consisting of 2k factorial nucleus (six replications of the central point and 2k axial points, where k is the number of factors evaluated) was used to evaluate the effect of Mw pre-treatment over sludge solubilisation. The content of total solids (TS) and the energy applied were the factors selected. These factors were denoted as X_1 (0.6 – 2.4 g/L) and X_2 (140 – 1 000 kJ/L), respectively. The experimental design was analysed using response surface methodology (RSM) using a second order polynomial function (Equation 7-3). The responses selected for the analysis were the content of total organic carbon (TOC) as Y_1 and ammonium release per unit of TS as Y_2.

$$\mathbf{Y} = \beta_0 + \beta_1 \mathbf{X_1} + \beta_2 \mathbf{X_2} + \beta_{12} \mathbf{X_1 X_2} + \beta_{11} \mathbf{X_1^2} + \beta_{22} \mathbf{X_2^2}$$

Equation 7-3

7.2.2.4 Batch digestion

Batch experiments of raw and pre-treated WAS were performed to determine the biochemical methane potential (BMP). Experiments were carried out in Erlenmeyer flasks of 250 mL and were filled with inoculum and the corresponding amount of substrate in order to attain a substrate/inoculum ratio of 1, expressed in volatile solids (VS). Tap water was added to complete the volume. Two reactors were used for measuring gas production and composition. A batch reactor containing only inoculum was used as blank to subtract the background gas production. The temperature was set at 34 °C and controlled by a water bath. Agitation was provided by means of magnetic stirrers. Gas volumes were measured using bottle gasometers and corrected to standard temperature and pressure (STP, 0 °C and 760 mmHg). Digestion systems were denoted as follows: untreated WAS (U_WAS) and Mw

pre-treated WAS (MwP_WAS, followed by the energy value). The energy applied was in the range of 488 – 2700 kJ/L. Samples selected for analysing particle size and total organic carbon (TOC) were denoted by the term Initial and Digested based on the sampling time to indicate substrate samples and digested material. Samples selected for thermal analyses also followed this nomenclature.

7.2.2.5 Semi-continuous digestion

The digestion process was carried out in two completely mixed reactors provided with mechanical stirrers and working volume of 3 L (mesophilic conditions, 34 °C). The feedstock was sewage sludge composed by a mixture of PS and WAS with a volumetric proportion of 30:70 (in accordance with the volumetric production of sewage sludge in the WWTP). Reactors were run on a semi-continuous basis and manually fed once a day. Sampling was performed three times a week and was carried out before feeding the reactors and after complete homogenisation. Daily gas production was measured using a reversible liquid displacement device and a wet-tip counter.

WAS sample was irradiated at energy values of 975 kJ/L based on the results obtained from the previous batch tests. Reactors were operated at hydraulic retention times (HRTs) of 25, 20, 10 and 5 days and were designated in accordance with the substrate being digested in each case as RU_SS and RMwP_SS. The first one, indicating the digestion of SS, and the second one for the digestion of pre-treated SS. This later substrate was comprised by the mixture of pre-treated WAS and PS.

7.2.2.6 Analytical techniques

Nitrogen concentration was determined by the Kjeldahl method (MAPA, 1994) and organic matter was analysed in accordance with the Walkey-Black method (Walkley and Black, 1934). Chemical oxygen demand (COD), TS, VS, ammonium and pH were determined in accordance with APHA Standard Methods (APHA et al., 1989). The content of total organic carbon (TOC) of the supernatant obtained after centrifugation of the sample was analysed using Analytikjena Multi N/C_3100 system by thermocatalytic oxidation.

Biogas composition was analysed as described by Martínez et al. (2012), using a gas chromatograph (Varian CP3800 GC) equipped with a thermal conductivity detector. A packed column (HayeSep Q 80/100; 4 m) followed by a molecular sieve column (1 m) was used to separate CH_4, CO_2, N_2, H_2 and O_2. Volatile fatty acids (VFAs) were determined as

described by Cuetos et al. (2008), using the same gas chromatograph and a flame ionisation detector (FID) equipped with a Nukol capillary column (30 m × 0.25 mm × 0.25 μm) from Supelco. The detection limit for VFA analysis was 5.0 mg/L. The system was calibrated with a mixture of standard volatile acids from Supelco (for the analysis of fatty acids C2 to C7). Samples were previously centrifuged (10 min, 3500 × g) and the supernatant was filtered through 0.45 μm cellulose filters.

Particle size analysis was carried out using a Laser Diffraction Particle Size Analyser LS 13 320 Beckmann Coulter. This analysis allowed the determination of the specific surface area (SSA) of particles in a liquid sample. Samples were previously diluted in tap water for analysis. Ten measurements were performed for each sample. Samples analysed were those taken from the inoculum, substrates, pre-treated WAS and digestates obtained from batch digestion assays.

Non-isothermal thermogravimetric analysis was performed using a Setaram TGA92 analyser at atmospheric pressure. Approximately 5 mg of sample was placed in a crucible of height 2 mm and with a circular base 5 mm in diameter for each experiment. The analyses were carried out under a 50 mL/min air flow at a heating rate of 10 °C/min from room temperature to 700 °C. The mass loss (TG) and derivative curves (DTG) of the samples were represented as a function of temperature. An MSC200 quadrupole mass spectrometer (MS) from Balzers linked to the thermobalance was used for evolved gas analysis. The gas lines between the TG and the MS were heated to 200 °C in order to avoid cold points and thus prevent the condensation of some of the gaseous products. The MS operated on 100 eV of ionisation energy, using a Channeltron detector (1000 V). The signals were normalised to the total mass loss of the sample during the TG analysis and to the maximum of the total intensity of the experiment in order to compare the peak height of the same compound released from different samples. The mass spectrometer signals studied during the experiments were the 44, 30, 27 and 15 m/z signals. Hydrocarbons give several m/z signals in the MS corresponding to their fragments. The main m/z signals observed for hydrocarbons were m/z 15 and 27, corresponding mainly to the CH_3^+ and $C_2H_3^+$ ions, respectively. The 44 and 30 m/z signals correspond mainly to CO_2^+ and NO^+ ionic species, respectively. For simplicity, these mass spectrometer signals will be assigned to the gaseous species CO_2 and NO, respectively. Samples subjected to TG and MS analyses were: WAS sample, pre-treated WAS samples at 975 and 2700 kJ/L, and digested samples obtained at the end of batch digestion tests.

7.2.3 Results and discussion

7.2.3.1 Response surface methodology: effect of Mw pre-treatment of WAS at low irradiation energies

Responses were adjusted to a second order polynomial equation (Equation 7-2). The models obtained were the following (Equations 7-3 and 7-4):

$$Y_1 = -378 + 639.4\ X_1 + 1.22\ X_2 - 0.19\ X_1X_2 - 28.2\ X_1^2 - 8.25E\text{-}4\ X_2^2$$

Equation 7-3

$$Y_2 = 58.4 - 9.3\ X_1 + 9.88\ E\text{-}2\ X_2 - 5{,}04E\text{-}3\ X_1X_2 + 0.36\ X_1^2 - 7.75E\text{-}5\ X_2^2$$

Equation 7-4

Table 7.5 presents the ANOVA results for the estimated models. The energy input was the main factor for both responses evaluated (X_2).

Table 7.5 ANOVA results obtained for responses, TOC (Y_1) and ammonium release (Y_2)

	TOC (Y_1)			Ammonium release (Y_2)		
Parameter	Error	t-Value	Prob>|t|	Error	t-Value	Prob>|t|
β_0	96.11	-3.93	8.21E-04	9.93	5.88	<0.0001
β_1	69.49	9.20	<0.0001	7.18	1.29	0.21
β_2	0.23	5.28	<0.0001	2.39E-04	4.13	2.30E-04
β_{12}	7.48E-02	-2.51	0.021	7.73E-05	0.65	0.52
β_{11}	12.15	-2.32	0.031	1.25	0.28	0.78
β_{22}	1.75E-04	-4.72	1.30E-04	1.81E-09	4.29	1.47E-04
R^2		0.985			0.707	
R^2 adj		0.980			0.662	

In the case of TOC (Y_1) values, the polynomial representation indicated the presence of curvature. On the other hand, for the ammonium released (Y_2), only the energy input was found to present a significant effect over the response. This effect is associated with thermal solubilisation of particulate materials, especially proteins. At low energy levels, an initial increase in this response was observed with the increase in energy. However, when values

were about 600 kJ/L, a decrease in the response (ammonium concentration) was observed with further increases in the energy applied with the pre-treatment. In addition, ammonium release was less effective when the solid content of the sludge was increased. The graphical representation of Equations 7-3 and 9-4 are presented in Figure 7.5.

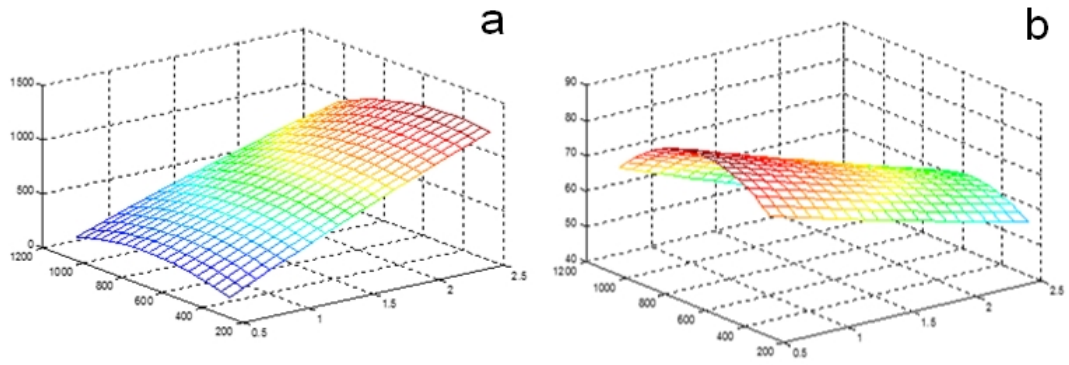

Figure 7.5 Graphic representation of responses a) TOC (Y_1) and b) ammonium release (Y_2)

Samples obtained from the different pre-treatment experiments were submitted to particle size analysis. The specific surface area (SSA) parameter was calculated and an increase in the range of 8 to 14% was observed within the energy range applied. The SSA was in average 3 550 cm^2/g for all pre-treated samples, while this value was 3 183 cm^2/g for the untreated sludge sample. A subsequent experimental stage with higher energy range was studied to evaluate the effect over batch digestion.

