UNIVERSITAT DE BARCELONA
DEPARTAMENT D'ENGINYERIA QUÍMICA

Biodegradation of Organic Micropollutants in Themophilic and Mesophilic Anaerobic Digestion of Sewage Sludge

Memoria presentada por

Toufik Benabdallah El-Hadj

Para optar al grado de Doctor por la
Universitat de Barcelona

Barcelona, Junio de 2006

UNIVERSITAT DE BARCELONA
DEPARTAMENT D'ENGINYERIA QUÍMICA

Biodegradation of Organic Micropollutants in Themophilic and Mesophilic Anaerobic Digestion of Sewage Sludge

Memoria presentada por

Toufik Benabdallah El-Hadj

Para optar al grado de Doctor por la
Universitat de Barcelona

Barcelona, Junio de 2006

UNIVERSITAT DE BARCELONA
DEPARTAMENT D'ENGINYERIA QUÍMICA

JOAN MATA ÁLVAREZ, CATEDRATICO DE INGENIERÍA QUÍMICA DEL DEPARTAMENT D'ENGINYERIA QUÍMICA DE LA UNIVERSITAT DE BARCELONA,

CERTIFIQUE: Que TOUFIK BENABDALLAH EL HADJ, alumno del programa de doctorado d'Enginyeria del Medi Ambient i del Producte, ha realizado en los laboratorio de este departamento y bajo mi dirección el trabajo que tiene como título

BIODEGRADATION OF ORGANIC MICROPOLLUTANTS IN THERMOPHILIC AND MESOPHLIC ANAEROBIC DIGESTION OF SEWAGE SLUDGE

presenta en esta memoria que consititue su Tesis Doctoral para optar al grado de Doctor por la Universitat de Barcelona.

Y para que así conste, firma el presente informe en Barcelona , el 10 de Junio del 2006.

Dr. Joan Mata Álvarez

Table of Contents

Abbreviations and symbols………………………………………………………….. AS-1

Tables and figures list…………………………………………………………... TF-1

Resumen…………………………………………………………………..………… R-1

Summary…………………………………………………………………………… S-1

1. Introduction……………………………………………………………………... 1

1.1. Sewage sludge…………………………………………………………… 1

1.1.1. Definition and types of sludge…………………………….. 1

1.1.2. Sludge generation and production………………………... 3

1.1.3. Sludge characteristics…………………………………….. 4

1.1.4. Sludge reuse and disposal………………………………… 9

1.1.5. Anaerobic digestion and sludge stabilization (mesophilic vs. thermophilic conditions)……………………………………... 10

1.2. References……………………………………………………………….. 15

2. Objectives……………………………………………………………………... 19

3. Materials and Methods……………………………………………………. 21

3.1. Experimental set-up…………………………………………………… 21

3.2. Analytical methods……………………………………………………. 22

3.2.1. Conventional parameters………………………………… 22

3.2.2. Organic Micropollutants analysis………………………… 27

3.3. References……………………………………………………………….. 35

4. Start-up and HRT influence in thermophilic and mesophilic anaerobic digesters seeded with waste activated sludge…………………………………….. 39

4.1. Introduction…………………………………………………………… 40

4.1.1. Anaerobic digesters start-up (thermophilic inoculum availability)…………………………………………………… 40

4.2. Materials and Methods……………………………………………... 42

4.2.1. Experimental set-up……………………………………….. 42

4.2.2. Analytical methods………………………………………... 42

4.2.3. Substrate and inoculum…………………………………… 42

4.2.4. Start-up procedure………………………………………... 43

4.2.5. HRT reduction procedure………………………………… 44

4.3. Results and discussion………………………………………………… 44

4.3.1. Start-up experience……………………………………….. 44

4.3.2. HRT reduction…………………………………………….. 46

4.4. Conclusions…………………………………………………………… 52

4.5. References. …………………………………………………………… 53

5. Biodegradation of PAH and DEHP micro-pollutants in mesophilic and thermophilic anaerobic sewage sludge digestion………………………………… 57

5.1. Introduction…………………………………………………………… 58

5.1.1. Polycyclic Aromatic Hydrocarbons (PAH)………………….. 58

5.1.2. Di-2-(Ethyl-Hexyl)-Phthalate (DEHP)……………………… 61

5.2. Materials and methods………………………………………………... 64

5.3. Results and discussion…………………………………………………. 66

5.3.1. PAH biodegradation………………………………………... 66

5.3.2. DEHP biodegradation……………………………………... 70

5.4. Conclusions…… 71
5.5. References…… 72
6. PCB and AOX removal in mesophilic and thermophilic anaerobic sewage sludge digestion…… 75
6.1. Introduction…… 76
6.1.1. AOX…… 76
6.1.2. PCB…… 78
6.2. Materials and methods…… 81
6.2.1. Experimental set-up…… 81
6.2.2. Analytical methods…… 82
6.3. Results and Discussion…… 82
6.3.1. AOX biodegradation…… 82
6.3.2. PCB biodegradation…… 83
6.4. Conclusions…… 87
6.5. References…… 87
7. NPE and LAS removal in mesophlic and thermophilic anaerobic sewage sludge digestion…… 93
7.1. Introduction…… 94
7.1.1. NPE…… 94
7.1.2 LAS…… 98
7.2. Materials and methods…… 103
7.2.1. Experimental set-up…… 103
7.2.2. Analytical methods…… 103
7.3. Results and discussion…… 104
7.3.1. NP/E degradation…… 104
7.3.2. LAS degradation…… 107
7.4. Conclusions…… 111
7.5. References…… 112
8. Effect of ultrasound pretreatment in mesophilic and thermophilic anaerobic digestion with emphasis on naphthalene and pyrene removal 117
8.1. Introduction…… 118
8.2. Materials and methods…… 120
8.2.1. Ultrasonic treatment…… 120
8.2.2. Biodegradability test…… 123
8.2.3. Lab-scale reactors…… 124
8.2.4. Naphthalene and Pyrene analysis…… 125
8.3. Results and discussion. …… 125
8.3.1. Ultrasonic pretreatment…… 125
8.3.2. Biodegradability test …… 128
8.3.3. Anaerobic digestion performance…… 129
8.3.4. PAH removal in ultrasonic pretreatment and anaerobic digestion…… 130
8.4. Conclusions…… 132

8.5. References…………………………………………………………….. 132
9. Conclusions and recommendations……………………………………………. 137
9.1. Conclusions and recommendations…………………………………….... 137
9.2. Summary Tables...…………………………………………………….. 146
Annexes……………………………………………………………………….. A-1
Annex Tables and annex Figures list.…………………………………....... A-1
Annex I for Chapter 4…………………………………………………… A-3
Annex II for Chapter 5………………………………………………….. A-11
Annex III for Chapter 6…………………………………………………. A-17
Annex IV for Chapter 7…………………………………………………. A-17

Abbreviations and Symbols

Abbreviations and Symbols

AD	Anaerobic Digestion
AGV	Ácido Grasos Volátiles
Alk	Alkalinity (mg $CaCO_3$/L)
AOX	Adsorbed Organ Halogen compounds
AOX	Compuestos organohalogenos adsorbidos
C10_LAS	LAS con el alquilo lineal de 10 carbonos
C11_LAS	LAS con el alquilo lineal de 11 carbonos
C12_LAS	LAS con el alquilo lineal de 12 carbonos
C13_LAS	LAS con el alquilo lineal de 13 carbonos
C_{18}	Octadecylsilica
CAPEC	Alkylphenol Dicarboxylates
COD	Chemical Oxygen Demand (g COD/L)
COD_{NaOH}	CODs obtained by alkaline hydrolysis
CODp	Particulated COD
COD_r	COD removal (%)
CODs	Soluble COD (g COD/L)
CODt	Total COD (g COD/L)
d	Days
d	Días
DD_{COD}	Degree of Disintegration (%)
DEHP	Di(2-ethylHexyl)Phthalate
DEHP	Di(2-etilohexilo)ftalato
dm	Dry matter
DQO	Demanda Química de Oxígeno
DQO_a	Demanda Química de Oxígeno agregada
DQO_e	Demanda Química de Oxígeno eliminada (%)
dw	Dry Weight
EE	Energetic Excess (kJ/L)
E_s	Specific Energy
EU-PAH	PAHs, which their content in the sludge proposed to be regulated in EU
FCU	Forming Colonies Units
FID	Flame ionisation Detector
GC	Gas Chromatography
HAc	Acetic Acid
HCB	Highly Chlorinated Biphenyls
HMWPAH	High Molecule Weight PAH
HPC	Hydroxyphenylcarboxylic acids
HPLC	High Performance Liquid Chromatography
HRT	Hydraulic Retention Time (d)
K_{oc}	Organic carbon (adsorption) Coefficient
K_{ow}	Octanol/Water Coefficient
LAA	Lodo Aerobio activado
LAS	Linear Alkylbenzene Sulfonates
LAS	Sulfanatos del alquilobenceno lineal
LCB	Lightly Chlorinated Biphenyls
LD	Lodos de Depuradora
LMWPAH	Low Molecule Weight PAH

L_r	Litre of reactor (L)
M%	Metanization yield (%)
m/z	Mass-to-charge ratio
MPN	Most Probable Number
MS	Mass Spectrometry
ms	materia seca
NH_4^+-N	Ammonia Nitrogen
NP	Nonilfenol
NP	Nonylphenol
NP1EO	Nonilfenolmonoetoxilado
NP1EO	Nonylphenol monoethoxyalte
NP2EO	Nonilfenoldietoxilados
NP2EO	Nonylpheno diethoxyaltes
NPE	Nonilfenol, Nonilfenolmonoetoxilado y Nonilfenoldietoxilados
NPE	Sum of NP, NP1EO and NP2EO
NPEC	Nonylphenol Carboxylates
NPEO	Nonilfenolethoxilados
NPEO	Nonylphenol polyethoxylates
PAE	Phthalate Acid Ester
PAH	Hidrocarbonos policíclicos aromáticos
PAH	Polynuclear Aromatic Hydrocarbons
PAH-AB	PAH con efectos abióticos
PAH-APM	PAH de Alto Peso Molecular
PAH-BPM	PAH de Bajo Peso Molecular
PAH-EU	PAHs seleccionados en 3^{er} borrador de la directiva Europea para controlar su contenido
PCA	Phenylcarboxylic acids
PCB	Bifenilos policlorados
PCB	PolyChlorinated Biphenyls
PCB-AC	PCB altamente clorados
PCB-LC	PCB ligeramente clorados
PCDD/F	Dibenzodioxinas/ furans policlorados
PCDD/F	PolyChloroDibenzoDioxins/Furans
PEB	Producción Especifica de Biogás (mL/ g)
PNLD	Plan Nacional de Lodos de Depuradoras
RI	Recovery Index
RSD	Relative Standard Deviation (%)
RSS	Raw Sewage Sludge
SBP	Specific Biogas Production (mL biogas/g VS or mL biogas/g COD)
S_{COD}	COD Solubilization (%)
SD	Standard Deviation
SIM	Selection Ion Mode
SMP	Specific Methane Production (mL CH_4/g VS or mL CH_4/ g COD)
SPC	SulfoPhenil Carboxylate
SRT	Solid Retention Time
ST	Sólidos Totales
STP	Sewage Treatment Plant
SV	Sólidos Vólatiles
SV_a	Sólidos Volátiles agregados
SV_e	Sólidos Volátiles eliminados (%)

TA	Total Acidity (mg HAc/L)
TCD	Thermal Conductivity Detector
TRH	Tiempo de Retención Hidráulico (días)
TS	Total Solid
TSS	Total Suspended Solids
UV	Ultraviolet
VFA	Volatile Fatty Acids
VS	Volatile Solids
VS_f	VS fed (g VS)
VS_r	VS removal (%)
VSS	Volatile suspended Solids
WAS	Waste activated sludge
WWTP	Wastewater Treatment Plant
X_{VS_s}	VS solubility improvement (%)

Tables and Figures List

Tables List

Resumen

Tabla R.1. Parámetros operacionales de la eficiencia del digestor mesofílico
Tabla R.2. Parámetros operacionales de la eficiencia del digestor termofílico
Tabla R.3. Parámetros operacionales de los digestores sin el pretratamiento del ultrasonido
Tabla R.4. Parámetros operacionales de los digestores durante el uso del ultrasonido como pretratamiento
Tabla R.5. Eliminación de naftaleno y pireno (%) bajo diferentes tratamientos aplicados
Table R.6. La mejora (%) en los parámetros operacionales y en la eliminación de naftaleno y pireno al suministrar sustrato sonicado en los digestores mesofílico y termofílico