7.2.3.2 Batch digestion: high irradiation energies

The destruction of flock structures, probably associated with microbial cell disruption, and its effect over the SSA parameter can be observed in Figure 7.6(a). This figure shows the SSA parameter *vs.* the energy applied with the pre-treatment. Initial_SSA is referred to the SSA values obtained after submitting the WAS sample to the Mw pre-treatment. The SSA parameter keeps relation with the size of flock structures. Figure 7.6(a) shows that an increase in SSA occurred with the increase in the applied energy, which would be due to the destruction of flock structures caused by the pre-treatment.

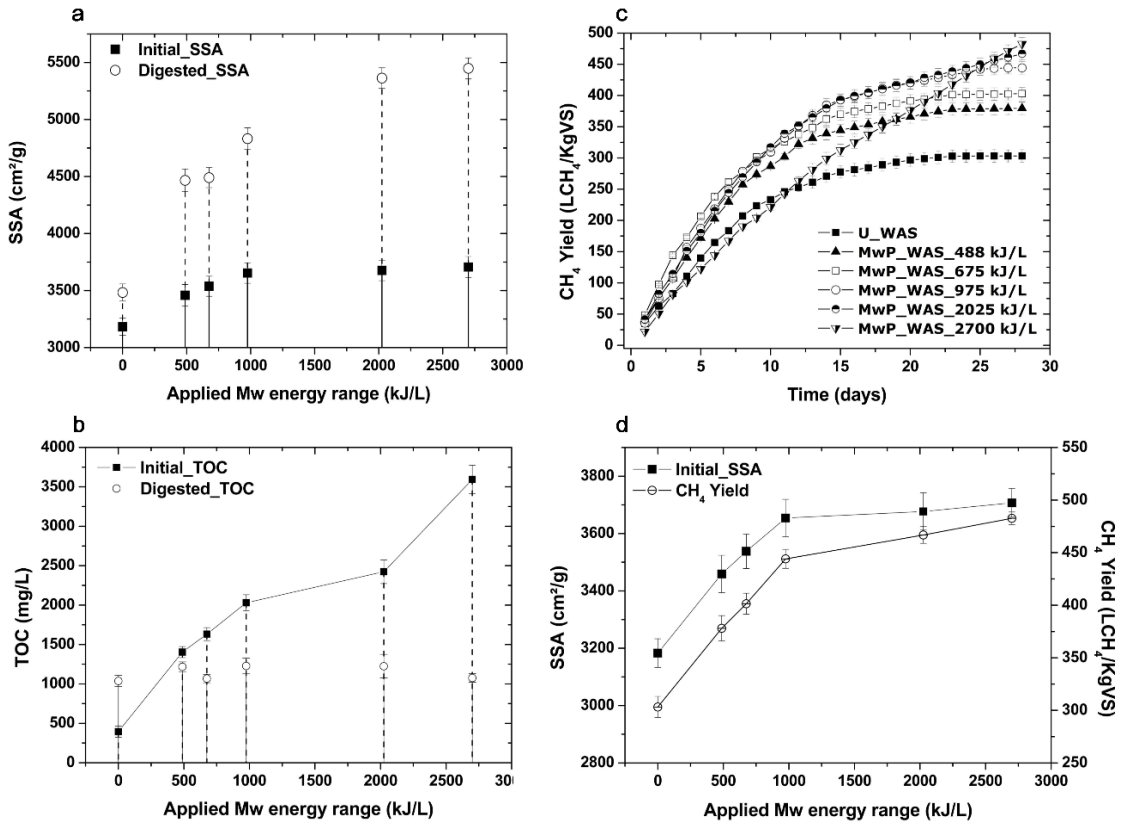

Figure 7.6 Effect of Mw pre-treatment over digestion tests: a) SSA values before and after pre-treatment of WAS (Initial_SSA), and SSA values after the digestion process (Digested_SSA); b) TOC values before and after pre-treatment of WAS (Initial_TOC), and TOC values after the digestion process (Digested_TOC); c) Methane yield of WAS obtained from batch digestion tests; and d) Initial_SSA values and methane yield

On the other hand, Figure 7.6(b) shows the enhancement on solubilisation of the organic matter. This is reflected by the rise in TOC values which were measured after the application of the pre-treatment to the WAS samples. As in the case of the SSA parameter, these values were denoted as Initial_TOC, i.e. the values obtained from samples taken right after the application of the Mw pre-treatment. The steep increase in TOC values obtained with the increase in the energy applied to the sample may be explained by the lysis of cells and so the release of the intracellular organic materials. These results demonstrate that the applied energy during Mw pre-treatment plays an important role in the organic matter solubilisation and an increase in the amount of readily available material for degradation is therefore obtained, which is in accordance with previous studies in the literature (Eskicioglu et al., 2009; Kuglarz et al., 2013; Uma Rani et al., 2013).

Results obtained from the batch digestion experiments are also presented in Figure 7.6. Figure 7.6(a) and Figure 7.6(b) show the results obtained from particle size and TOC analyses for the samples taken at the end of the digestion process and were denoted as Digested_SSA and Digested_TOC, respectively. When the samples obtained before and after the digestion process are compared, a decrease in TOC values in the range of 13-70% is observed, except for the U_WAS sample (without any Mw pre-treatment). In this later case, the sample experiments an increase in TOC values at the end of the digestion process. This behaviour is explained by the release of EPS and intermediary metabolites excreted by anaerobic consortia, therefore resulting in an effluent containing high concentration of suspended organic matter and poor quality (Chan et al., 2009). In any case, TOC values measured for the different experiments were similar for all samples obtained after batch digestion, thereby indicating that the pre-treatment applied was not causing significant effects over the organic content of supernants obtained after centrifugation.

Figure 7.6(c) shows the methane yield curves obtained for the different experiments. An increase in methane yield can be clearly associated with the application of the Mw pre-treatment. An energy value of 448 kJ/L improved methane yield about 25% over an initial value of U_WAS of 303 L CH_4/ KgVS. In addition, the increase obtained in SSA values was associated with higher methane yields (Figure 7.6(d)). The highest SSA values were obtained when the energy input during the Mw pre-treatment was higher than 975 kJ/L, obtaining a 46% increment in methane yield. However, no significant improvement in the SSA parameter was obtained in the range of 975-2700 kJ/L of applied energy and the methane production followed a similar trend. Thus, a good correlation was obtained when SSA and CH_4 yield values were fitted to a linear regression model (R^2= 0.967).

7.2.3.3 Thermogravimetric and mass spectrometric analyses

Figure 7.7 shows the results obtained from thermogravimetric analysis of WAS and pre-treated WAS samples at 975 and 2700 kJ/L (optimum and maximum energy inputs, respectively), as well as digested samples obtained from the corresponding assays at the end of digestion. From the TG profiles (Figure 7.7(a) and (b)), it can be seen that the mass loss during the combustion tests was higher for the initial samples (~92%) than for the digested ones (~66%).

In order to study the effect of the Mw pre-treatment, the DTG profiles of the initial samples were represented (Figure 7.7(c)). Samples present the characteristic three main peaks associated with the oxidation of organic material under combustion which have been

described by several authors (Klammer et al., 2008; Otero et al., 2011; Provenzano et al., 2013). It can be seen that an initial mass loss occurred around the temperature of 100 °C, which was due to moisture evaporation. After that, the thermal profile is characterised by three mass loss regions, which indicates that the sample combustion took place in three steps. The first region (150 - 350 °C) can be associated with the degradation of labile compounds and highly biodegradable material. The second region (350 - 475 °C) corresponds to the oxidation of either organic polymers already present in the original material or those generated during the pre-treatment process. Also, the thermal oxidation of more complex material which could have been generated during reactions taking place at lower temperatures could occur in this stage. Condensation reactions taking place at temperatures of 200 – 300 °C lead to the devolatilisation of organic material, but they can also form polymers which are oxidised at higher temperatures. Finally, the third region (475 – 550 °C) is associated with the organic fraction formed by complex materials with low biodegradability and material with high aromatic content which is hardly degradable.

The Mw pre-treated samples show narrower peaks and higher mass loss rates when compared to the U_WAS initial sample. The effect of the Mw pre-treatment at 975 and 2 700 kJ/L was similar on the first peak, which was slightly moved to higher temperatures which is explained by the removal of the more labile material during the pre-treatment. Moreover, this peak became much thinner, which indicates that the organic matter in the pre-treated samples presents a more uniform composition in labile compounds. On the other hand, the Mw pre-treatment at 975 kJ/L also generated narrower peaks in the second and third mass loss regions and a much higher peak in the second region compared to the pre-treatment at 2 700 kJ/L.

Figure 7.7(d) shows the DTG profiles of the digested samples. After digestion, samples showed a more similar thermal behaviour. The peak corresponding to the lower temperature region (150 – 350 °C) notably decreased. However, a slightly higher peak can be seen for the sample MwP_WAS 2700 kJ/L digested, which is also reflected by the higher mass loss detected in the TG curve (Figure 7.7(b). This may indicate that the Mw pre-treatment at very high energy may have caused the formation of toxic compounds or the generation of complex organic molecules which slightly inhibited the degradation process. Moreover, all digested samples showed a heavy decrease in the intensity of the peak in the second temperature region (350 – 475 °C), which may be explained by the degradation of this biodegradable material.