Chapter 1

Table 1.1. 1998 sludge production and 2005 predicted sludge generation in SpAin (Plan Nacional de LD-EDAR 2001-2006; BOE 166, 2001)
Table 1.2. Draft Directive (2000) setting **cut-off limits** for sludge contaminants (CEC, 2000)
Table 1.3. Dioxins Toxic Equivalent Factors (TEF) and relacionated compounds (Eljarrat et al., 2002)
Table 1.4. Catalonian sludge survey vs. the cut-off limits contaminants (Ortiz S., 2004. ACA)
Table 1.5. Sludge classes and Pathogen reduction
Table 1.6. Operational conditions and yields of mesophilic and thermophlic digesters traiting sewage sludge

Chapter 3

Table 3.1. typical retention time and factors calibration of Acid standards mixture
Table 3.3. LAS homologues separation condition in liquid chromatography

Chapter 4

Table 4.1. Waste activated sludge (WAS) and raw sewage sludge (RSS) characterisation
Table 4.2. Synthetic and real RSS feed mixture composition in the second step of the start-up

Chapter 5

Table 5.1. PAH degradation rates (mg/kg.d) under various reduction conditions (Chang et al., 2003)
Table 5.2. DEHP phyisico-chemical data (CEC, 2000)
Table 5.3. DEHP content in the WWTP sludge (mg/kg dm)
Table 5.4. Ratio of PAHs recovery in the seconds fraction (%)
Table 5.5. PAHs identification (typical retention time)
Table 5.6. Recovery indexes and RSD for PAH mixture and DEHP analysis (%)
Table 5.7. Inlet and outlet sludge PAH content at different conditions (mg/kg dw)
Table 5.8. LMW PAH content in the total PAH content in sludge (%)
Table 5.9. Meatabolites resulting from anaerobic degradation of PAH

Chapter 6

Table 6.1. Characteristics of some PCB congeners (Gusev et al., 2005)
Table 6.2. Arcolor 1260 biodegradtion in anaerobic-aerobic sequencing treatment (Master et al., 2002).
Table 6.3. Dechlorination rate of sludge under anaerobic conditions (Chang et al., 1999).
Table 6.4. PCB identification at GC-MS analysis .
Table 6.4. PCB content in the fresh and the treated sludge (mg/kg dw) at the studied HRTs.
Table 6.5. PCB content composition according to chlorination level in fresh and digested anaerobic sludge under thermophilic and mesophilic conditions.

Chapter 7

Table 7.1. Methane production from NP1-2EO (2 mg/l) incubation under anaerobic condition (Ejlertsson et al., 1999)

Table 7.2. Effects of temperature incubation factors on NP anaerobic degradation rate constants (k) and half-lives ($t_{1/2}$) in the sludge samples (Chang et al., 2005)
Table 7.3. stream waste LAS concetration in some Eropean contries (CEC, 2000).
Table 7.4. LAS content in sewage sludge in some European countries and USA (mg/kg dw) (CEC, 2000).
Table 7.5. Sludge LAS content (mg/kg dw) in Spain
Table 7.6. NPE identification in GC/MS analysis.
Table 7.7. Portion of recuperated NP/NPEOs in the third fraction relatively to the total amount recuperated.
Table 7.8. Recovery grade (RI) and relative Standard deviation (RSD) from NP analysis at tested HRTs.

Chapter 8

Table 8.1. Pretreatment advantages and disadvantages (Weemaes and verstraete, 1998).
Table 8.2. Average feed and digested sludge characteristics and operational parameters.
Table 8.3. PAH compounds identification at GC/MS analysis.
Table 8.4. Average treated sludge characteristics and operational parameters with combined ultrasonic and anaerobic digestion treatment.
Table 8.5. Average naphthalene and pyrene content in sludge samples within the non-sonicated substrate feeding period and within the pretreated substrate feeding period.

Chapter 9

Table 9.1. Yield parameters values in the mesophilic digester
Table 9.2. Yield parameters values in the thermophilic digester
Table 9.3. Operational parameters of anaerobic digestion without ultrasonic pretreatment
Table 9.4. Operational parameters with combined ultrasonic and anaerobic digestion treatment
Table 9.5. Naphthalene and pyrene removal (%) under the different applied treatments
Table 9.6. Improvement (%) in operational yield and naphthalene and pyrene removal when using sonicated substrate *in the mesophilic and thermophilic digester*

Figures List

Chapter 1

Figure 1.1. Generation, treatment, use, and disposal of sewage sludge (USEPA, 1999)
Figure 1.2. Flow diagram of Gavá wastewater treatment plant (primary and seconadry treatments).
Figure 1.3. Typical sludge organic micropollutant content(CEC, 2000).
Figure 1.4. Organic micropollutants octanol-water partition coefficient (K_{ow}). Log K_{ow} < 2.5: low sorption potential, Log K_{ow} > 2.5 and < 4.0: medium sorption potential and Log K_{ow}> 4.0: high sorption potential. (CEC, 2000).
Figure 1.5. WWTPs sludge destination, (a)in EU, (b) 1998 state in Spain and (c) 2005 predicted in Spain. (BOE 166, 2001).
Figure 1.6. Complex molecules reaction sequence during anaerobic digestion (van Haandel and Lettinga, 1994).
Figure 1.7. Methanogenics growth temperature dependence (van Lier et al., 1997).

Chapter 3

Figure 3.1. lab-scale anaerobic digestion installation.
Figure 3.2. Biogas recollection (a) and boigas measuring device(b).
Figure 3.3. (a) Soxlet extraction and (b) solid phase extraction dispositives.
Figure 3.4. Scheme of PAH, DEHP, PCB and NPE analysis procedure.
Figure 3.5. Scheme of LAS sludge analysis procedure.
Figure 3.6. Scheme of AOX analysis [DIN 38414-S18 (modified)].

Chapter 4

Figure 4.1. Biogas production within synthetic substrate feeding step in (a) thermophilic and (b) mesophilic digester. (AC: acetate feeding; GLUC: glucose feeding).
Figure 4.2. Biogas production per unit organic matter and methane composition in the (a) thermophilic and (b) mesophilic digester within RSS substitution feed step
(■ mL biogas/g CODt, ♦ mL biogas/g CODs, × methane content (%))
Figure 4.3. Biogas production per organic matter unit added (VS_f) and methane content in both digesters at HRT reduction period. (□ Mesophilic specific biogas production; ■ thermophilic specific biogas production; Δ Methane biogas content in the mesophilic digester; ○ methane biogas content in the thermophilic digester).
Figure 4.4. Evolution of volatile solids removal (%) at each HRT. (□Mesophilic digester; ■ thermophilic digester).
Figure 4.5. Total VFAs amount and Acetic and Propionic acids content in the thermophilic digester effluent. (□Total VFA; ▲ Acetic Acid; ж Propionic Acid).
Figure 4.6. pH evolution in the mesophilic (□) and the thermophilic (■) digester at HRT reduction period
Figure 4.7. Ammonia nitrogen evolution in the mesophilic (□) and the thermophilic (■) digester at HRT reduction period.
Figure 4.8. Methanization yield (M%) in the mesophilic (□) and the thermophilic (■) digester at HRT reduction period.

Chapter 5

Figure 5.1. PAHs chemical structures which their sum content in the sludge proposed to be regulate in EU, EU-PAH (CEC, 2000).
Figure 5.2. Thermodynamics of naphthalene degradation in anaerobic digester at 25 ºC, with naphthalene at saturation concentration, and 1 mM CO_2. Shared regions show simultaneous decalate (C_{10}) oxidation, and hydrogen conversion are possible (Christensen et al., 2004).
Figure 5.3. Naphthalene removal as a function of temperature in enriched samples under anaerobic digester (Christensen et al., 2004)
Figure 5.4. DEHP structure formula.
Figure 5.5. Structural formula of some compounds related with anaerobic phthalate biodegradation (Kleerebezem et al., 1999)
Figure 5.6. Proposed pathways for anaerobic biodegradation of dimethylterephthalate

Figure 5.7. Anaerobic removal efficiencies of (a) total PAH, (b) HMWPAH, (c) LMW PAH and (c) PAH with abiotic characters (phenanthrene and fluorene) under thermophilic(■) and mesophilic(□) conditions at the tested HRTs.

Figure 5.8. Proposed activation reaction for naphthalene by addition of CO_2 to generate 2-naphthoic acid

Figure 5.9. Proposed scheme of the upper pathway of anaerobic 2-methylnaphthalene degradation (1*) to the central intermediate 2-naphthoic acid (8*), (2*) fumaric acid, (3*) naphthyl-2-methyl-succinic acid, (4) naphthyl-2-methyl-succinyl-CoA, (5*) naphthyl-2-methylene-succinyl-CoA, (6) naphthyl-2-hydroxymethyl-succinyl CoA, (7) naphthyl-2-oxomethyl-succinyl-CoA. Compounds marked with (*) have been identified as pure substance or free acids.

Figure 5.10. Proposed reductive 2-naphthoic acid pathway of anaerobic PAH degradation. (I) 2-naphthoic acid, (II) 5,6,7,8-tetrahydro- 2-naphthoic acid, (III) hydroxydecahydro-2-naphthoic acid, (IV) b-oxo-decahydro-2-naphthoic acid, (V) $C_{11}H_{16}O_4$-diacid, (VI) 2- carboxycyclohexylacetic acid

Figure 5.11. DEHP content in the fresh (▨), thermophilic (■) and mesophilic (□) sludge at the tested HRTs.

Figure 5.12. DEHP removal efficiencies at the tested HRTs under thermophilic (■) and mesophilic (□) conditions.

Chapter 6

Figure 6.1. Reductive dehalogenation in anaerobic systems (Magnuson et al., 2000).

Figure 6.2. Chemical structure and correspondent nomencalture of PCBs proposed to be regulate their content in sludge destined to agricultural use.

Figure 6.3. Proposed pathway for anaerobic dechlorination of 2,3,4,5,6-CB by anaerobic microbiological granules. Chlorine removal was observed in the order meta, ortho and parafollowed by ortho dechlorination (Natarajan et al., 1996)

Figure 6.4. AOX content in the fresh sludge (▨), thermophilic (■) and mesophilic (□) treated sludge and AOX removal efficiency (%) in the thermophilic (-●-) and mesophilic (-○-) digester at the tested HRTs.

Figure 6.5. Total PCB (a), LCB (b) and HCB (c) removal efficiencies (%) in the thermophilic(■) and mesophilic (□) digester at the applied HRTs.

Figure 6.6. Removal efficiencies (%) of PCB 28 (a), PCB 52 (b), PCB 101 (c), PCB 138 (d), PCB 153 (e) and PCB 180 (f) in the thermophilic (■) and mesophilic(□) digester at the tested HRTs.

Chapter 7

Figure 7.1. NP and NPEO chemical structure.

Figure 7.2. NP content in influent and effluent of catalonian WWTP during 1998-2003 period (Barceló and Petrovic, 2004).

Figure 7.3. NP content in sludge (CEC, 2000)

Figure 7.4. Structures of nonylphenol ethoxylates (NPEO) and their metabolites, including nonylphenol (NP), nonylphenol carboxylates (NPEC), and alkylphenol dicarboxylates (CAPEC). Numbers in parentheses denote the range in ethoxy chain lengths. Microbial biotransformation pathways, including ethoxy chain shortening (solid lines) and oxidation (dashed lines) (Ferguson and Brownawall, 2003)

Figure 7.5. LAS chemical structure

Figure 7.6. Anaerobic LAS biodegradation pathways. LAS: linera alkybezensulfonate, SPC: Sulfophenylcarboxylic acids, HPC: Hydroxyphenylcarboxylic acids, PCA: Phenylcarboxylic acids (Sarrazin et al., 1997)

Figure 7.7. Schematic representation of the proposed degradation route of dodecylbenzene sulfonates (A) and 4-hydroxyphenylpropionate (B) by P. aeruginosa W51D. TCA, tricarboxylic acid cycle (Campos-García et al., 1999).

Figure 7.8. Fresh (▨), thermophilic (■) and mesophilic (□) sludge content and the removal efficiency in the thermophilic (-Δ-) and the mesophilic (-○-) digesters of (a) total NPE, (b) NP, (c) NP1EO and (d) NP2EO at tested HRTs.