The peak corresponding to the higher temperature region remarkably increased after the digestion of all samples and it became thinner, indicating an increase in the resistant organic matter fraction with a more uniform composition. This increase was lower in the case of the Mw pre-treated sample at 2700 kJ/L. Enrichment in aromatic and complex components takes place during the digestion process resulting in a digested material with lower biodegradability. In the case of the digested sample pre-treated at the highest energy input, the lower intensity of the peak in this region seems to keep relation with the application of such high energy levels and therefore interfere at some extent with the degradation process. Results demonstrate that the digestion process was capable of removing the organic compounds located in the 400 – 500 °C region, and this preferential degradation favoured the accumulation of aromatic structures. The changes experienced in the region associated with the contribution of complex compounds may explain the increase in methane yield and the rate of biogas production during the initial stages of batch digestion tests.

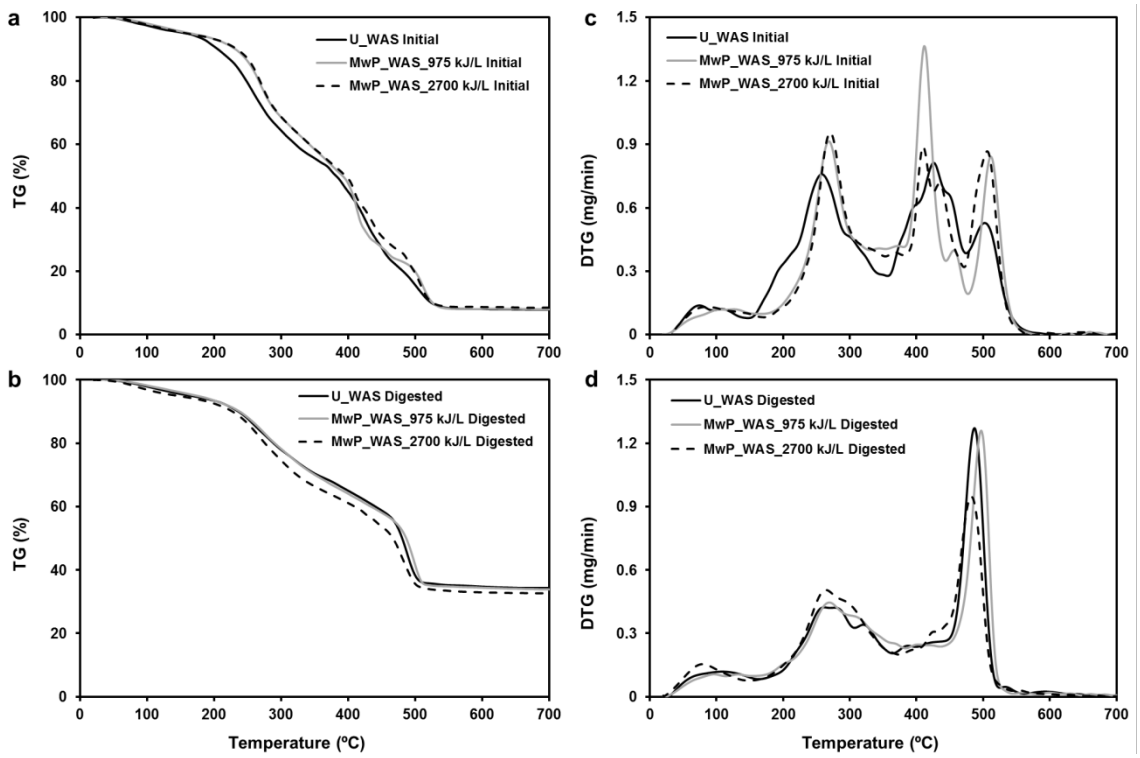

Figure 7.7 Results from thermogravimetric analysis of untreated (U_WAS) and pre-treated (MwP_WAS) samples before and after batch digestion tests: (a) TG curves for initial samples, (b) TG curves for digested samples, (c) DTG curves for initial samples and (d) DTG curves for digested samples

Results from thermal analysis showed that the application of the maximum energy during the Mw pre-treatment resulted in sample presenting a slightly higher mass loss and a broadening of the thermal profile in the high temperature region. Results obtained from the digestion test of this pre-treated WAS sample (energy input of 2 700 kJ/L) showed a decrease in the rate of methane production, although the effect over methane yield was not so evident. This behaviour may be explained by the transformation of the organic matter during the Mw pre-treatment, which indicates that not only the solubilisation and formation of labile compounds can take place, but also the generation of complex compounds which may hinder the biological degradation process.

Figure 7.8 shows the results obtained from mass spectrometric analysis of initial WAS and pre-treated WAS samples at 975 and 2 700 kJ/L (optimum and maximum energy inputs, respectively), as well as samples obtained at the end of digestion. Evolved gas analysis by mass spectrometry is a useful technique for determining the nature of the volatile products formed during combustion of samples. The compounds evaluated and their main m/z signals were: CO_2 (m/z 44), $C_2H_3^+$ (m/z 27), NO (m/z 30) and CH_3^+ (m/z 15). Emissions of CO_2 (m/z 44) are shown in Fig. 3(a), where the maximum emission is observed at high temperatures. These emissions are associated with the third peak represented in the DTG curves ascribed to complex compounds and aromatic structures. It is significant the high release of CO_2 associated with these structures, in particular in digested samples, therefore confirming the hypothesis of accumulation of these compounds due to the preferential degradation of labile components.

The emissions associated with light hydrocarbons such as $C_2H_3^+$ (m/z 27) are represented in Figure 7.8(b). The results indicated higher emissions of the vinyl radical from non-digested samples. The degradation process reduced the amount of readily degradable material and digested samples were therefore characterised by lower emission of light hydrocarbons over the whole temperature range.

Figure 7.8(c) shows the emission profile of NO (m/z 30). The formation of this compound was associated with the oxidation of biomass bound-nitrogen. The sample pre-treated at 975 kJ/L presents the lowest emission along the temperature profile, both before and after digestion. This indicates the beneficial effect of the Mw pre-treatment on the degradation of the proteinaceous material of the sample. However, the application of higher energy input during the Mw pre-treatment caused a lower effect on the decrease of the nitrogenous material. If the initial and digested samples are compared, it can be seen that the digestion

of pre-treated samples caused a very higher decrease on the NO emissions, which indicates that Mw irradiation favoured the degradation of proteinaceous material by anaerobic microflora and it was more notable after irradiation with 975 kJ/L than with 2700 kJ/L. This could explain the behaviour of the methane production curve obtained from the digestion of this sample (as shown in Figure 7.6(c)). In general, the highest emission was obtained from the U_WAS digested sample, evidencing the difficulty in degrading the biological material without Mw pre-treatment.

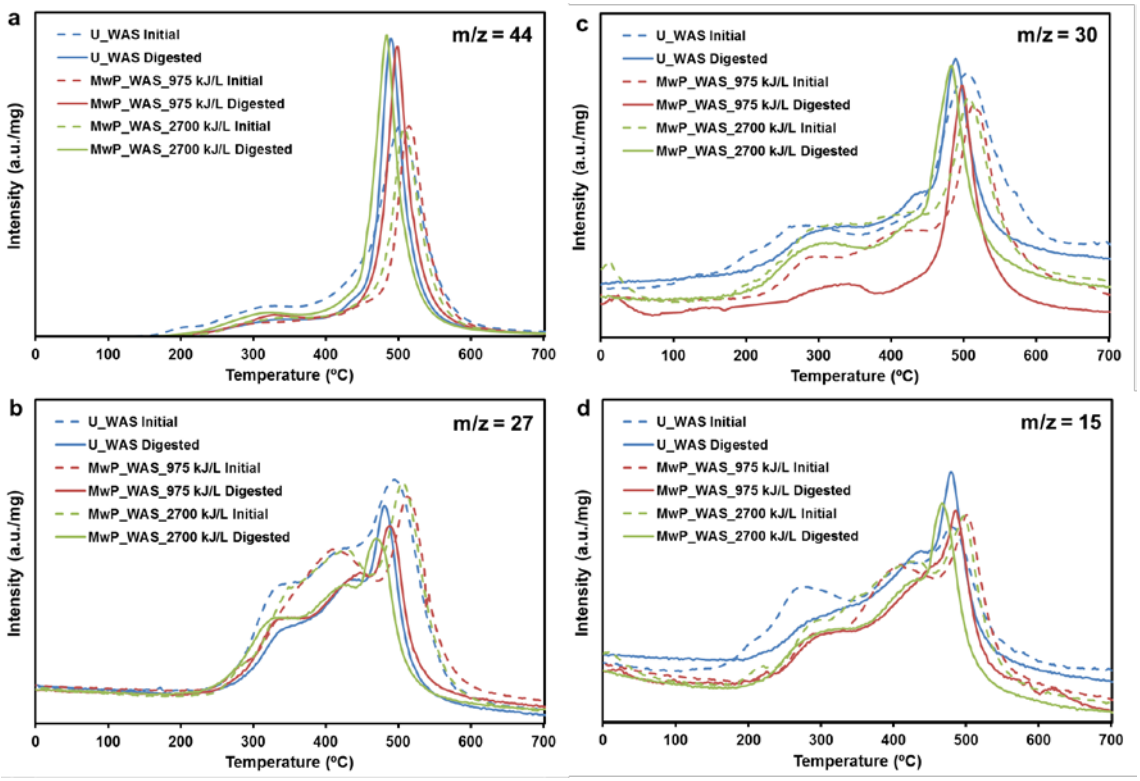

Figure 7.8 Results from mass spectrometric analysis of untreated (U_WAS) and pre-treated (MwP_WAS) samples before and after batch digestion tests: (a) evolution profile of m/z 44 (CO_2), (b) evolution profile of m/z 27 (light hydrocarbons such as C_2H_3+), (c) evolution profile of m/z 30 (NO) and (d) evolution profile of m/z 15 (light hydrocarbons such as CH_3+)

The emissions of light hydrocarbons such as CH_3^+ (*m/z* 15) are represented in Figure 7.8(d). A significant release of this gaseous component was detected for the U_WAS sample at the low temperature range, for both initial and digested samples. The application of the Mw pre-treatment reduced this emission and a further reduction was attained after the digestion process. This behaviour is in accordance with the elimination of the more labile compounds and highly biodegradable material after the pre-treatment that was seen in Figure 7.7(c).