Figure 7.9. Total LAS amount in fresh Bulk Feed (▨), Thermophilic (■) and Mesophilic (□) anaerobic Bulk effluent and the removal efficiency in the thermophilic (-Δ-) and the mesophilic (-○-) digesters at the HRTs applied.

Figure 7.10. Total LAS amount in fresh supernatant feed (▨) Thermophilic (■) and Mesophilic (□) anaerobic supernatant effluent at tested HRTs.

Figure 7.11. Proportional weight distribution (%) in liquid phase of LAS homologues (a) C10, (b) C11, (c) C12 and (d) C13 in the fresh feed (▨), Thermophilic (■) and Mesophilic(□) anaerobic effluent at the tested HRTs.
Figure 712. Total LAS amount in dried matter of fresh Feed (■) Thermophilic (□) and Mesophilic (▨) anaerobic effluent and the removal efficiency in the thermophilic (-Δ-) and the mesophilic (-○-) digesters at tested HRTs.
Figure 7.13. Content of LAS homologues [C10 (a), C11 (b), C12 (c) and C13 (d)] in dried sludge of fresh Feed (▨), Thermophilic (■) and Mesophilic (□) anaerobic effluent and the removal efficiency in the thermophilic (-Δ-) and the mesophilic (-○-) digesters at tested HRTs.

Chapter 8

Figure 8.1. Model of sludge desintegration with increasing ultrasonic specific energy.
Figure 8.2. Proposed mechanisms causing enhanced PAH bioavailability (Tiehm, 1999).
Figure 8.3. SONOPLUS BANDELIN (Ultrasound apparatus).
Figure 8.4. Biodegradability test installation.
Figure 8.5. Particles size distribution for the selected specific energies applied.
Figure 8.6. Effect of ultrasound E_S on aqueous phase turbidity.
Figure 8.7. Effect of ultrasonic pretreatment on TS (■) and VS (□) concentration and TS (▲) and VS (Δ) solubilization yield at Es doses tested.
Figure 8.8. Solubilization (■) and degree of disintegration (●) of COD at the tested specific energies.
Figure 8.9. Methane accumulated volume in the biodegradability test under (a) mesophilic and (b) thermophilic conditions. ((○) Blank; (Δ) Es = 0 kJ/kg TS; (■) Es = 5000 kJ/kg TS; (◊) Es = 8000 kJ/kg TS; (▲) Es = 11000 kJ/kg TS; (●) Es = 15000 kJ/kg TS).
Figure 8.10. Improvement in biogas production, COD removed and specific biogas production when using pretreated sludge in the mesophilic (□) and the thermophilic (■) digestion.
Figure 8.11. Naphthalene (□) and pyrene (■) removal in dry matter sludge after the studied treatments. (Son+M_dig: combined sonication and mesophilic anaerobic digestion treatment;
Son+T_dig: combined sonication and thermophilic anaerobic digestion treatment).

Resumen

Resumen

Durante las últimas décadas, la estrategia del desarrollo de los tratamientos de aguas residuales se ha caracterizado por dos aspectos. El primero es el esfuerzo continuo para mejorar la calidad del efluente modernizando las plantas de tratamiento existentes así como el diseño y la instalación de nuevos procesos de tratamiento más eficaces. El segundo aspecto es el hecho preocupante del incremento de los lodos residuales en las estaciones depuradoras de aguas residuales (EDAR) y los problemas asociados. Estos problemas son el incremento permanente de la producción de los lodos, el alto coste del tratamiento de los fangos y los riesgos que pueden representar para el medio ambiente y el ser humano. Por otra parte, la aplicación de los lodos como abono a los suelos agrícola está regulada por legislaciones cada vez más restrictivas.

Acondicionar las tierras con fangos puede suponer una introducción excesiva o desequilibrada de nutrientes, metales pesados, contaminantes orgánicos y patógenos que pueden afectar negativamente el medio y sus elementos. Si la carga patogénica puede reducirse por debajo de los lindares mínimos de salubridad con un tratamiento térmico o alcalino entre otros, los demás tipos de contaminantes requieren en muchos casos tratamientos específicos dependiendo de los elementos o los compuestos presentes.

La mejora de la calidad de los fangos en términos de metales pesados puede llevarse a cabo mediante tres vías: (1) control de la fuente generadora (prevención de la descarga de los contaminantes a las corrientes residuales); (2) la remoción de partículas coloidales y suspendidas como etapa primaria de tratamiento; y/o (3) la remoción de los metales pesados de los lodos con la lixiviación química (con ácidos orgánicos e inorgánicos) o con agentes de coagulación o con la lixiviación microbiológica.

La reducción de la contaminación orgánica acostumbra a ser lo que más complicación conlleva. Esto es debido a que, bajo este tipo de contaminación, se puede incluir todo tipo de compuestos sintéticos o naturales, que pueden ser utilizados o incorporados voluntaria o involuntariamente, tales como productos de limpieza, de desinfección, fármacos, pinturas, hidrocarburos, etc. Estos productos son de uso habitual y cotidiano tanto en los hogares como en los lugares públicos.

Los compuestos orgánicos que pueden suponer un riesgo al medio ambiente comprenden numerosas clases químicas, con estructuras muy variadas. Muchos de ellos son altamente bioactivos, a menudo con múltiples grupos funcionales. En este trabajo se ha optado por los microcontaminantes listados en el 3er borrador presentado a la comisión del medioambiente de la Unión Europea (CEC, 2000). La mayoría de los compuestos citados para limitar su concentración en los lodos (hidrocarburos aromáticos policíclicos (PAHs); dietilhexilftalatos (DEHP); bifenilos policlorados (PCBs); organohalogenados adsorbidos (AOX); los nonilfenoles y nonilfenoles etoxilados (NPE); alquilobencenosulfonatos lineales (LAS)) se encuentran a niveles bajos (mg/kg) lo cual dificulta su detección mediante técnicas analíticas convencionales y complica su monitorización en el medio. Debido a su alto índice de hidrofobicidad y/o lipofilicidad, estos compuestos son bioacumulables en la materia sólida y, especialmente, en los fangos por su alto contenido en materia orgánica afín a estas sustancias.

La mayoría de estos compuestos, bien por arrastre de aguas y escorrentías, o porque son deshechos de un proceso industrial, entran en el medio normalmente en las Estaciones de Depuración de Aguas Residuales (EDARs). En este sentido, las depuradoras actuales se han diseñado para ser herramientas muy efectivas en lo que respecta a tratar los problemas relacionados con la contaminación carbonada, nitrogenada y microbiana. Sin embargo, especialmente en las zonas urbanas, las aguas residuales pueden contener una multitud de compuestos sintéticos y naturales que no han sido considerados en el diseño y operación de los procesos de depuración, resultando en eliminaciones (transformaciones) parciales y permitiendo finalmente la llegada de estos compuestos a los diferentes compartimientos ambientales receptores (agua, aire y suelo). La eficacia de la eliminación en las EDARs varía en función de la estructura y concentración de los microcontaminantes orgánicos y del tipo de tratamiento empleado.

El hecho de que los contaminantes orgánicos puedan entrar continuamente en el medioambiente, les confiere la característica de “persistentes”, ya que aunque a veces tuviesen una baja estabilidad ambiental, su posible eliminación/transformación (mediante biodegradación, hidrólisis, fotolisis, etc.) está continuamente contrarrestada por su reposición.

La posibilidad de efectos continuados pero indetectables o imperceptibles durante su largo ciclo de vida sobre elementos del medioambiente, hace más que necesaria la actuación inmediata.

Para reducir los riesgos de los efectos impredecibles sobre el medioambiente y la salud humana y evitar la introducción en las cadenas vitales dichos compuestos, el impulso de nuevas vías tecnológicas y la adopción de una visión global del problema serán el camino idóneo para afrontar a estas situaciones y otros posibles escenarios en el futuro.

En esta dirección, este trabajo se ha desarrollado para estimar los contenidos de estos contaminantes en los lodos de depuradora de una área metropolitana como Barcelona, perfeccionando métodos analíticos para su determinación en compartimientos sólidos, como son los fangos, y valorando la influencia del uso de un tratamiento avanzado como es la digestión anaerobia sobre los microcontaminantes orgánicos y la estabilización de los fangos en general.

Para la consecución de estos objetivos se ha optado por un estudio comparativo entre la digestión anaerobia bajo las condiciones mesófilas y termófilas persiguiendo los siguientes objetivos concretos:

i) Estudiar la puesta en marcha de los digestores anaerobios, prestando una atención especial al digestor termofílico.

ii) Comparar los parámetros operacionales (el contenido en materia orgánica expresada como sólidos volátiles (SV) o demanda química de oxígeno (DQO), producción y composición de biogás, los ácidos grasos volátiles (AGV), acidez, alcalinidad, pH, nitrógeno amoniacal $N\text{-}NH_4^+$) estimando la eficiencia de cada digestor en términos convencionales (reducción de materia orgánica (SV y DQO) y producción específica de biogás por unidad de materia orgánica agregada o eliminada y el contenido del biogás en metano) durante la reducción del Tiempo de Retención Hidráulico (TRH).

iii) Reportar los rendimientos de ambos digestores en función de la eliminación o biotransformación de los microcontaminantes orgánicos.

iv) Estudiar la aplicación de ultrasonidos como pretratamiento de la digestión anaerobia en ambos rangos de temperatura y observar de su efecto sobre los rendimientos en términos convencionales y sobre la reducción del contenido de naftaleno y pireno en los lodos.

Para ello, este trabajo se ha estructurado de acuerdo con los siguientes capítulos:

El ***Capítulo 1*** ha servido como una introducción bibliográfica donde se ha descrito los tipos de los lodos, su generación, sus características generales, sus rutas de disposición y el papel de la digestión anaerobia.

En el ***Capítulo 3***, se han detallado los métodos analíticos utilizados en este estudio, diferenciando los convencionales (alcalinidad, acidez, producción y composición del biogás, materia orgánica, nitrógeno amoniacal, pH, sólidos y ácidos grasos volátiles (AGV)) de los específicos para los análisis de los microcontaminantes orgánicos (PAHs, DEHP, PCBs, NPE, AOX y LASs) que se han puesto a punto durante este trabajo.

En el ***Capítulo 4***, se ha estudiado el arranque de los digestores, utilizando Lodo Activado Aerobio (LAA) y empleando sustrato sintético de fácil degradación biológica como es el acetato y la glucosa. La sustitución gradual de la glucosa con la mezcla de lodos, ha permitido alcanzar el estado estacionario tras 60 y 85 días, registrando 35 y 30 días como TRH en el digestor mesofílico y el termofílico, respectivamente.

Tras arrancar los digestores, se ha procedido a la reducción del TRH. Aumentando la carga orgánica diaria, y manteniendo la concentración de los sólidos en la mezcla de lodos alrededor de 40 g/L, se ha podido alcanzar un TRH de 8 d como el mínimo en el reactor termofílico, mientras se ha optado por no seguir con el proceso de reducción de TRH en el mesofílico a partir del TRH de 18 d, debido a las problemas de acidificación en estas últimas condiciones.

La reducción de la atracción de vectores (roedores, insectos, etc.) se ha llevado a cabo con casi el mismo grado en ambos digestores, rebajando la parte de los sólidos volátiles (SV) respecto al contenido de los sólidos totales (ST) de más del 75% a menos del 50%.

Por otra parte, la reducción de la materia orgánica ha sido, en general, ligeramente superior al 50% en el mesodigestor, mientras que ha permanecido en alrededor del 50% en el termodigestor, durante el periodo de la reducción del TRH. Esto es debido a la alta tasa de eliminación de la materia orgánica a temperaturas en el rango termofílico y su efecto tan marcado que ha enmascarado en cierto grado el efecto de TRH en términos de eliminación de la materia orgánica. Asimismo, la pérdida en la eficiencia de la eliminación de materia orgánica respecto al término de la DQO ha sido relativamente mayor en el mesodigestor (pasando de 59% a 47%), al rebajar el TRH de 35 a 18 d, que en el termodigestor (pasando de 60% a 55%), al rebajar el TRH de 30 a 8 d.