Summarizing, at the low temperature range (150 – 350 °C) the digestion process mainly reduced the gaseous components corresponding to *m/z* 30 and 15, i.e. NO and light hydrocarbons like CH_3^+. At the intermediate temperature range (350 – 450 °C), the digestion process also decreased the above mentioned components, but in this region the gaseous components corresponding to *m/z* 27, i.e. light hydrocarbons like $C_2H_3^+$ were noticeablely reduced. Finally, at the higher temperature range (450 – 550 ºC), the gaseous emissions associated with *m/z* 44, 30 and 15 (CO_2, NO and light hydrocarbons like CH_3^+ respectively) increased after the digestion process, but those related to *m/z* 27 decreased.

On the other hand, the effect of the Mw pre-treatment of the initial samples is less marked in the mass spectrometic profiles. However, it can be seen that the gaseous components corresponding to *m/z* 30 (NO) decreased along all the temperature range when the samples were Mw treated. Moreover, the components associated with *m/z* 27 (light hydrocarbons like $C_2H_3^+$) decreased after pre-treatment at the lower and higher temperature ranges, whereas those related to *m/z* 15 (light hydrocarbons like CH_3^+) also decreased at the lower temperature region. In addition, the decrease was slightly higher when the Mw energy applied during the pre-treatment was 975 kJ/L than in the case of 2700 kJ/L.

7.2.3.4 Semi-continuous digestion

Results of biogas production are presented in Figure 7.9 for the digestion of the untreated sewage sludge (RU_SS) and Mw pre-treated sewage sludge at 975 kJ/L (RMwP_SS). Table 7.6 shows the digestion data for these semi-continuous experiments. An increase in biogas production was observed for all periods tested for the Mw pre-treated system when comparing with RU_SS. The decrease in HRT (i.e. increase in OLR) resulted in a decrease in methane yield for both of the reactors studied. This behaviour was accompanied by a poor removal of VS, which resulted in higher VS content, as it can be seen in Table 7.6. Lowering HRT adversely affected sludge mineralisation, although pH measurements did not reflect digestion problems and VFA measurements indicated that process imbalances were only experienced in the last period of the test. Sludge mineralisation is the other important feature of the digestion process, and this phenomenon should not be set apart when high digestion efficiency should to be attained.

Table 7.6 also shows the increment in methane yield obtained (for all HRT tested) when the Mw pre-treatment was applied (comparison was made using data from the RU_SS reactor at an equivalent HRT). The best results were obtained when HRT was in the range of 25 – 10 days. The evaluation of reactors at 5 d HRT resulted in poor performance (detriments in

methane yields) although digestion systems were capable of keeping high pH values and the VFA accumulation was not severe enough to be the cause of inhibitory problems. The results obtained were in accordance with those previously reported in literature (Park et al., 2004; Toreci et al., 2009).

Table 7.6 Digestion data for the semi-continuous state fed with untreated (RU_SS) and pre-treated (RMwP_SS) sludges

	RU_SS				RMwP_SS			
HRT (d)	25	20	10	5	25	20	10	5
OLR[a] (gVS/L*d)	1.0 ± 0.2	1.6 ± 0.2	2.1 ± 0.1	4.1 ± 0.2	1.0 ± 0.2	1.6	2.1	4.1
CH_4 yield (L CH_4/Kg VS)	226 ± 25	213 ± 19	209 ± 18	166 ± 12	324 ± 32	308 ± 30	295 ± 28	214 ± 22
Yield Increment	--	--	--	--	43	45	41	29
Gas Prod. (L/d)	1.1 ± 0.1	1.6 ± 0.2	2.1 ± 0.2	3.3 ± 0.2	1.6 ± 0.2	2.4 ± 0.1	3.1 ± 0.2	4.3 ± 0.2
VS (g/L)	16.6 ± 1.0	19.3 ± 0.9	18.0 ± 0.8	18.3 ± 0.7	13.5 ± 1.0	15.9 ± 0.9	16.5 ± 0.9	17.5 ± 1.0
TS (g/L)	21.4 ± 0. 9	24.6 ± 0.6	23.2 ± 1.0	23.2 ± 0.9	20.2 ± 0.6	22.2 ± 0.9	23.4 ± 0.6	24.6 ± 0.7
VS removal (%)	35 ± 1.6	43 ± 1.4	16 ± 1.5	12 ± 1.2	47 ± 1.3	49 ± 1.3	23 ± 1.5	16 ± 1.4
NH_4^+ (mg/L)	1385 ± 85	1360 ± 89	1277 ± 53	908 ± 24	1665 ± 83	1500 ± 90	1617 ± 89	1077 ± 79
VFA total (mg/L)	62.9 ± 2.9	59.1 ± 1.1	35.8 ± 0.7	456.1 ± 9.1	55.4 ± 2.7	37.5 ± 0.5	22.7 ± 0.3	450.1 ± 8.5
pH	7.4 ± 0.1	7.3 ± 0.1	7.2 ± 0.1	7.0 ± 0.1	7.4 ± 0.1	7.3 ± 0.1	7.3 ± 0.1	7.0 ± 0.1

Eskicioglu et al. (2007) reported low improvements in biogas production when applying Mw pre-treatment at temperatures below the boiling point of sludge while operating at 5 d HRT. However, Woon-Ji and Johng-Hwa (2011) reported a 55% increase in methane yield when operating at similar conditions. Operating at higher sludge temperatures may have a significant effect in the behaviour of the digestion process. Toreci et al (2009) reported an improvement of 84 and 47% when operating at 175 °C and heating rates of 3.75 °C/min and 1.25 °C/min, respectively. In the present study, the pre-treatment was applied reaching the boiling point of sludge and this may explain the high improvement obtained when operating in the HRT range of 24 – 10 d. Operation at lower HRT values resulted in poor performance of both systems but the improvement in biogas production was still significant when compared to the untreated digestion system.

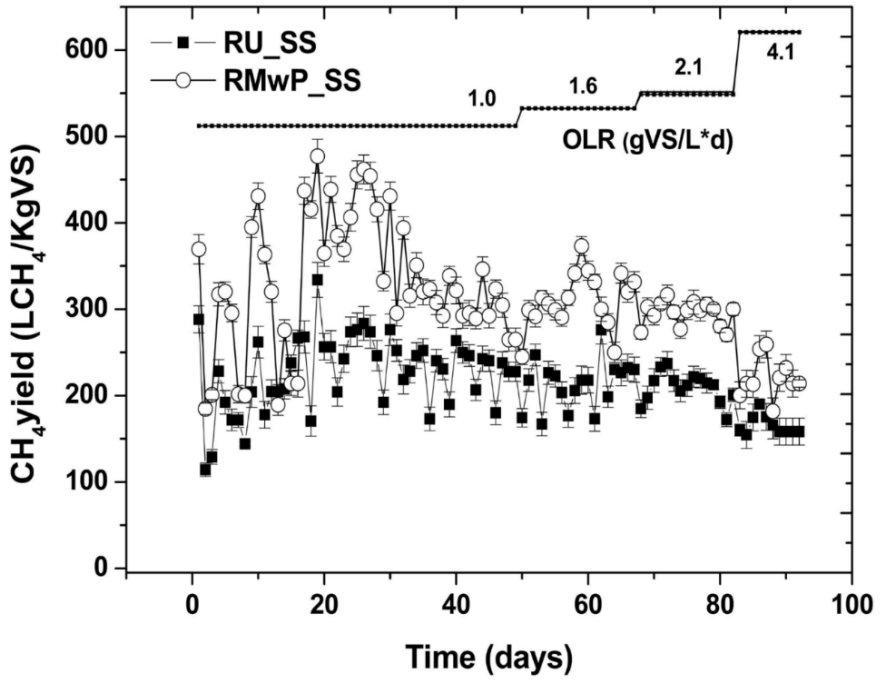

Figure 7.9 Semi-continuous digestion of untreated sewage sludge (RU_SS) and microwave pre-treated sewage sludge (RMwP_SS_975 kJ/L)

The pre-treatment favoured the mineralisation of organic matter. This effect can be deduced from the higher removal of VS obtained from the RMwP reactor, along with the higher ammonium values registered. The concentration of ammonium in the effluent was higher for all periods evaluated, which indicates that the application of the Mw pre-treatment facilitated the hydrolysis of proteins making complex compounds available for anaerobic microorganisms. The fact that ammonium values were higher in the pre-treated system (for all HRTs evaluated) indicated that complex proteins have become available thanks to the

effects associated to the pre-treatment; otherwise ammonium values should have been similar at high HRT values in both reactors.

7.2.4 Conclusion

Microwave pre-treatment (MwP) was studied as a means for increasing biogas production from sewage sludge and reducing the time needed for stabilisation. The application of MwP resulted in an increase in biogas production (methane yield). The pre-treatment affected the specific surface area (SSA) of the organic particles, evidencing a linear correlation with methane yields obtained from batch tests. TG analysis revealed that a complexation of the organic material took place after pre-treatment, probably being the cause of the lower biogas production rate during the initial digestion stage along with the accumulation of these compounds in the digested sample.

A significant increase in biogas production was obtained when evaluating the digestion process under semi-continuous operation at hydraulic retention times (HRTs) of 25 – 10 d (43% increment in methane yield).

7.2.5 References

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Capítulo/Chapter 8

8. Anaerobic digestion of an agroindustrial waste (brown juice) in UASB reactor

Abstract

The aim of this work was to study the digestion of an agro-industrial biorefinery waste called brown Juice. The digestion process was studied under batch conditions evaluating the effect of nutrient supplementation and under continuous operation when using an up-flow anaerobic sludge blanket (UASB) reactor. The results obtained from batch tests showed a good performance at all substrate/inoculum ratio tested (0.25-1.25). The methane yield values did not show any significant differences between them and were close to the theoretical value estimated, about 500 L CH_4 Kg VS^{-1}. Regardless of the positive results obtained from batch tests, the anaerobic digestion in UASB reactor showed a poor performance obtaining relatively low methane production and reduced COD removal due to substrate overloading problems and bulking phenomena.

Keywords: Brown Juice, anaerobic digestion, up-flow anaerobic sludge blanket, nutrient supplementation.

Edition of this manuscript was in accordance with the scientific journal

8.1 Introduction

The Green biorefinery is nowadays a well-known concept for using the completely green biomass as raw material for the production of industrial products like proteins, lactic acids, fibres and energy. (Kromus et al., 2004). Such transformation processes generate sub-products and/or wastes that must be properly managed to prevent future environmental problems. The biorefinery process usually starts with a fractionation of the green biomass into a solid and a liquid fraction, called press cake and green juice, respectively.

The green juice contains non-denatured proteins which are suitable for the development of protein feed additives. The protein isolation takes place using ecological and sustainable technologies, without the use of inorganic acid or any organic solvents. This residual fraction obtained after protein precipitation is called brown juice (Kamm et al., 2000). Brown juice (BJ) contains mainly water-soluble carbohydrates, proteins and minerals. It is suitable for ruminant feedstock, fertilizer and feedstock for anaerobic digester to produce biogas, which is converted into electricity and heat (O'Keeffe et al., 2011).

Anaerobic digestion is an attractive waste treatment technology intended for the sustainable management of wastes and energy recovery. This process involves the degradation and stabilization of organic materials under anaerobic conditions by microbial organisms and leads to the production of biogas.

The implementation of an Up-flow Anaerobic Sludge Blanket (UASB) reactor has been applied successfully throughout the world to a wide range of industrial and domestic wastewaters like beverage, brewery, food and tannery industries (Lettinga and Hulshoff Pol, 1991). This reactor has several advantages over other anaerobic reactors due the potential to its simple design, low capital investment, easy construction, maintenance and flexibility to face fluctuations in temperature, pH and substrate concentration (Lettinga and Hulshoff Pol, 1991; Alvarez et al., 2006; Hinken et al., 2014). The key feature of UASB reactor is that it allows the use of high volumetric chemical oxygen demand (COD) loadings compared to other reactor types like the conventional continuous stirred-tank reactor (CSTR). The UASB reactor is the most robust high-rate anaerobic reactor designed to operate at short hydraulic retention times (HRT) and long solids retention times (SRT) therefore incorporating large amounts of high-activity biomass (von Sperling and Chernicharo, 2005).

In an UASB reactor, the biomass is retained in the form of granules. These granules consist of dense particles formed by anaerobic microorganisms working as functional units capable of performing themselves the complete methanogenic degradation of the organic matter. The granule structure has been described as a central core of acetoclastic methanogens surrounded by a layer of bacteria that hydrolyse and acidify complex organic matter (Harmsen et al., 1996; Liu et al., 2003; McHugh et al., 2003; Zhen et al., 2006). This aggregation of biomass sustains good activity, which allows settleability and promotes mass transport between granules and media solution conferring the high throughput characteristic of UASB reactors

The stable and well function of the UASB reactor is mainly dependent on the growth and maintenance of the sludge granules. The size of the granules depends by several factors, such as composition of wastewater, operating conditions (HRT, OLR), pH and alkalinity, temperature and concentration of essential macro- and micro-nutrients. (Lau & Fang, 1997; Gonzalez et al., 1998; Ahn et al., 2002; Ghangrekar et al., 2005).

In the present study, the biogas potential and the effect of nutrient supplementation in brown juice digestion was studied in batch experiments. Furthermore, the treatment of the brown juice was tested in a lab-scale UASB reactor for evaluating biogas production at different OLR along with the overall stability of the process.

Considering the importance of granular sludge in UASB reactor, an additional purpose of this study was to evaluate the particle size of granules as a characteristic parameter to measure the effect of brown juice concentration on granular sludge.

8.2 Material and methods

8.2.1 Substrates

The granules used as inoculum in the batch tests and UASB reactor were collected from a wastewater treatment plant (WWTP) treating industrial food waste and located in Haribo, Demark,. The total solid (TS) content of the inoculum was 120 g L^{-1}, of which 45 g L^{-1} were volatile solids (VS).

Brown juice used as substrate was obtained after lactic acid fermentation of the green juice after mechanical fractionation of fresh biomass. This green biomass was a mixture of red clover and clover grass. The biomass was manually harvested on the 20th of May, 2014 in

Orten (Denmark). Right after harvest, a mechanical pressing was done using a Vincent CP4 screw press obtaining two fractions. The green juice was inoculated with an overnight cultured Lactobacillus salivarius BS 1001 in a concentration of 0.02 L/L of green juice. It was then incubated at 38 °C for one day until the pH reached 4.3 units, indicating a proper fermentation. To obtain the brown juice, a centrifugation step was performed during 10 min at 3 800 rpm at 5 °C and afterwards was stored at -18 °C. The frozen substrate was thawed at 4 °C for 2–3 days before use. Chemical composition of this substrate is presented in Table 8.1.

Table 8.1 Brown Juice characterization

Parameters	Brown Juice
pH	4.23
Total Solids g l^{-1}	46.47±0.38
Volatile Solids g L^{-1}	36.01±0.33
Ash g L^{-1}	10.60±0.05
CODt g L^{-1}	54.8±0.71
TKN g L^{-1}	0.80±0.01
Total VFA g L^{-1}	2.47±0.14
Free Sugar g L^{-1}	27.45±2.44
Lactic Acid g L^{-1}	13.49±1.25
Citric Acid g L^{-1}	1.23±0.19
Succinic Acid g L^{-1}	1.04±0.25

The anaerobic basic medium (ABM) used for batch tests was prepared as indicated in Angelidaki & Sanders (2009).The medium was added as a source of nutrients and micronutrients, growth factors vitamins and trace metals necessary for growth of the microorganisms.

8.2.2 Methane potential batch test

Batch assays were performed for obtaining the biochemical methane potential of the substrate. The experiments were carried out in closed vessels as described in Angelidaki et al., (2009). The vessels were filled with 30 mL of the mixture with brown juice diluted with 10%, 20%, 30%, 40%, and 50% of ABM or water and inoculated with 10 mL of granules in order to attain the desired proportion of VS between substrate and inoculum of 0.25, 0.50, 0.75, 1.00 and 1.25 respectively. Three vials were set up for each composition. Blanks containing 10 mL of inoculum and filled with 30 mL of ABM or water and were set-up in triplicate to determine the endogenous methane production of the anaerobic inoculum.

$NaHCO_3$ at a concentration of 15 g L^{-1} was added to the vials for pH correction. The vials were gassed with a mixture of 80% N_2 and 20% CO_2 under the filling procedure, they were sealed with a rubber stopper and a metal cramp and incubated at 37 °C. The process was monitored by measuring the methane production in the headspace of each vial using gas chromatography (Biswas et al., 2012). The vessels were denoted as BJ:ABM and BJ:water at the corresponding substrate/inoculum ratio being treated

Methane production was fitted to the modified Gompertz equation (Cuetos et al., 2013) Equation 8-1:

$$P_{(t)} = P_{\max}.\exp\left[\ -\exp\left[\ \frac{R_{\max}.e}{P_{\max}}(\lambda - t)+1\ \right]\ \right]$$

Equation 8-1

Where $P_{(t)}$ is the cumulative methane production (L kg VS^{-1}); P_{max} is the methane production potential (L kg VS^{-1}), R_{max} is the maximum methane production rate (L kg VS^{-1} d^{-1}), λ is lag-phase time (d) and e is 2.718.

Data analysis was performed using Origin 6.1 software. A modification to the model was proposed when an extended lag phase was observed (Fernandez et al., 2014). This modification considers the addition of a new parameter corresponding to the initial methane production obtained from the experiment.

8.2.3 UASB reactor

The UASB bioreactor consisted of a double glass column with an internal diameter of 4 cm and a height of 35 cm, with a working volume of 400 mL. The bioreactor was heated by circulating water at 37 °C through the water jacket. The reactor was equipped with a sieve on the top, which separated gas and granule particles from the liquid, preventing the granules being washed out. The influent was pumped by a peristaltic pump into the reactor and pumped upwards through the sludge bed, leaving the reactor by the upper end in order to promote uniform fluidization of media and the granules. The UASB bioreactor was tested with an up-flow velocity of 1.5 m h^{-1}. The produced biogas was registered using a volumetric Miligascounter (Ritter ®).

The reactor was inoculated with 120 mL (30% of the reactor working volume) of granules and operated at 37 °C using a pH controller (7.1-7.5) with the addition of $NaHCO_3$ solution as a buffer.