Por otro lado, la Producción Especifica de Biogás (PEB) respecto a los sólidos volátiles agregados (SV_a) ha oscilado entre 446 y 295 mL/g SV_a entre los TRHs de 35 y 18d en el mesodigestor, y entre 502 y 289 mL/g SV_a entre los TRHs de 30 y 8d en el termodigestor. Asimismo, se puede constatar que los valores de PEB observados en este estudio son similares a los registrados por Cecchi y Traverso (1986) [350 mL/g SV_a en el termofilico con carga orgánica de 1,6 kg SV_a/L y tiempo de retención celular equivale a 33d], Rimkus et al. (1982) [320 mL/g SV_a a TRH de 17d en el mesofilico y 400 mL/g SV_a a TRH de 11,3d en el termofílico] y Speece (1988) [250-500 mL/g SV_a en el termofílico].

El efecto de la reducción de TRH ha sido aún más pronunciado sobre el contenido de metano del biogás generado bajo las condiciones termófilas, ya que se ha rebajado desde el 72% (TRH de 30d) hasta casi el 50% (a TRHs $\leq$ 11d). Por otro lado, el digestor mesofílico ha generado, siempre, un biogás con un alto contenido de metano (alrededor o superior al 70%) dentro del periodo de estabilidad. Esto debido al lento crecimiento de las metanogénas y la necesidad de un tiempo suficiente para el desarrollo óptimo de su actividad.

Además de los parámetros relacionados con el biogás, el contenido de los AGV en los digestores anaerobios se considera un indicador idóneo de su estabilidad, sobretodo con la metodología del presente trabajo donde al reducir el TRH, la sobrecarga puede provocar un estado de inhibición. Como era de esperar, el recorte del TRH ha inducido a un aumento de la concentración de los AGV. El funcionamiento estable del mesodigestor se ha producido hasta concentración de 414 mg AGV/L. El aumento de la carga orgánica diaria del termodigestor ha conducido a la acumulación sostenida de los AGV hasta los 1398 mg/L, con un importante incremento en el contenido de los ácidos acético y propiónico (494 y 335 mg/L, respectivamente). Más allá de estos valores, se han notado sensibles alteraciones en el digestor termofílico.

Desde la estimación de la tasa de metanización (M%) de la materia orgánica introducida se ha podido observar un descenso pronunciado en los valores de este término al rebajar el TRH. Los bajos TRHs (menores o iguales a 11d) han conducido a una conversión metanogénica de la materia orgánica agregada inferior al 30%. Esto es debido a la baja eficiencia de degradación registrada a TRHs cortos y al importante descenso del contenido del metano en el biogás.

En el ***Capítulo 5***, se ha analizado la evolución de los PAH y el DEHP tras el tratamiento anaerobio. Para los PAHs, se ha observado una alta eficiencia de eliminación (50-65%) en el rango termofílico respecto a la digestión anaerobia en el rango mesofílico (38-44%). El recorte del TRH ha influido negativamente en la eliminación de los PAH para ambos rangos de temperatura, ya que el promedio de los valores de eficiencia de eliminación descendió del 70% al 52% en el rango termofílico, y desde el 56 al 52% en el rango mesofílico de temperatura. Los resultados obtenidos muestran una alta eliminación para los PAH de Bajo Peso Molecular (BPM) respecto al de Alto Peso Molecular (APM) debido, probablemente, al bajo índice de hidrofobicidad y su facil asimilación por el consorcio anaerobio. Por otra parte, los PAH fluorescentes (fenantreno, fluoreno y antraceno) se han eliminado con un promedio superior al registrado con los PAH de BPM. Esta diferencia en la eficiencia de eliminación ha sido mayor en el rango termofílico. Esto se debe a la acentuación de los efectos abióticos con el aumento de la temperatura.

Para el dietilhexilftalatos (DEHP), se ha observado el no cumplimiento en los fangos generados de las condiciones de salubridad propuestas en el tercer borrador propuesto por la Comisión Europea. Por otro lado, la digestión anaerobia bajo condiciones termófilas ha resultado más eficiente al reducir el contenido del DEHP (45,4-46,7%) respecto a la digestión anaerobia mesofílica (21,7-37,8%) aplicando los mismos TRHs (18, 22 y 26d). La reducción de TRH a 8 y 12d ha afectado negativamente la eficiencia del termodigestor disminuyendo la remoción del DEHP hasta el 31,7-32,3%, lo mismo se ha observado en el mesodigestor donde la eliminación de DEHP ha descendido desde 37,8 a 27,7 y 21,7 al bajar el TRH desde 26 a 22 y 18d, respectivamente.

En el ***Capítulo 6*** se ha estudiado la biodegradación y/o biotransformación de los compuestos halogenados expresados como AOX y PCB bajo las condiciones anaerobias.

Tras los análisis de las muestras frescas y digeridas, se ha podido estimar que la eficiencia de eliminación de los organohalogenos adsorbidos (AOX) en el rango termofílico ha sido mayor (40,4-50,3%) que en el mesofílico (30,2-43,1%). Las concentraciones de entrada y los rendimientos de los digestores han permitido generar un lodo anaerobio con contenido de AOX por debajo del lindar máximo (500 mg/kg ms) permitido en la tercera propuesta. Por otra parte, en el digestor mesofílico, se ha podido notar un efecto negativo del recorte del TRH. Sin embargo, este efecto no ha sido observado bajo las condiciones termófilas.

Para los 6 congéneres de PCBs, se ha registrado una concentración en el rango 1,6-2,9 mg/kg ms en el fango fresco. La aplicación del tratamiento anaerobio ha permitido reducir el contenido inicial en un 32,0-58,4% y 59,4-83,5% bajo las condiciones mesófilas y termófilas, respectivamente. Lo que ha conducido a concentraciones en el rango de 0,65-1,61 mg/kg ms en el lodo mesofílico y en el rango de 0,26-0,77 mg/kg ms en el lodo termofílico, teniendo en cuenta que 0,8 mg/kg ms es la máxima concentración propuesta para los lodos destinados al uso agrícola.

Por otro lado, el efecto de TRH ha sido notable, registrando las máximas eficiencias a altos TRHs y bajos rendimientos a TRHs cortos en ambos rangos de temperatura.

Por otra parte, se ha podido diferenciar dos categorías de congéneres de PCB. La primera categoría la constituyen los PCBs ligeramente clorados (LCB) con 3 mientras que 4 átomos de Cl y la segunda categoría incluye los PCBs altamente clorados (ACB) con más de 4 átomos de Cl. La aplicación de la digestión anaerobia ha reducido en mayor grado el contenido de los ACBs [43,3-69,3% (condiciones mesófilas) y 79,2-94,8% (condiciones termófilas)] en relación con los LCBs [-39,1- -4,7% (condiciones mesófilas) y 7,3-18,4% (condiciones termófilas)]. Esto se debe a la alta velocidad de biodegradación/biotransformación de los ACB y la inhibición o baja velocidad de la eliminación de los LCB causando su acumulación y que se ha reflejado por los valores negativos. Asimismo, se ha constatado que los altos rendimientos registrados para la eliminación de los PCB son debidos principalmente a la eficiencia de la digestión anaerobia en eliminar o transformar los ACBs de una parte y al alto contenido del fango fresco en ACBs, por otra parte. La acumulación de una importante cantidad de LCBs en el fango mesofílico conduce a pensar que la degradación de PCBs sigue la decloración reductiva como mecanismo de biotransformación.

En el ***Capítulo 7***, se ha analizado el comportamiento de los detergentes noiónicos (NPE) e iónicos (LAS) bajo las condiciones anaerobias.

Para los nonilfenoles y nonilfenoles etoxilados (NPE), los contenidos de los fangos analizados han superado ampliamente el límite propuesto (50 mg/kg ms) para su uso en los suelos agrícolas. El fango fresco ha tenido una concentración que oscila entre 1097 y 2100 mg/kg ms. La digestión anaerobia ha permitido reducir el contenido de NPE a 773-1369 mg/kg ms en el rango termofílico y 1013-1827 mg/kg ms en el rango mesofílico. De hecho, la eficiencia de eliminación de NPE ha sido relativamente mayor bajo las condiciones termófilas (9,4-34,8%) respecto en condiciones mesófilas (7,7-21,9%). Debido a la alta hidrofobicidad del NP, su grado de eliminación y/o biotransformación ha sido el más reducido en comparación con los nonilfenoles mono y dietoxilados (NP1EO y NP2EO). Por otra parte, la alta cantidad de NP en relación de NP1EO y NP2EO ha influido negativamente en la eficiencia de la eliminación total de NPE. El efecto del TRH se ha detectado en el rango termofílico diferenciando altos TRHs (26, 22 y 18 d), donde la eficiencia de biodegradación/biotransformación ha sido superior al 25%, y bajos TRHs (12 y 8d), donde la eficiencia ha sido inferior al 17%. Sin embargo, en el rango mesofilico la influencia del TRH no ha sido apreciable. Salvo la desetoxilación de los nonilfenoles etoxilados (NPEO) que se lleva a cabo durante la digestión anaerobia, ni los mecanismos ni los compuestos intermediarios de la biodegradación/biotransformación de los nonlifenoles bajo las condiciones anaerobia se han podido determinar.

Para los alquilobencenosulfonatos lineales (LAS) se han podido detectar altas concentraciones en la fase sólida de los fangos. En el lodo fresco se ha observado un contenido que varía entre 4477 y 5977 mg/kg ms. Esta concentración ha disminuido a

4048-4922 mg/kg ms en el fango mesofílico y a 821-1650 mg/kg ms en el rango termofílico. También, se ha registrado un efecto negativo del recorte del TRH sobre la eficiencia de los digestores anaerobios pasando de una eliminación (biotransformación) del 86% y 17% (a TRH de 26d) a 67% y 9% (a TRH de 8 y 18 d) en el digestor termofílico y el mesofílico, respectivamente. De hecho, la acción anaerobia a altas temperaturas (55ºC) ha sido muy efectiva reduciendo la longitud de la cadena lineal de los LAS desde 11,76-11,82 a 10,36-11,01, mientras a 35ºC se han generado biosólidos conteniendo LAS con un promedio del número de carbonos que oscila entre 11,56 y 11,80.

Se han citado dos mecanismos de la biodegradación anaeróbica de los LAS (Balson and Felix, 1995; Campos-Garcia et al., 1999; Sarrazin et al., 1997), el primero consiste en la degradación sucesiva de la cadena lineal del alquilo, del grupo sulfonato y del anillo del benceno, mientras el segundo consiste en la desulfonación, seguida de la carboxílación del alquilo y finalmente la mineralización del anillo aromático. Sin embargo la biodegradación de los LAS necesita más aclaración, sobre todo a la luz de los resultados obtenidos en este trabajo. Es importante conocer si ambos mecanismos pueden ocurrir simultáneamente o por separado y si se llevan a cabo con la misma intensidad para los LAS de cadena larga y corta.

En el ***Capítulo 8***, se ha estudiado el efecto de los ultrasonidos como pretratamiento sobre la eficiencia de la digestión anaerobia en condiciones mesófilas y termófilas, y poniendo énfasis en su efecto sobre la reducción del contenido de contaminantes como son el naftaleno y el pireno.

Tras la monitorización de la variación de la distribución volumétrica del tamaño de las partículas, el grado de desintegración de la materia orgánica, el grado de solubilización y la producción del metano en el test de biodegradabilidad, se ha concluido que 11000 kJ/kg ST representa la energía específica óptima, a la frecuencia de 20 kHz, para llevar a cabo el pretratamiento de la mezcla de lodos, empleada en este trabajo como sustrato para los digestores anaerobios.

El suministro del alimento sonicado ha mejorado la eficiencia de los digestores anaerobios (el mesofílico y el termofílico) en los siguientes términos: producción de biogás (30,9 y 16,4%), eliminación de SV (12,4 y 6,4%) y producción específica de biogás por unidad de SV removidos (16,2 y 9,5%). La mejora registrada durante el periodo de la alimentación con sustrato sonicado se debe, en gran parte, al aumento de la porción de la materia orgánica soluble y a la mejora en el grado de hidrólisis durante la digestión anaerobia. Debido al alto nivel en el que la hidrólisis se lleva a cabo en la digestión anaerobia termofilíca en comparación con la mesofílica, la mejora de los parámetros de rendimiento ha sido mayor en el digestor anaerobio mesofílico

La biodegradación de naftaleno ha pasado del 33,6% al aplicar únicamente la digestión anaerobia mesofílica a más del 53,9% al combinar el ultrasonido con la digestión anaerobia. La mejora en la eliminación del naftaleno ha sido menor en el rango termofílico donde se ha registrado 50,2% de eliminación/biotransformación sin pretratamiento y 63,5% al aplicar previamente el ultrasonido al sustrato. La mejora de la biodegradación del naftaleno, empleando el ultrasonido como pretratmiento, se debe, en general, al efecto del ultrasonido que permite una solubilización avanzada de la materia orgánica, y de hecho compuestos orgánicos específicos como es el naftaleno, y hace más disponible al consorcio de microorganismos que pueden asimilar dicho compuesto. Por otra parte, el alto rendimiento observado con el tratamiento combinado en condiciones mesófilas en comparación con las condiciones termófilas puede ser por el hecho de que la digestión termofílica por si sola afecta considerablemente la disponibilidad del naftaleno y le hace más asimilable.

Para la biodegradación del pireno, a pesar del 11% de su contenido que se ha difundido en la parte acuosa al sonicar el sustrato, su eliminación se ha mantenido casi estable tras la aplicación del tratamiento combinado en relación con la digestión anaerobia como tratamiento único. Esto puede explicarse por el alto índice de hidrofobicidad del pireno y por la dificultad de su asimilación por los organismos degradantes presentes en el medio anaerobio.

Como conclusiones principales de este trabajo se pueden mencionar las siguientes:

Los resultados experimentados en este trabajo han mostrado viabilidad del Lodo Aerobio Activado (LAA) como inóculo para el arranque de los digestores anaerobios. Una estabilización en periodo corto y las altas eficiencias registradas, comparado con los datos de la bibliografía, permite confirmar que LAA se puede considerar una buena alternativa para el arranque de los digestores, sobre todo en el rango termofílico de la temperatura.

A partir de la producción especifica de biogás y la reducción de materia orgánica se ha determinado el óptimo TRH para el digestor anaerobio mesofílico en el rango 18-22 días y para el digestor anaerobio termofílico en el rango 12-18 días.

Se ha constatado la biodegradación/biotransformación de la mayoría de los microcontaminantes seleccionados para estudiar en este trabajo. Dependiendo de las características de cada compuestos o clase de compuestos, su composición y las condiciones operacionales (TRH y temperatura) aplicadas se ha podido clasificar los microcontaminantes según su tasa descendente de biodegradación/biotransformación como lo siguiente: PAH, PCB, AOX, DEHP, LAS y NPE en las condiciones mesófilas y LAS, PCB, PAH, AOX, DEHP y NPE en las condiciones termófilas.

Se han observado altos contenidos de los LAS en los fangos, sin embargo los nonilfenoles (NP) han mostrado la alta recalcitrancia sea en condiciones mesófias o termófilas. Así, se ha identificado los NPE (NP), el DEHP y los LAS como los compuestos más refractarios en la digestión anaerobias de los lodos de EDARs.

En este sentido, los pretratamientos incorporados en los procesos de la estabilización de los lodos de EDARs pueden jugar un papel fundamental para proveer un alto potencial a la digestión anaerobia para llevar a cabo la eliminación/transformación de los contaminantes orgánicos. A la luz de los resultados obtenidos en este trabajo, además de la mejora de la eficiencia del tratamiento anaerobio en términos convencionales (producción de biogás y eliminación de materia orgánica) constatada, la incorporación del tratamiento por ultrasonidos ha conducido a la reducción del contenido de los contaminantes (naftaleno y pireno) en los lodos sonicados (una probable difusión de los contaminantes a la fase líquida). La aplicación de la digestión anaerobia como tratamiento posterior al ultrasonico ha mejorado sustancialmente la degradación del naftaleno y el pireno en comparación con la aplicación de la digestión anaerobia como tratamiento único. La mejora ha sido mayor para el naftaleno y más pronunciada bajos las condiciones mesófilas. Estos resultados impulsan un estudio más profundo de los pretratamientos para confiramar los resultados obtenidos y estimar su efectos sobre otros tipos de contaminantes. La exploración de nuevos tratamientos , químicos, térmicos, biológicos o combinación de ellos aparece como una vía muy prometedora para producir un fango de calidad mejor y de uso sostenible.

Los mecanismos de biodegradación/biotransformación para muchos contaminantes no quedan bien claros y necesitan elucidaciones. Ejemplo de ello, son la biodegradación de los PAH de alto peso molecular, el DEHP, los LAS y el NP. La aclaración de los mecanismos permite identificar los supuestos compuestos intermediarios y los metabolitos, determinar su toxicidad y elaborar estrategias para mejorar la degradación y/o disminuir su efecto en los procesos biológicos, como es la digestión anaerobia.

La monitorización de los metabolitos, también, permite entender mejor las etapas de biodegradación/biotransformación de los microcontaminates, evitar la liberación al medio receptor compuestos más nocivos y con alta tendencia de acumulación y adoptar el tratamiento más adecuado para eliminar dichos compuestos.

Tabla R.1. *Parámetros operacionales de la eficiencia del digestor mesofílico*

Parámetro /TRH (d)		26	22	18	12	8
SV_e (%)	-	51.7 ± 0.2	50.7 ± 0.8	49.9 ± 1.2	-	-
DQO_e (%)	-	47.4 ± 0.9	46.9 ± 0.3	46.8 ± 0.4	-	-
PEB (mL/ g SV_a)	-	295 ± 14	328 ± 2	308 ± 10	-	-
Contenido de CH_4 en el biogás (%)	-	72.6 ± 0.9	74.9 ± 1.4	73.2 ± 0.9	-	-
PAH-EU eliminación (%)	PAH-BPM	57.07 ± 1.2	56.71 ± 2.3	52.60 ± 1.5	-	-
	PAH-APM	41.17 ± 0.8	35.25 ± 1.1	39.18 ± 0.6	-	-
	PAH-AB	56.13 ± 0.9	53.96 ± 1.5	49.97 ± 0.5	-	-
	PAH-EU	43.20 ± 3.6*	36.56 ± 2.3	41.67 ± 0.7	-	-
DEHP eliminación (%)	-	37.8 ± 2.1*	27.7 ± 2.5*	21.7 ± 3.1*	-	-
PCB eliminación (%)	PCB-LC	-4.67 ± 1.5	-16.14 ± 2.1	-39.11 ± 3.1	-	-
	PCB-AC	69.30 ± 2.3	67.03 ± 3.5	43.27 ± 2.3	-	-
	PCB total	58.40 ± 4.1	47.02 ± 4.6*	33.02 ± 4.1*	-	-
AOX eliminación (%)	-	38.2 ± 3.5	43.2 ± 7.1	30.2 ± 1.4	-	-
NPE eliminación (%)	NP	-3.6 ± 1.0	12.8 ± 1.6	11.2 ± 0.9	-	-
	NP1EO	12.5 ± 3.6	42.4 ± 4.3	15.2 ± 0.3	-	-
	NP2EO	71.4 ± 9.4	-35.6 ± 4.5	29.5 ± 1.5	-	-
	NPE	7.7 ± 1.3*	21.9 ± 1.9*	13.0 ± 1.8*	-	-
LAS eliminación (%)	C10-LAS	5.5 ± 0.3	12.9 ± 0.6	11.2 ± 0.6	-	-
	C11-LAS	-5.7 ± 0.6	9.0 ± 0.0	10.5 ± 0.0	-	-
	C12-LAS	28.4 ± 3.2	21.1 ± 2.1	4.6 ± 0.0	-	-
	C13-LAS	35.7 ± 2.1	18.1 ± 1.6	11.4 ± 0.2	-	-
	LAS total	17.7 ± 2.2*	15.8 ± 1.2*	9.6 ± 0.5*	-	-

*: No cumplimiento de las condiciones de salubridad para el uso agrícola (CEC, 2000).

Tabla R.2. *Parámetros operacionales de la eficiencia del digestor termofílico*

Parámetro /TRH (d)		26	22	18	12	8
SV_e (%)	-	50.9 ± 0.6	51.7 ± 0.4	52.3 ± 1.2	50.9 ± 0.6	50.3 ± 1.0
DQO_e (%)	-	58.6 ± 0.5	57.0 ± 3.9	59.6 ± 0.4	56.9 ± 1.1	55.1 ± 1.0
PEB (mL/ g SV_a)	-	461 ± 15	412 ± 16	420 ± 31	337 ± 6.0	290 ± 18
Contenido de CH_4 en el biogás (%)	-	71.5 ± 3.5	67.7 ± 3.7	60.7 ± 2.1	54.1 ± 1.6	54.0 ± 1.6
PAH-EU eliminación (%)	PAH-BPM	81.72 ± 2.1	75.65 ± 3.5	72.03 ± 3.6	60.95 ± 2.9	59.82 ± 3.1
	PAH-APM	63.08 ± 1.9	53.72 ± 2.4	62.44 ± 1.3	54.02 ± 5.6	48.52 ± 2.1
	PAH-AB	83.50 ± 1.8	84.69 ± 1.3	74.53 ± 3.4	71.60 ± 6.3	62.17 ± 2.8
	PAH-EU	65.46 ± 1.7	55.06 ± 2.5	64.22 ± 2.6	59.07 ± 4.6	51.01 ± 3.3
DEHP eliminación (%)	-	45.4 ± 3.1	46.2 ± 2.5*	46.7 ± 3.5	31.7 ± 5.1*	32.3 ± 2.3*
PCB eliminación (%)	PCB-LC	18.42 ± 1.2	11.55 ± 0.5	14.11 ± 2.5	7.82 ±1.4	7.26 ± 2.4
	PCB-AC	94.80 ± 2.1	92.52 ± 2.3	88.39 ± 3.4	82.16 ± 4.1	79.20 ± 2.3
	PCB total	83.54 ± 1.8	73.04 ± 2.2	79.14 ± 2.9	69.67 ± 3.9	59.41 ± 2.4
AOX eliminación (%)	-	50.2 ± 4.1	44.0 ± 3.2	45.7 ± 2.2	49.6 ± 3.5	40.4 ± 5.2
NPE eliminación (%)	NP	10.8 ± 3.2	16.5 ± 3.5	20.6 ± 3.3	-3.4 ± 1.1	-6.8 ± 1.4
	NP1EO	59.6 ± 5.2	43.9 ± 4.5	73.6 ± 6.5	63.8 ± 5.1	59.2 ± 4.2
	NP2EO	65.3 ± 6.3	20.0 ± 2.1	88.4 ± 7.2	34.2 ± 3.0	78.3 ± 9.2
	NPE	29.5 ± 3.3*	26.4 ± 2.8*	34.8 ± 5.2*	9.4 ± 1.3*	16.5 ± 2.8*
LAS eliminación (%)	C10_LAS	57.8 ± 3.5	56.4 ± 4.7	-54.6 ± 6.2	-50.3 ± 6.2	-34.6 ± 4.1
	C11_LAS	86.1 ± 6.3	81.2 ± 5.6	87.5 ± 4.6	85.0 ± 5.4	86.1 ± 9.2
	C12_LAS	88.6 ± 4.5	87.7 ± 6.1	90.7 ± 8.1	88.6 ± 3.8	80.8 ± 5.5
	C13_LAS	96.9 ± 6.3	95.5 ± 5.5	97.8 ± 7.7	96.2 ± 4.6	86.7 ± 6.1
	LAS total	86.3 ± 9.6	84.5 ± 7.2	72.0 ± 8.3	69.2 ± 5.7	67.3 ± 9.5

*: No cumplimiento de las condiciones de salubridad para el uso agrícola (CEC, 2000).