During the first period (Period I), the granules were adapted with ABM dilution at an increasing OLR from 2.5 to 7.0 g COD $L^{-1}d^{-1}$ and an HRT of 1 day for 20 days to ensure enough nutrients for the growth of microorganisms. Thus, preparing the reactor for higher organic rates. The next stages of the experiment (Period II-IV) were evaluated with water dilution to attain the desired OLR (12-14 g COD $L^{-1}d^{-1}$), the HRT was set from 1 to 3 days.

8.2.4 Particle size analysis of granules

Particle size analysis was carried out using a Laser Diffraction particle Size Analyser LS 13 320 Beckmann Coulter. This is a light scattering instrument, which operates on the principle of the Fraunhofer diffraction theory. Samples were diluted in tap water for analysis. 10 measurements were performed for each sample. The size distribution was based on volume and the average size was quoted as the *median* based on volume equivalent diameter.

One-way analysis of variance (ANOVA) (α = 0.05) of repeated measures was used to determine statistically significant differences among particle size parameters between the different proportions of brown juice used during the batch tests. T-student analysis was used to compare the data obtained from UASB reactor. Statistical analysis of data was performed using Origin 6.1 software.

8.2.5 Analytical techniques

Samples were analysed for total solid (TS), volatile solids (VS), chemical oxygen demand (COD), ammonium and pH according to APHA Standard Methods (1989). Biogas composition was analysed using a gas chromatograph (SRI GC model 310), equipped with a Porapak Q column of 182.88 cm length and 2.1 mm i.d. Nitrogen was used as carrier gas with a pressure of 196 kPa. The injector and detector temperatures were 80 °C; the temperature of the oven was constant on 80 °C. The retention time for methane with these parameters was about 0.4 min. As standard gas, a mixture of 30% CH_4 and 70% N_2 was utilized.

Volatile fatty acids (VFAs) was analysed using a gas chromatograph (Varian CP 3800 GC) equipped with a flame ionization detector (FID) and Nukol capillary column (30m×0.25mm×0.25m) from Supelco. Injector and detector temperatures were 220 and

250 °C, respectively. The oven temperature was set at 150 °C for 3 min and thereafter increased to 180 °C. Samples were previously centrifuged (10 min, 14 000 rpm) and the supernatant filtrated through 0.45 μm cellulose filters.

8.3 Results and discussion

8.3.1 Methane potential test

The accumulated methane yield obtained during batch experiments are presented in Figure 8.1. Two batch experiments for different concentrations of brown juice were carried out BJ:ABM and BJ:water to observe the effect of the lack of nutrients in BJ. Thus, different substrate/inoculum ratios were tested the results of the batch experiments show that the process was partially inhibited and brown juice was degraded.

The batch systems at Figure 8.1 presented a sigmoid gas production curve, which was adjusted to the modified Gompertz model by considering the initial volume of methane produced as an additional parameter. Parameters obtained from the model are shown in Table 8.2.

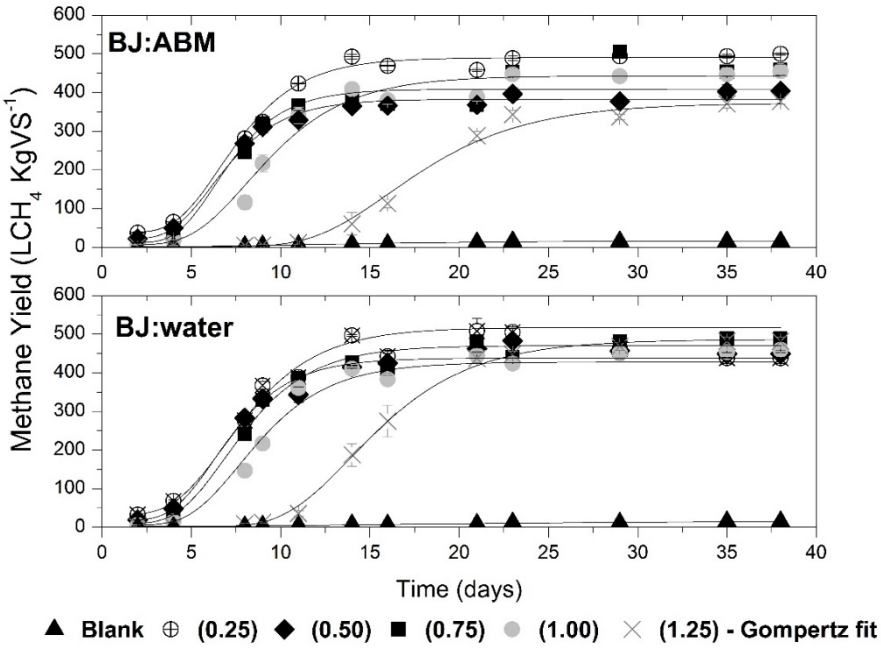

Figure 8.1 Batch digestion of brown juice diluted with anaerobic basic medium (BJ:ABM) and water (BJ:water) at different substrate/inoculum ratios

A lag phase with the increase of brown juice concentration from 3 to 11 days was observed (Table 8.2). This delay could be due to an adaptation of microorganisms to the substrate. After the lag phase period was overcome, the system seemed to show the capacity to degrade the substrate.

Table 8.2 Results and parameters estimated from anaerobic batch tests

Substrate/inoculum ratio ***BJ:ABM***	**Final Methane Yield (LCH_4 $KgVS^{-1}$)**	**Estimated P_{max} (LCH_4KgVS^{-1})**	**Initial value**	**R_{max} (LCH_4 $KgVS^{-1}$ d^{-1})**	**λ (d)**	**R^2**
Blank	15.22±0.8	16.5±0.8	0.02±0.0	0.81±0.1	3.6±0.2	0.993
0.25	500.24±25.1	452.4±22.6	38.1±1.9	67.6±3.3	4.1±0.2	0.996
0.50	404.2±20.2	361.1±18.0	21.4±1.0	60.0±3.0	3.9±0.2	0.989
0.75	458.7±22.9	397.8±19.8	11.2±0.5	69.3±3.4	4.2±0.2	0.998
1.00	455.4±22.7	437.8±21.8	5.4±0.2	51.8±2.5	5.0±0.3	0.998
1.25	376.6±18.8	371.4±18.5	1.8±0.1	32.2±1.6	11.8±0.6	0.993
BJ : water						
Blank	15.10±0.8	15.1±0.7	0.0±0.0	1.5±0.1	3.4±0.4	0.994
0.25	437.8±21.8	484.9±24.2	32.3±1.6	62.7±3.1	4.0±0.2	0.998
0.50	448.7±22.4	419.8±20.9	18.4±0.9	66.3±3.3	3.9±0.2	0.989
0.75	489.6±24.4	461.0±23.0	9.3±0.4	64.2±3.2	4.2±0.2	0.989
1.00	459.4±22.9	421.8±21.0	5.7±0.2	55.9±2.7	4.9±0.2	0.999
1.25	481.6±24.0	484.2±24.2	2.1±0.1	47.7±2.3	10.2±0.5	0.997

Pmax is the methane production potential, Rmax is the maximum methane production rate, λ is lag-phase time

Nges and Björnsson (2012) found the possibility of nutrient deficiency for this kind of substrates for mono-digestion of energy crops, but the similar results of P_{max}, $R_{max,}$(Table 8.2) for both batch experiments at this work indicate that brown juice could be a proper substrate for methane production without external nutrient addition.

The results of methane yield obtained under batch tests showed a high methane yield around 500.2 L CH_4 Kg VS^{-1}, from BJ:ABM at 0.25 substrate/inoculum ratio and 489.6 L CH_4 Kg VS^{-1} with BJ:water at 0.75 substrate/inoculum ratio. These values did not show significant differences and were close to the theoretical yield of the BJ (500 L CH_4 Kg VS^{-1}).

The development of the cumulative methane yield for all substrate/inoculum ratios showed that the microorganisms were not highly inhibited and were well adapted to the BJ.

8.3.2 Effect of brown juice concentration on granular sludge size

In order to evaluate the effect of brown juice concentration on granular sludge. Particle size distribution (PSD) of the granules before (initial) and after the batch experiments are shown in Figure 8.2. The PSD was expressed as a volume fraction. Results show a binomial distribution of particle sizes in the range from 0 to 2000 µm.

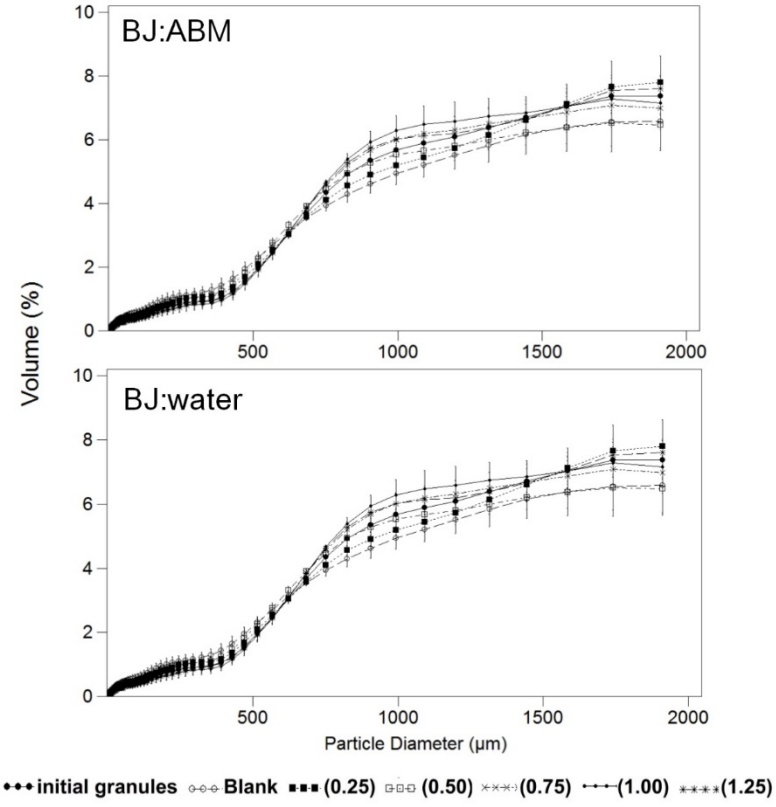

Figure 8.2 Particle size distribution (PSD) of batch test of brown juice diluted with anaerobic basic medium (BJ:ABM) and water (BJ:water) at different substrate/inoculum ratio

The median particle diameter and percentiles (d10, d50 and d90) were used to compare the size of granule obtained at different substrate/inoculum ratios of BJ diluted with ABM and with water (Table 8.3). The d10, d50 and d90 values indicate that 10%, 50% and 90% of the particles measured were less than or equal to the size stated.

Table 8.3 Particle size parameters of batch test

BJ:ABM	Median (µm)		d10 (µm)		d50 (µm)		d90 (µm)	
Initial	986.9±62.7	(ab)	164.1±56.8	(ab)	986.9±62.7	(ab)	1761.9±25.4	(ab)
Blank	893.8±83.4	(c)	109.36±40.1	(c)	893.8±83.4	(c)	1733.8±30.4	(b)
0.25	977.2±61.9	(ab)	149.8±37.7	(bc)	977.2±61.9	(ab)	1773.2±24.0	(a)
0.50	924.4±50.2	(ab)	139.4±29.7	(bc)	924.4±50.1	(ab)	1731.8±26.6	(b)
0.75	987.4±57	(ab)	192±59.7	(ab)	987.4±56.9	(ab)	1751.4±19.5	(ab)
1.00	1011.4±45.8	(a)	230.4±73.1	(a)	1011.4±45.8	(a)	1758±9.7	(ab)
1.25	1002.2±52.9	(ab)	208.6±68.9	(ab)	1002.2±52.8	(ab)	1770.2±11.0	(a)
ANOVA								
Sum Sq	112704.0		106766.3		112704.0		16163.7	
Mean Sq	18784.0		17794.4		18784.0		2694.0	
Variance	0.06		0.32		0.06		0.01	

BJ:water	Median (µm)		d10 (µm)		d50 (µm)		d90 (µm)	
Initial	986.9±62.7	(b)	164.1±56.8	(b)	986.9±62.7	(b)	1761.9±25.4	(c)
Blank	1040±57.7	(ab)	208.8±49.5	(ab)	1040±57.7	(ab)	1807±8.6	(a)
0.25	1012.8±56.4	(ab)	234.6±83.4	(ab)	1012.8±56.4	(ab)	1772±12.1	(c)
0.50	1024.8±61.8	(ab)	217.2±72.9	(ab)	1024.8±61.8	(ab)	1784±8.8	(ab)
0.75	1032.4±43.9	(ab)	246.4±87.6	(ab)	1032.4±43.9	(ab)	1778.8±6.5	(bc)
1.00	1005.2±44.6	(b)	180±37.8	(b)	1005.2±44.6	(b)	1792.2±9.4	(ab)
1.25	1079.8±49.7	(c)	285.6±105.4	(c)	1079.8±49.7	(c)	1796.2±5.5	(a)
ANOVA fit stadistics								
Sum Squares	52685.3		100700.1		52685.3		14021.3	
Mean Square	8780.9		16783.4		8780.9		2336.9	
Variance	0.05		0.34		0.05		0.01	

Results marked by the same letter (a, b or c) are not significantly different.

The Blank sample in the batch tests BJ:ABM showed a significant decrease in particle size compared with the initial granules indicating a possible negative effect of the media on the granular sludge. Nevertheless, the median diameter obtained for the final granules corresponding to conditions tested (0.25-1.25 substrate/inoculum) were similar to the initial granules.

With regard to the batch tests using water as diluent (BJ:water), the statistical analysis presented in Table 8.3 showed no significant differences between particle sizes of the Blank sample and final granules corresponding to the different conditions tested (0.25-1.00 substrate/inoculum ratio) compared with the initial granules used as inoculum. However, statistical analysis shows a significant increase in particle size of the sample with 1.25 substrate/inoculum ratio in the batch tests with water.

These results are similar to previous research, which found that the average particle size of granular sludge is dependent of multiple factors comprising nutrient supply, substrate limitation inside the granules and production of extracellular biopolymers (Grotenhuis et at., 1991; Ismail et al., 2010).

8.3.3 UASB reactor

The UASB reactor was tested over a period of 70 days. Figure 8.3 shows a stable performance of the reactor with a mild increase in the methane yield with the increase in the OLR. Methane yield was very low during period I. A steep rise in this parameter was observed as the time of operation progressed (periods II - IV). The increase may probably due to the increase in organic loading. However, the methane yield gas was relatively low compared to the results obtained in the batch test. The low pH value and the high protein and sugar contents in these substrate may cause a system overloading, and the inhibition of methane production (Table 8.4).

A significant deterioration of the granular sludge was observed at Period IV, the granules significantly decreased in size, and their color changed from gray to yellowish. Bulking sludge is one of the most common problems encountered in UASB reactors. Bulking sludge is generally defined as the sludge which settles slowly and compacts poorly because of an excessive growth of filamentous and/or Zoogloea organisms (Martins et al., 2004). Bulking severely affects the treatment, often leading to a complete loss on performance in UASB reactors. This phenomenon has been reported by Yamada et al. (2001) when working with mesophilic UASB reactors treating high-strength organic wastewater.

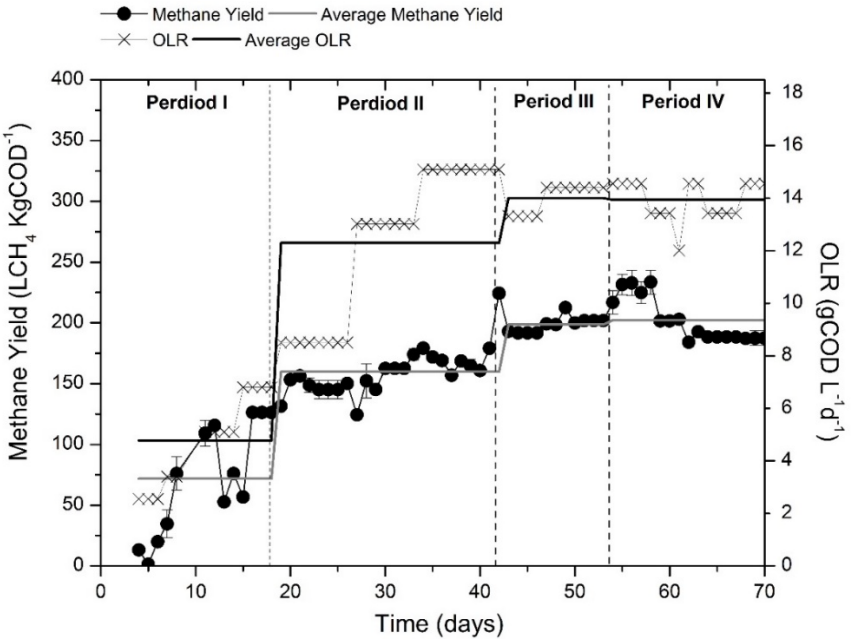

Figure 8.3 UASB reactor performance

Results from the adaptation period showed a poor performance in COD reduction, with this value falling down to 33%. The high COD loading cannot be managed by the bacteria at the short retention time applied. The efficiency in COD removal increased about 50%-55% in periods II and III. The poor performance regarding COD removal is presumably caused by the low HRT applied and the sudden increase in OLR causing a shock loading. Finally in Period IV a significant deterioration of the granular sludge is observed leading to reactor failure.

Table 8.4 Parameters of anaerobic digestion at UASB reactor

Period	I	II	III	IV
HRT (days)	1.0±0.1	1.1±0.1	2.2±0.1	3.0±0.1
OLR (g COD L^{-1})	4.8±0.2	12.3±0.6	14.0±0.7	13.9±0.7
Biogas production (L d^{-1})	0.4±0.02	1.4±0.07	1.8±0.07	2.0±0.08
Methane concentration (%)	40±0.5	50±0.5	61.5±0.5	55.8±0.5
Methane yield (L CH_4 Kg COD^{-1})	95.3±4.7	165.4±8.7	198.7±9.9	202.4±10.1
COD removal (%)	33.0±2.0	50.5±2.5	55.2±2.7	n.m
Ammonia (mg L^{-1})	239.5±11.9	43.0±2.1	57.8±2.8	90.8±4.5

n.m not measured

Figure 8.4 shows the particle size distribution (PSD) of the granules samples from the UASB reactor at the beginning (initial UASB granules) and at the end of the process (End UASB granules). After 70 days of operation, the granular sludge obtained from the UASB reactor presented a decrease in the particle size compared to that of the inoculum.