Experimento del ultrasonido como pretratamiento

Tabla R.3. *Parámetros operacionales de los digestores sin el pretratamiento del ultrasonido*

Parámetro	***Mesofílico***	***Termofílico***
TRH (d)	20	15
SV_e (%)	41.60 ± 0.5	46.34 ± 0.7
DCO_e (%)	43.89 ± 1.7	49.21 ± 1.6
Producción diaria de biogás (L_{BIOGAS}/(L_r·día))	0.360 ± 0.021	0.578 ± 0.034
Contenido de metano (%)	63.6 ± 1.9	67.6 ± 2.6
PEB (L_{BIOGAS}/g SV_e)	0.76 ± 0.035	0.821 ± 0.026
PEB (L_{BIOGAS}/g DQO_e)	0.36 ± 0.008	0.388 ± 0.011

Tabla R.4. *Parámetros operacionales de los digestores durante el uso del ultrasonido como pretratamiento*

Parámetro	***Mesofílico***	***Termofílico***
TRH (d)	20	15
SV_e (%)	46.76 ± 1.2	49.30 ± 0.8
DCO_e (%)	51.93 ± 2.1	52.53 ± 1.9
Producción diaria de biogás (L_{BIOGAS}/(L_r·día))	0.470 ± 0.020	0.673 ± 0.012
Contenido de metano (%)	63.9 ± 2.1	67.8 ± 3.5
PEB (L_{BIOGAS}/g SV_e)	0.883 ± 0.032	0.898 ± 0.28
PEB (L_{BIOGAS}/g DQO_e)	0.399 ± 0.014	0.423 ± 0.016

Tabla R.5. *Eliminación de naftaleno y pireno (%) bajo diferentes tratamientos aplicados*

	Mesofílico	***Termofílico***	***Sonication***	***Son+Meso***	***Son+Termo***
Naftaleno	33.61 ± 0.6	50.23 ± 0.4	24.19 ± 0.2	53.91 ± 0.3	63.54 ± 0.7
Pireno	31.27 ± 0.9	55.99 ± 1.2	11.13 ± 01	32.58 ± 0.5	53.12 ± 0.9

Son+Meso: tratamiento combinado del ultrasonido y de la digestión anaerobia mesofílica
Son+Termo: tratamiento combinado del ultrasonido y de la digestión anaerobia termofílica

Table R.6. *La mejora (%) en los parámetros operacionales y en la eliminación de naftaleno y pireno al suministrar sustrato sonicado en los digestores mesofílico y termofílico*

Parámetro	***Mesofílico***	***Termofílico***
SVe	12.40 ± 0.5	6.39 ± 0.4
DQO_e	18.32 ± 0.8	6.69 ± 0.9
Producción de biogás	30.88 ± 1.1	16.44 ± 0.7
Eliminación de naftaleno	60.38 ± 2.3	26.52 ± 1.5
Eliminación de pireno	4.20 ± 0.2	-5.14 ± 0.6

Summary

Summary

Activated sludge is the most commonly biochemical process used for municipal and industrial wastewater treatment. However, a great amount of excess sludge resulting from activated sludge processes in wastewater treatment plants (WWTPs) is becoming a serious issue, especially in developed countries. In addition, this huge amount is expected to increase in the next few years. The excess sludge should be disposed off in the most effective manner, which can imply the anaerobic digestion step. This process yields a significant reduction in the amount of sludge and produces methane gas, which is can be used to produce electricity and heat. Urban Waste Water Treatment Directive (91/271/EEC) encourages the use of sludge in agricultural land whenever appropriate.

Nowadays, mesophilic anaerobic processes (under 35ºC) predominate but they show to be unable to accomplish the new specifications. Consequently, only the migration to higher temperature conditions, i.e. thermophilic operation, seems to be capable of producing stabilized sludge that meets the new restrictions related to the presence of harmful bacteria. In addition, the effect of anaerobic digestion on organic micropollutants has scarcely been studied. Moreover, the temperature impact on these compounds in anaerobic digestion of sewage sludge has also not been elucidated.

The overall objective of this work is to study the fate of some organic micropollutants during raw sewage sludge (RSS) anaerobic digestion and the effect of thermophilic temperature conditions on treatment efficiency, when compared with mesophilic anaerobic. To this purpose, a mesophilic and thermophilic anaerobic digester were operated using Waste Activated Sludge (WAS) as inoculum and the effects of Hydraulic Retention Time (HRT) reduction and its influence on the fate of the selected organic micropollutants by the 3rd draft of Directive presented to the European Comission (CEC, 2000) were studied. Furthermore, the effect of a complementary treatment, namely the ultrasonic pretreatment, on the biodegradability of the organic feed and the behaviour of the subsequent anaerobic digestion was also examined, focussing on the micropollutants reduction.

In Chapter 1, a literature overview related to sewage sludge, including types of sludge, generation and production rates, characteristics, disposal routes and anaerobic digestion background is presented.

In Chapter 3, the experimental set-up and the analysis methods used in this study are described. It includes the conventional parameters used for sludge characterization (alkalinity, acidity, biogas production and composition, organic matter, nitrogen ammonia, pH, solids and volatile fatty acids) and the organic micropollutants (Polycyclic Aromatic Hydrocarbons (PAH), Di-2-(Ethyl-Hexyl)-Phthalate (DEHP), PolyChlorinated Biphenyls (PCB), Nonyphenols (NPE), Linear AlkylBenzene Sulphonates (LAS) and Organic Halogenated compounds (AOX)) analysis.

In Chapter 4, the possibility of using waste activated sludge (WAS) as a seed for start-up of both mesophilic (35 ºC) and thermophilic (55 ºC) anaerobic digesters, fed with a real sludge waste (primary and secondary sludge mixture) is evaluated. Based on the gradual substitution of synthetic substrate by real feed “RSS”, both anaerobic digesters were rapidly stabilized within 60 and 85 days at an HRT of 35 and 30 days with 450 and 520 mL biogas/g COD added and 65% and 72% as methane content under mesophilic and thermophilic conditions, respectively. Moreover, HRT was progressively reduced in order to assess the maximum organic load that can be treated in the thermophilic reactor. The minimum HRT reached was 8 days with a VS removal efficiency of 50.32% and a biogas yield index of 440 mL biogas/g VS added (54% as methane content).

In Chapter 5, a performance of the anaerobic digestion under mesophilic and thermophilic conditions at different HRT is assessed and the removal efficiencies of two important compounds or family compounds (PAH and DEHP) are evaluated. A positive effect of thermophilic temperature was observed on both micropollutants biodegradation. For PAH biodegradation and/or biotransformation, 50-65% and 38-44% are recorded as the removal efficiencies ranges under thermophilic and mesophilic conditions, respectively. HRT reduction led to a reduction of the mean removal values from 70% to 52% under thermophilic temperature conditions and from 56% to 52% under mesophilic temperature conditions. Besides, Low Molecular Weight PAHs showed high ability to be removed through an anaerobic process than High Molecular

Weight PAHs. On the other hand, it was observed an important abiotic removal rate, especially for fluorescent PAHs (phenanthrene, fluorene and anthracene), where due to light, high temperature, agitation or pH modification at the optimum conditions can lead to accentuate the PAH removal.

From DEHP monitoring, it was recorded a high content in the treated sludge and non-fulfilment of the cut-off limit proposed in the 3rd draft (CEC, 2000). In the thermophilic digester, DEHP elimination efficiency was increased by 25% to 50% with respect to mesophilic anaerobic conditions. Furthermore, high HRTs had a clear positive effect on DEHP biodegradation. However, the micropollutant removal enhancement did not always satisfy the health and safety conditions proposed in EU for sludge destined to be reused in the agricultural soil.

In Chapter 6, the behaviour of PCBs and AOX during anaerobic digestion of sewage sludge is assessed. The total PCB removal efficiency was in the range of 59.4-83.5% under thermophilic conditions and 33.0-58.0 % under mesophilic conditions. HRT played an important role in the digester performance since high working HRTs implied more reduction of the total PCB amount in the sludge. However, a bioaccumulation of Lightly Chlorinated PCBs was detected in the mesophilic digester, which indicated that PCB biodegradation was performed following a reductive dechlorination mechanism.

The AOX removal efficiency was in the range of 40.4-50.3 % for thermophilic conditions and 30.2-43.2% for mesophilic conditions. The AOX content in the treated sludge of both thermophilic and mesophilic digesters did not exceed the cut-off limit proposed in the 3rd draft (CEC, 2000). Moreover, high HRTs promoted an improvement of the AOX removal capacity of the anaerobic digestion.

Anionic (as Linear alkylbenzensulphonate (LAS)) or nonionic (as nonylphenols (NPE)) surfactants and their degraded products are the highest residual sewer contaminants and, therefore, sludge pollutants, due to their frequent use. In Chapter 7, the influence of temperature and HRT under anaerobic condition on LAS and NPE is analysed. A biological removal efficiencies of mono- and diethoxylate nonylphenol (NP1EO and NP2EO) were reached up to 78% and 71%, repectively, in both thermophilic and mesophilic anaerobic digestion. However, for nonylphenol (NP) the anaerobic treatment had removal efficiency values below 20%. Moreover, the effect of decreasing the hydraulic retention time (HRT) was clearly observed under thermophilic conditions for the sum of NP, NP1EO and NP2EO (NPE) biodegradation. Their removal efficiency was reduced by a 43% when HRT decreased from 26 to 8 days. Besides, the HRT reduction effects were not clearly stated under mesophilic conditions.

The LAS content in the dried sludge decreased substantially under thermophilic conditions, which released a biosolids with 67-86% below the initial LAS content. In contrast, the mesophilic treatment had LAS removal efficiency below 20% at all the HRTs applied. Hence, the HRT reduction led to an increase in the LAS content of the digested sludge.

The hydrolysis of the organic matter has been identified as the rate limiting step in the many anaerobic digestion processes of sewage sludge. Most common cell disruption processes are carried out thermally, physically, chemically, biologically or by their combinations. The mechanical disruption process, which is part of the physical process, is the most applied process in sludge disruption. The ultrasound pretreatment is the most applied mechanical cell disruption due to its energy balance advantage and easy integration in any exiting industrial plant. In Chapter 8, the influence of ultrasonic pretreatment of RSS on its biodegradability and the consequent enhancement of both naphthalene and pyrene removal efficiencies after anaerobic treatment is assessed. An optimal specific energy of 11,000 kJ/kg TS promoted the best solubilization yield and enhanced biogas production in the subsequent anaerobic stabilization under both mesophilic and thermophilic conditions. The high efficiency of thermophilic anaerobic digestion was slightly improved when it was combined with ultrasonic pretreatment. However, the feeding of a pretreated sludge highly favoured the biogas production in mesophilic anaerobic digestion when compared with the performance without pretreatment.

The diffusion of Polycyclic Aromatic Hydrocarbons (PAH) compounds to the aqueous phase was stated by a reduction in the sonicated sludge pollutants content. High naphthalene removal efficiencies were recorded by the combined ultrasonic-thermophilic anaerobic digestion treatment. However, the naphthalene removal enhancement by combined ultrasonic and anaerobic digestion treatment was more pronounced under mesophilic conditions. On the other hand, the obtained pyrene removal was nearly the same with and without ultrasonic pretreatment.

Chapter 1

Introduction

Chapter 1

1. Introduction

1.1. Sewage sludge

During the last twenty years, developments in municipal wastewater treatment strategies are characterised by two aspects (Rulkens, 2004). The first aspect is a continuous effort to improve the quality of the effluent by upgrading existing treatment plant and the designing and implementation of new more effective treatment plants (Carballa, 2005). The second aspect is an increasing awareness of the problems associated with the sewage sludge produced in the wastewater treatment process. These problems are a continuous increase in sludge production, the high costs of sludge treatment and the risks sewage sludge may have to the environment and human health. Due to this, the original application of the sludge as a fertiliser in agricultural systems has become increasingly under pressure (Campbell, 2000). Parallel to this development, the government policy and regulations regarding the application of sludge in agriculture have changed considerably (Spinoza, 2001).

1.1.1. Definition and types of sludge

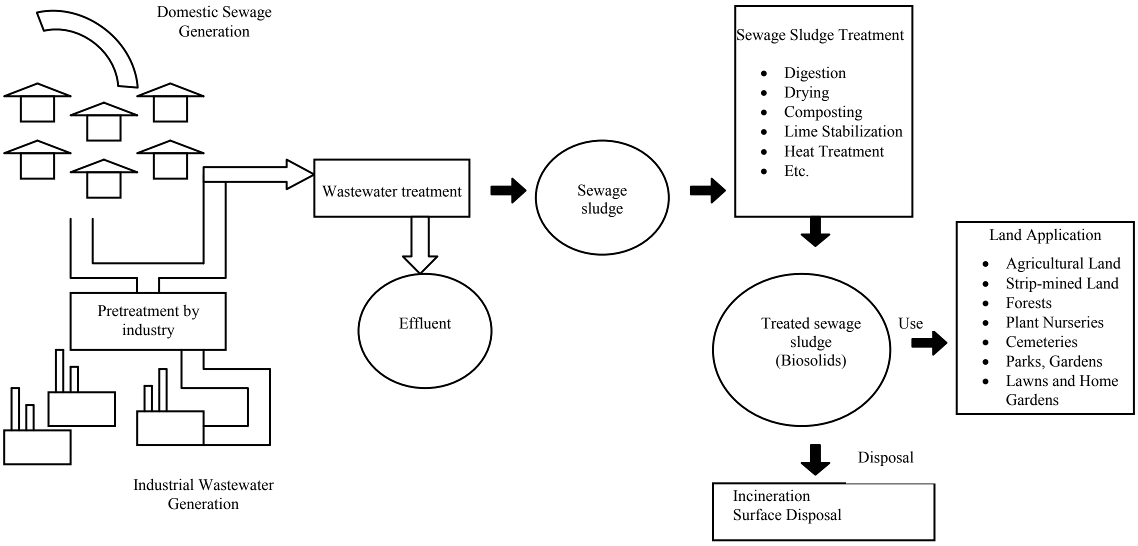


Figure 1.1. *Generation, treatment, use, and disposal of sewage sludge (USEPA, 1999)*

Sludge is by-product of the wastewater treatment process (see Figure 1.1). There are three main categories of sludge (Carballa, 2005):

- Sludge originating from treatment of urban wastewater.
- Sludge originating from treatment of industrial wastewater.
- Sludge from drinking water treatment.

Sludge from conventional sewage treatment plant is derived from primary, secondary and tertiary treatment process. The Figure 1.2 shown the flow diagram in Gavá plant designed for primary and secondary treatment.

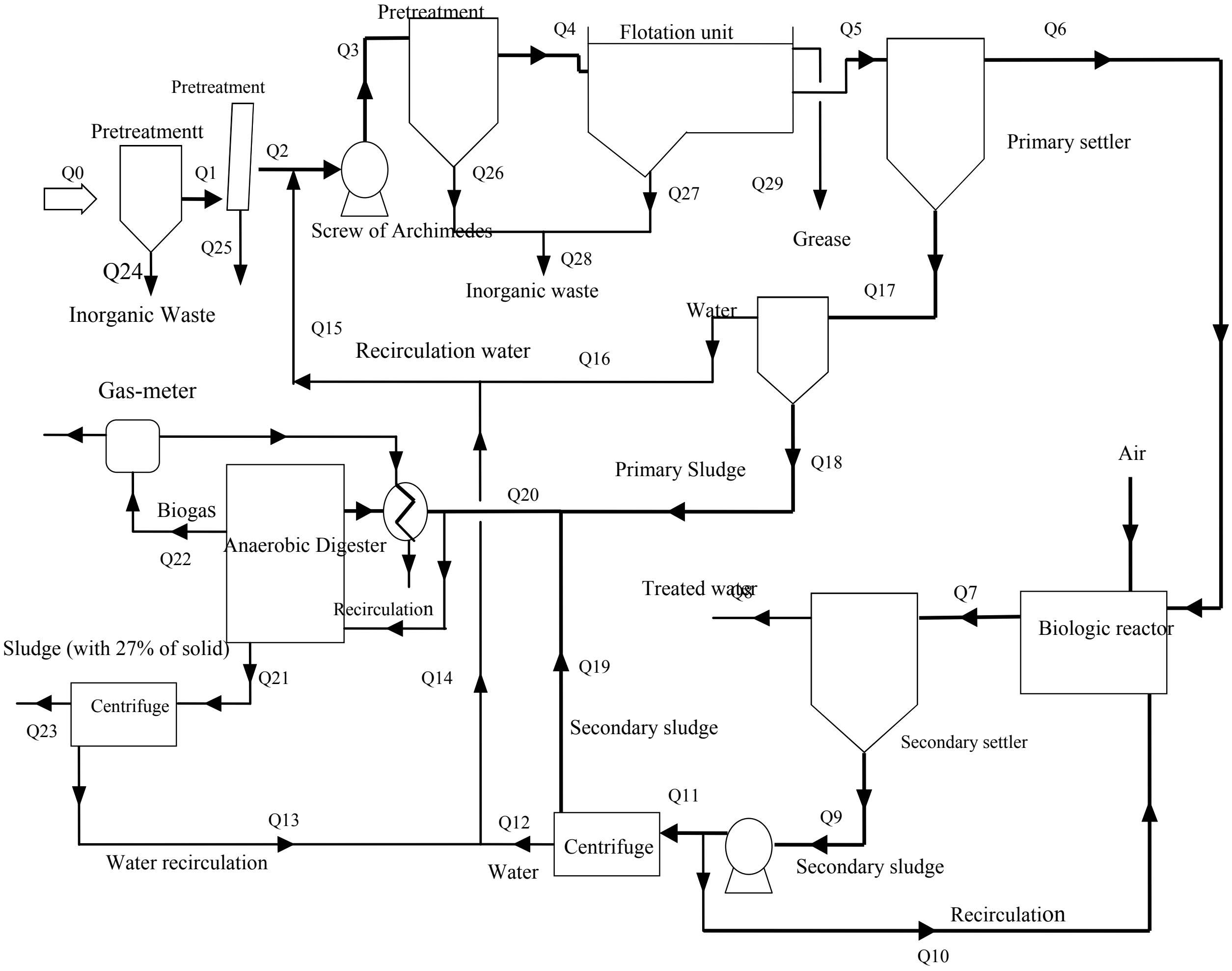


Figure 1.2. Flow diagram of Gavá wastewater treatment plant (primary and secondary treatments)

Pre-treatment

Pretreatment consists of various physical and mechanical operations, such as screening, sieving, blast cleaning, oil separation and fat extraction. It allows the removal of voluminous items, sand and grease. The residues from pre-treatment are not considered to be sludge and they are disposed in landfills.

Primary sludge

Primary sludge is produced following primary treatment, which consists of physical or chemical processes to remove matter in suspension (e.g. solids, grease and scum). Sedimentation and flotation are the most common processes used in primary treatment. They remove 50-70% and 25-40% of the suspended solid and BOD, respectively.

Secondary sludge

Secondary sludge is generated from the use of specially provided decomposers to break down the remaining organic materials in wastewater after primary treatment. The active agents in these systems are microorganisms, mostly bacteria, which need the available organic matter to grow. There are various techniques, such as lagooning, bacterial beds, activated sludge and biofiltration processes.

Mixed sludge

The primary sludge and secondary sludge can be mixed together prior to sludge treatment generating a type of sludge referred to as mixed sludge.

Tertiary sludge

Tertiary sludge is generated when carrying out tertiary treatment, which is an additional process, designed to remove remaining nutrients (mainly nitrogen and phosphorus) through high performance bacterial or chemical processes.

Treated sludge

After water purification, additional treatments need to be performed on sludge, in order to reduce its water content, pathogen load, volume and global mass, stabilise its organic matter and reduce the generation of odours.

Several treatments can be applied to achieve those purposes and the final sludge is usually referred to as treated sludge.

1.1.2. Sludge generation and production

After the implementation of Urban Waste Water Treatment Directive 91/271/EEC, the majority of the EU population will be severed by Station Treatment Plants (STPs), by the year 2005. As results, the sludge quantities have increased in most countries during the 1990s.

In 1999, the EU-15 produced about 7 million tons of dry matter from STPs and the expected production for the year 2005 was 8.3 million tons by the year 2005 (Magoarou, 2000). The annual production in Spain was approximately 800,000 tons in 1998 and it was expected to increase up to 1.3-1.5 million by the year 2005 (see Table 1.1).

Table 1.1. *1998 sludge production and 2005 predicted sludge generation in Spain (Plan Nacional de LD-EDAR 2001-2006; BOE 166, 2001).*

Autonomic community	Tons dry matter / year	
	1998	2005
Andalusia	-	312,500
Aragon	-	41,000
Asturias	-	36,000
Canary Islands	-	54,000
Cantabria	-	18,000
Castile and Leon	23,906	81,000
Castile-La Mancha	-	56,000
Catalonia	244,805	200,000
Ceuta	-	1,200
Valencian community	-	130,000
Extremadura	-	36,000
Galicia	-	90,000
Balearic Islands	28,639	29,000
La Rioja	-	8,000
Madrid	162,278	178,000-342,862
Melilla	1,095	1,100
Murcia	32,740	37,000
Navarre	6,227	11,314
Basque Country	21,948	63,000
Total	800,000	1,547,976

1.1.3. Sludge characteristics

The characteristics of sludge depend on the original pollution load of the treated wastewater and also on technical characteristics of the treatment carried out. Conventional characterisation can be grouped in physical, chemical and biological parameters (Carballa, 2005):

- Physical parameters give general information on sludge processability and handlability.

- Chemical parameters are relevant to the presence of nutrients and toxic compounds, thus being important for the final disposal.
- Biological parameters give information on microbial activity and organic matter presence, thus affecting its suitability for beneficial use.

Therefore, sewage sludge contains some compounds of agricultural value, which may be usefully reused (organic matter, nutrients, etc.), whereas other substances are pollutants (heavy metals, pathogens and organic pollutants).

Organic matter

Sludge organic matter is mostly constituted of soluble matter, such as hydrocarbons, amino-acids, small proteins and lipids. Its content in urban sewage sludge is high (> 50%), but varies according to the treatment and conditioning carried out on sludge.

Organic matter is mainly used for the improvement of the physical properties of the soil, such as structure or the retention capacity of minerals and water. Other benefits are the increase of soil population, activity and mineralization capacity (Metcalf and Eddy, 1991).

Nitrogen and phosphorus content

The proportion of nitrogen and phosphorus in sewage sludge is comparable to that of animal manure, 20-80,000 and 10-90,000 mg/kg, respectively and it is influenced by the operation of the STP and the sludge storage conditions (Metcalf and Eddy, 1991).

Nitrogen is mostly found in the sludge under organic form and to a lesser extent as ammonia. The other mineral forms of nitrogen are found as traces. However, phosphorus is mostly present under mineral form (30-98% of total P) (Carballa, 2005)

They are used by the plant for its growth, the rigidity of its cell walls and for the development of its root system. However, as plants can only assimilate mineral forms, the agricultural value of the sludge is determined by the nitrogen and phosphorus availability, which is dependant on the sludge treatment as well as on external factors, such as temperature, humidity, pH and texture of the soil (Metcalf and Eddy, 1991).

Other compounds of agricultural value

Other compounds present in the sludge such as calcium, potassium, sulphur, magnesium, sodium and oligo-elements (boron, cobalt, selenium) may be of interest due to their positive impacts on the pH, structure and permeability of the soil as well as in crop production. However, they may appear in sludge under various forms, thus being their efficiency dependant on their availability.

Heavy metals

Heavy metals (Cd, Cr, Cu, Hg, Ni, Pb and Zn) are present in sludge between 0.3 and 2,000 mg/kg, approximately. There are three main origins for heavy metals in sewage sludge: domestic effluents, road runoff and industry. The proportion of each origin may be different for each compound (CEC, 2000)

Heavy metals may affect plant health and growth, soil properties and microorganisms, livestock and human health and accumulate in the environment. However, they can be beneficial certain soils, correcting trace elements deficiency (Metcalf and Eddy, 1991).

Pathogens

Sewage sludge contains various microorganisms, especially when biological treatments are carried out, and it can also contain plant pathogens. Only, some of them have health-related impacts (USEPA, 1999). The presence of pathogens in the sludge is related to the sanitary level of the population and the type of industry in the region. The types of pathogens usually considered are virus, bacteria, protozoa and helminths.

Organic pollutants

A wide variety of organic chemicals with diverse physical and chemical properties may be found in sludge. They also may affect soils, plants, animals and human health, and have impact on the environment.

Table 1.2. *Draft Directive (2000) setting* ***cut-off limits*** *for sludge contaminants (CEC, 2000).*

AOX mg/kg dm	**DEHP** mg/kg dm	**LAS** mg/kg dm	**NP/NPE** mg/kg dm	**PAH** mg/kg dm	**PCB** mg/kg dm	**PCDD/F** ng TEq/kg dm
500	100	2600	50	6	0.8	100

AOX: Adsorbed organohalogenous compounds.
DEHP: Di(2-ethylhexyl)phthalate.
LAS (C10-C13): Linear alkylbenzene sulfonates.
NP/NPE: NonylPhenol, nonylphenol mon- and diEthoxylates.

PAH: Polynocluear Aromatic Hydrocarbons. (Sum of Low Molecular Weigh "LMW" PAH [acenapthene, phenanthrene, fluorene, fluoranthene] and High molecular Weigh "HMW" PAH [pyrene, benzo(b+j+k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, indeno(1, 2, 3-c,d)pyrene]).