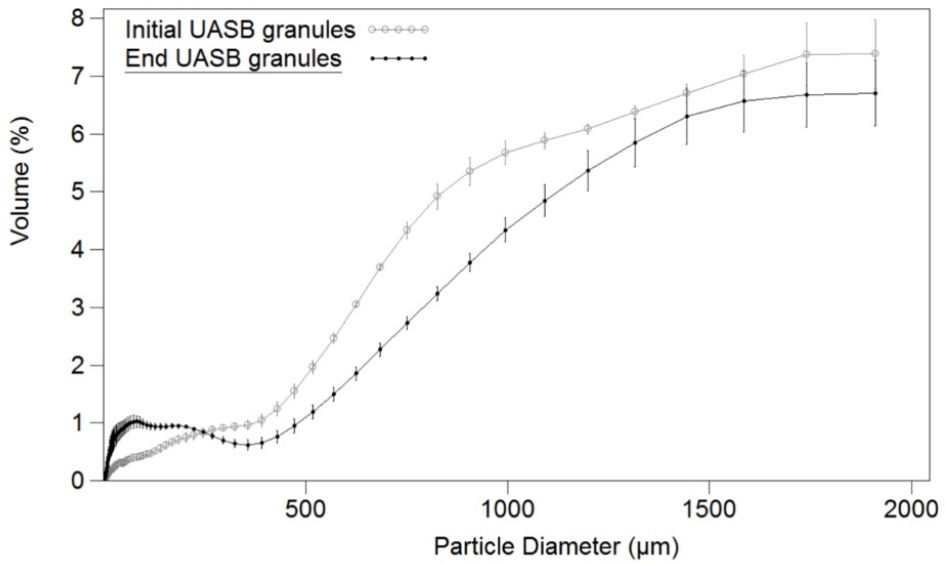

Figure 8.4 Particle size distribution of UASB granules at the end of reactor operation

The particle size distribution shows a significant decrease in the median value if compared with that one from the inoculum, with the latter having 50% of the particles (d50) a diameter lower than 978 µm. This value decreased to 860 µm for d50 (Table 8.5). This behaviour can partly be explained by a biomass shift caused by substrate overloading leading therefore to the bulking phenomena.

Table 8.5 Particle size analysis of granules at initial stage and at the end of the experiment under semi-continuous regimen (using UASB reactor) (t-Stadistic results)

UASB reactor granules		Difference	(t) Value	Alpha	Sig	LCL	UCL
Median (µm)							
Initial	994.8±59.4	134.4	3.5	0.05	1	212.1	56.7
End	860.4±134.3						
d10 (µm)							
Initial	154.8±52.9	123.1	8.9	0.05	1	151.5	94.6
End	31.7±8.8						

d50 (µm)							
Initial	978.9±62.6	118.5	3.1	0.05	1	196.9	40.1
End	860.4±134.3						
d90 (µm)							
Initial	1757.4±26.7	20.0	1.8	0.05	0	42.6	2.6
End	1737.4±33.7						

Sig (1) indicates that the means difference is significant at the 0.05 level.

Sig (0) indicates that the means difference is NOT significant at the 0.05 level.

LCL lower limits 95%

UCL upper limits 95%

8.4 Conclusions

The results obtained from batch tests showed a good performance for all substrate/inoculum ratios tested, with methane yields showing no significant difference between them and were close to the theoretical yield estimated (500 L CH_4 Kg VS^{-1}).

Despite the positive results obtained from the batch tests, the process in an UASB reactor showed a poor performance regarding methane production and COD removal. The main characteristics of brown Juice were a low pH value and high protein and sugar content. At the OLR tested the system suffered from overloading. The results of particle size analysis performed on UASB reactor samples showed the disintegration of larger granules. The treatment of brown juice in UASB reactor still needs to confront several challenges such as avoiding the appearance of the bulking phenomena and granule disaggregation, which causes detriments in reactor performance as it is foaming, flotation and washout of sludge. Thus, further investigation is still required.

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Capítulo/Chapter 9

9. Conclusiones generales/General conclusions

Las siguientes conclusiones pueden obtenerse de este estudio:

Referidas a la co-digestión del lodo de depuradora con residuos de alto contenido en lípidos (grasas):

1. No se observaron cambios en la producción especifica de metano al utilizar la mezcla de FOG como co-sustrato al 0.2% (V/V) con un tiempo de retención hidráulica (TRH) de 30 días. Sin embargo una continua disminución del TRH y un incremento en la adición del FOG (1.8%) resulto en un detrimento significativo en el rendimiento del proceso.

2. Los espectros de infrarrojo por transformada de Fourier (IR-TF) obtenidos de las muestras co-digeridas mostraron bandas de alta intensidad en la región alifática, las cuales son atribuidas a grasas-lípidos, asemejándose al espectro obtenido del FOG. Los resultados sugieren una posible absorción de los componentes lipídicos del FOG, lo que podría haber impedido la degradación del sustrato durante el tratamiento anaeróbico.

3. El proceso de digestión anaerobia del lodo de depuradora con la grasa de descarte de carnicería se llevó a cabo de manera satisfactoria operando en condiciones discontinuas y semi-continuas. La adición del co-sustrato mejoró la estabilidad del proceso de digestión y el rendimiento de biogás, debido al incremento en la carga orgánica. Sin embargo la digestión en termófilo se caracterizó por un efluente de mala calidad, asociado a un alto contenido en ácidos grasos volátiles.

4. Por otro lado, cuando se digirió grasa obtenida de una industria de procesamiento de leche, los resultados mostraron una operación satisfactoria en el reactor en semi-continuo operando con un tiempo de retención hidráulica de 40 días, a pesar de que se observaron inhibiciones en el proceso operando en discontinuo.

Referidas a la aplicación de pre-tratamiento:

5. La digestión anaerobia del lodo de depuradora mejoró de forma significativa con el pre-tratamiento de ultrasonidos al compararse con los resultados del lodo sin tratamiento. Se demostró que el tamaño de partícula juega un rol relevante en la disponibilidad del material orgánico para la degradación microbiana, la producción específica de metano aumentó con la aplicación de ultrasonidos gracias a la desaglomeración de los flóculos y el incremento del área de superficie específica (SSA). El pre-tratamiento con ultrasonidos también afectó a la deshidratabilidad del lodo digerido.

6. En los ensayos en discontinuo, la aplicación del pre-tratamiento con microondas (MWP) resultó en un incremento del rendimiento de metano y mostró una correlación lineal con el área de superficie específica de las partículas. Los análisis termogravimétricos revelaron que se llevó a cabo una complejación del material orgánico aplicar el pre-tratamiento con alta energía. Operando en régimen semi-continuo y con TRH de 25-10 d se obtuvo un incremento en la producción de biogás (sobre un 43% de producción específica de metano).

Referidas a la digestión anaerobia de un residuo agroindustrial de biorefineria en reactor UASB:

7. El residuo agroindustrial de biorrefinería llamado jugo de marrón podría ser un sustrato adecuado para la producción de metano sin la adición de externa de nutrientes externo. Este residuo no causó la inhibición del proceso de digestión anaeróbia. Los resultados en la prueba en discontinuo mostraron un buen desempeño en todas las relaciones sustrato/inóculo probadas, los valores de rendimiento de metano no mostraron diferencias significativas entre ellos y estaban cerca del valor de rendimiento teórico estimado alrededor de 500 L CH_4 Kg $VS^{-1.}$

   A pesar de los resultados positivos obtenidos en los ensayos en discontinuo, la digestión anaerobia en el reactor UASB mostró un pobre desempeño en relación con la producción de metano y la remoción de DQO. Las principales características del jugo marrón, bajo valor de pH y un alto contenido en proteínas y azúcares pudieron causar una sobrecarga del sistema. Los resultados de análisis de tamaño de partícula en el reactor UASB mostraron la desintegración de los gránulos más grandes.

The following conclusion can be drawn from the present study:

Regarding to the co-digestion of sewage sludge with agro-industrial wastes with high lipid content (fats):

1. The fat, oil and grease (FOG) mixture used as co-substrate resulted in no observable modifications in the specific methane production when a FOG content of 0.2% (V/V) was applied at a hydraulic retention (HRT) of 30 days. However, a continuous decrease in the HRT and an increase in the addition of FOG to 1.8% resulted in a significant detriment to performance of the process.

2. The FTIR spectra obtained from the co-digested samples showed high-intensity bands in the aliphatic region, which where ascribed to fat and lipids, resembling the spectra obtained from the FOG sample. The results showed the adsorption of lipid components of FOG, which impeded the degradation of the substrate during the anaerobic treatment.

3. The co-digestion process of sewage sludge (SS) with fat discarded from butcheries (F) was successfully performed under batch and semi-continuous conditions. The addition of the co-substrate improved digestion stability and biogas yields by the increase in the organic loading rate (OLR). However, thermophilic digestion was characterised by an effluent of poor quality associated with high VFA content.

4. When digesting fat obtained from a milk-processing factory, the results showed successful operation of the semi-continuous reactor operating with an HRT of 40 days, although high inhibition was initially reported from batch tests performed.

Regarding the application of pre-treatment:

5. Digestion of sewage sludge was significantly enhanced by ultrasound pre-treatment, when compared with results obtained from WAS samples. It was demonstrated that particle size plays a relevant role in the availability of this material to microbial degradation, specific methane production (SMP) was improved with the application of ultrasounds thanks to floc disagglomeration and increase in specific surface area (SSA). Ultrasound also affected sludge dewaterability from digested sludge.

6. The application of MwP resulted in an increase of the methane yield when tested under batch conditions. Methane yield was linearly correlated to the specific surface area (SSA)

of the organic particles. Thermogravimetric (TG) analysis revealed that a complexation of the organic material took place when the pre-treatment was applied at high energy inputs. A significant increase in biogas production was obtained when evaluating the digestion process under semi-continuous operation at hydraulic retention times (HRTs) of 25 – 10 d (43% increment in methane yield).

Regarding the digestion of biorefinery agro-industrial waste (Brown juice) in UASB reactor:

7. The agro-industrial biorefinery waste called brown juice could be a proper substrate for methane production. This waste did not cause inhibition of the anaerobic digestion process. The results from batch tests showed a good performance for all substrate/inoculum ratios tested. The methane yields showed no significant differences between them and were close to the theoretical value estimated (500 L CH4 Kg VS^{-1}).

   Despite the positive results obtained from batch test, the anaerobic digestion using a UASB reactor showed a poor performance with regard to the methane production and COD removal. The main characteristics of Brown Juice were low pH value and high protein and sugar content which caused process disturbance. The results of particle size analysis at UASB reactor showed the disintegration of larger granules.