PCB: PolyChlorinated Biphenils. (Sum of 6 congeners PCB 28, 52, 101, 138,153, 180. Tri-, tetra-, penta-, Hexa-, hexa-, heptachloro PCB, respectively).

PCDD/F: PolyChloro-DibenzoDioxins/Furans.

Table 1.3. *Dioxins and related compounds Toxic Equivalent Factors (TEF) (Eljarrat et al., 2002)*

PCDD congener	TEF	PCDF congener	TEF	PCB congener	TEF
2,3,7,8-TCDD	1	2,3,7,8-TCDF	0.1	***No-ortho***	
1,2,3,7,8-PeCDD	1	1,2,3,7,8-PeCDF	0.05	PCB No. 81	0.0005
1,2,3,4,7,8-HxCDD	0.1	2,3,4,7,8-PeCDF	0.5	PCB No. 77	0.0005
1,2,3,6,7,8-HxCDD	0.1	1,2,3,4,7,8-HxCDF	0.01	PCB No. 126	0.1
1,2,3,7,8,9-HxCDD	0.1	1,2,3,6,7,8-HxCDF	0.1	PCB No. 169	0.01
1,2,3,4,6,7,8-HpCDD	0.01	2,3,4,6,7,8-HxCDF	0.1	***Mono-ortho:***	
OCDD	0.0001	1,2,3,7,8,9-HxCDF	0.1	PCB No. 105	0.0001
		1,2,3,4,6,7,8-HpCDF	0.01	PCB No. 114	0.0005
		1,2,3,4,7,8,9-HpCDF	0.01	PCB No. 118	0.0001
		OCDF	0.0001	PCB No. 123	0.0001
				PCB No. 156	0.0005
				PCB No. 157	0.0005
				PCB No. 167	0.00001
				PCB No. 189	0.0001

The most common considered compounds are polynuclear aromatic hydrocarbons (PAH), plychlorinated biphenils (PCB), polychlorodibenzodioxins/furans (PCDD/F), the sum of organohalogenous compounds (AOX), nonylphenol and nonylphenolethoxylates (NPE), linear alkylbenzene sulfonates (LAS) and di(2-ethylhexyl) phthalate (DEHP).

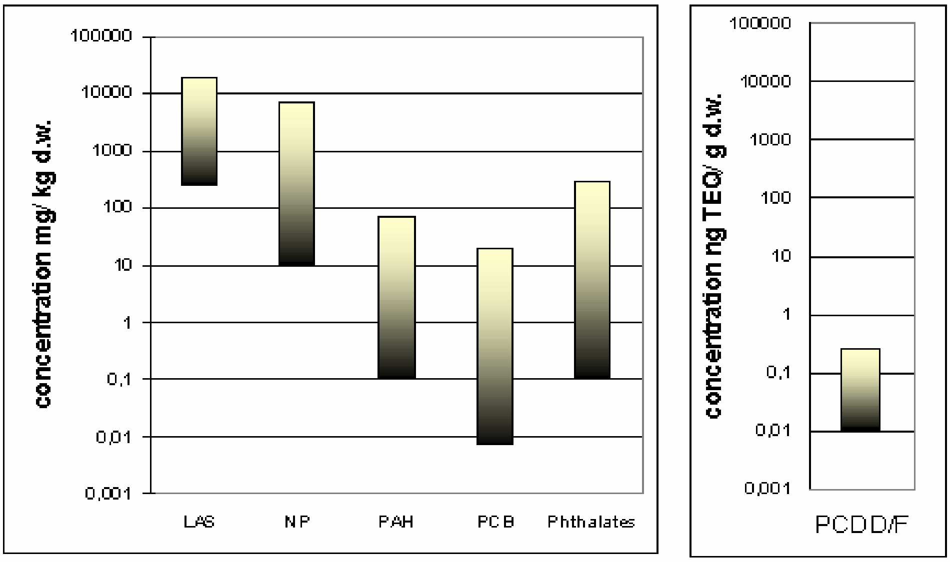


Figure 1.3. *Typical sludge organic micropollutant content (CEC, 2000).*

As they are not often mentioned in the national regulations, no survey has been regularly performed describing the organic pollutant content in sewage sludge. However, recently most concern has been targeted on the presence of these ubiquitous organic pollutants and the Working Document on sludge (CEC, 2000) proposes limit values in the sludge for use on agricultural land (see Table 1.2).

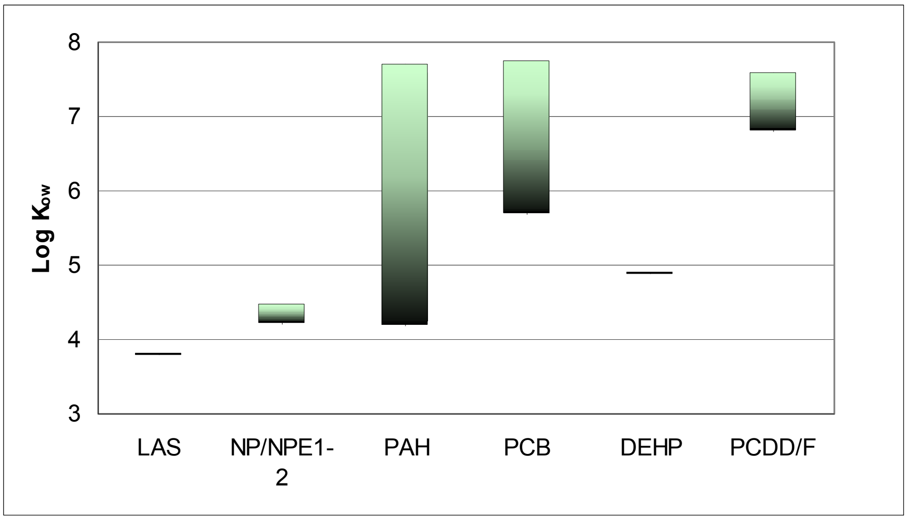


Figure 1.4. *Organic micropollutants octanol-water partition coefficient (K_{ow}). Log K_{ow} < 2.5: low sorption potential, Log K_{ow} > 2.5 and < 4.0: medium sorption potential and Log K_{ow}> 4.0: high sorption potential. (CEC, 2000)*

The higher content of organic xenobiotic compounds in sludge (Figure 1.3) and the accumulation tendency of organic compounds in solid matter, due to their higher hydrophobic index (see Figure 1.4), leads to consider this as the major limiting factor of soil sludge application (Barceló and Petrovic, 2004; Pavlostathis and Jaglal, 1991; Subramaniam et al., 2004).

Table 1.4. *Catalonian sludge survey vs. the cut-off limits contaminants (Ortiz S., 2004. ACA)*

Contaminant (s)	**Samples number**	**Non-fulfilment samples**	**% Non-fulfilment**
AOX	94	**54**	**57.4**
LAS	94	0	0.0
DEHP	62	7	11.3
NPE	62	**57**	**91.9**
PAH	62	5	8.1
PCB	62	1	1.6
PCDD/F	30	2	6.7

In Catalonia as well as other EU regions, higher sludge organic micropollutants content was recorded. Thus, it was frequently to detect a STPs produced biosolids that non-fulfilled the cut-off limit proposed in the Working Document on sludge, 3rd draft, (CEC, 2000) as showed in Table 1.4.

1.1.4. Sludge reuse and disposal

Sewage sludge production is a continuous process and requires a flexible and source range of outlets for its disposal to be economically and environmentally acceptable. The predominant disposal options available include landspreading, incineration and landfilling (see Figure 1.5).

Landspreading

Landspreading is a way of recycling the compounds of agricultural value present in sludge to land. All types of sludge (liquid, semi-solid or dried sludge) can be speared on land. However, the use of each of them induces practical constraints on storage, transport and spreading.

This route may be cheaper than other disposal routes. However, the presence of pollutants in sludge implies that practice should be carefully done and monitored. To this purpose, codes of practices and spreading schemes have been established in some countries, summarising the regulatory obligations.

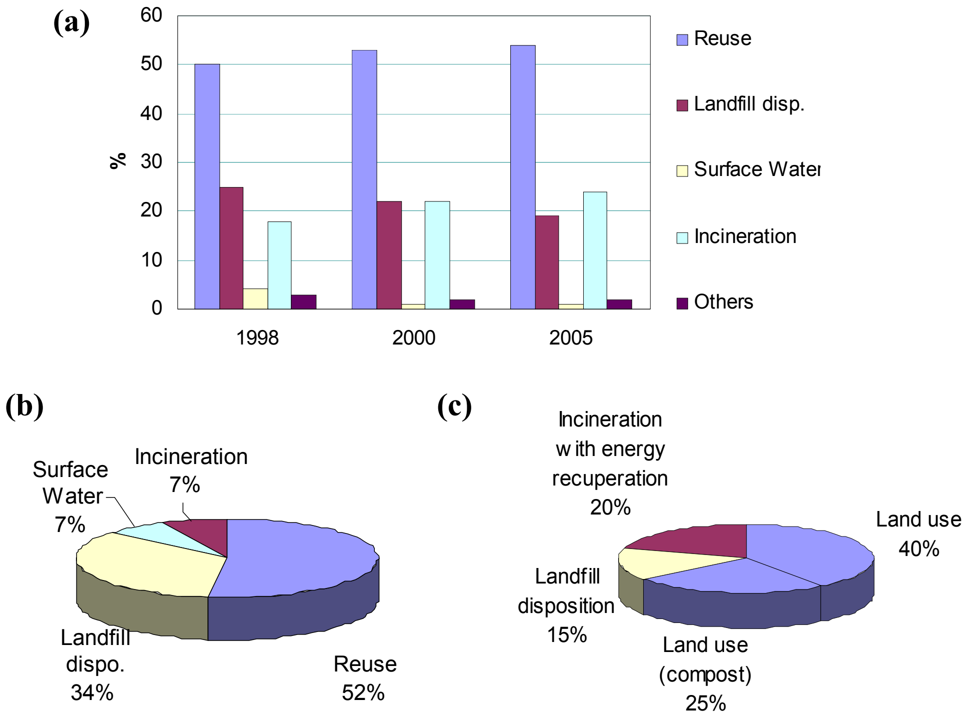


Figure 1.5. WWTPs sludge destination, (a) In EU, (b) 1998 state in Spain and (c) 2005 predicted in Spain (BOE 166, 2001).

Incineration

Incineration is a combustion reaction, which produces a residual solid waste and a flue gas. According to the Waste Incineration Directive 2000/76/EC, different types of incineration may be considered (mono-incineration, co-incineration, etc.).

Several technologies also involving thermal oxidation, such as wet oxidation and pyrolysis, are being developed and introduced in the market as an alternative to conventional combustion processes.

Landfilling

So far, landfilling has been the major route for sludge disposal. However, it should be a limited outlet in the future due to the implementation of the Landfill Directive 1999/31/EC, which states that this solution must be only chosen when no other ways exist.

There are two possibilities for landfilling sludge: mono-deposits (the landfill is only used for sludge) and mixed-deposits (the landfill is also used for municipal wastes). The conditions for disposal are set out in the regulations of each country.

Other routes

Other routes of sludge disposal are its use as a forest fertiliser, as a soil conditioner for the restoration of the disturbed soils, as a soil forming material for reclaiming derelict land, and for producing soil for use on green areas in the urban environment.

1.1.5. Anaerobic digestion and sludge stabilization (mesophilic vs. thermophilic conditions)

Sludge stabilization is obtained with treatments reducing their organic content (aerobic or anaerobic digestion) or blocking their fermentation ability (lime addition, heat treatment or composting) (Carballa, 2005). Among them, the anaerobic digestion (AD) process has been and continues to be established as the most widely used technology to stabilize sewage sludge (Ray et al., 1990), specially for STPs with more than 20,000-30,000 population equivalent (p.e) or 2,000 mg COD/ L (de la Rubia, 2000, de la Rubia et al., 2001). The interest in this process has been focussed on an increase in the process efficiency and a reduction in the investment and operation costs.

Anaerobic digestion is a complex biochemical reaction carried out without oxygen presence in a number of steps, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis (see Figure 1.6), by several types of microorganisms (Pavlostathis and Giraldo-Gómez, 1991). During the process, a gas principally composed of methane (CH_4) and carbon dioxide (CO_2), otherwise known as biogas, is produced.

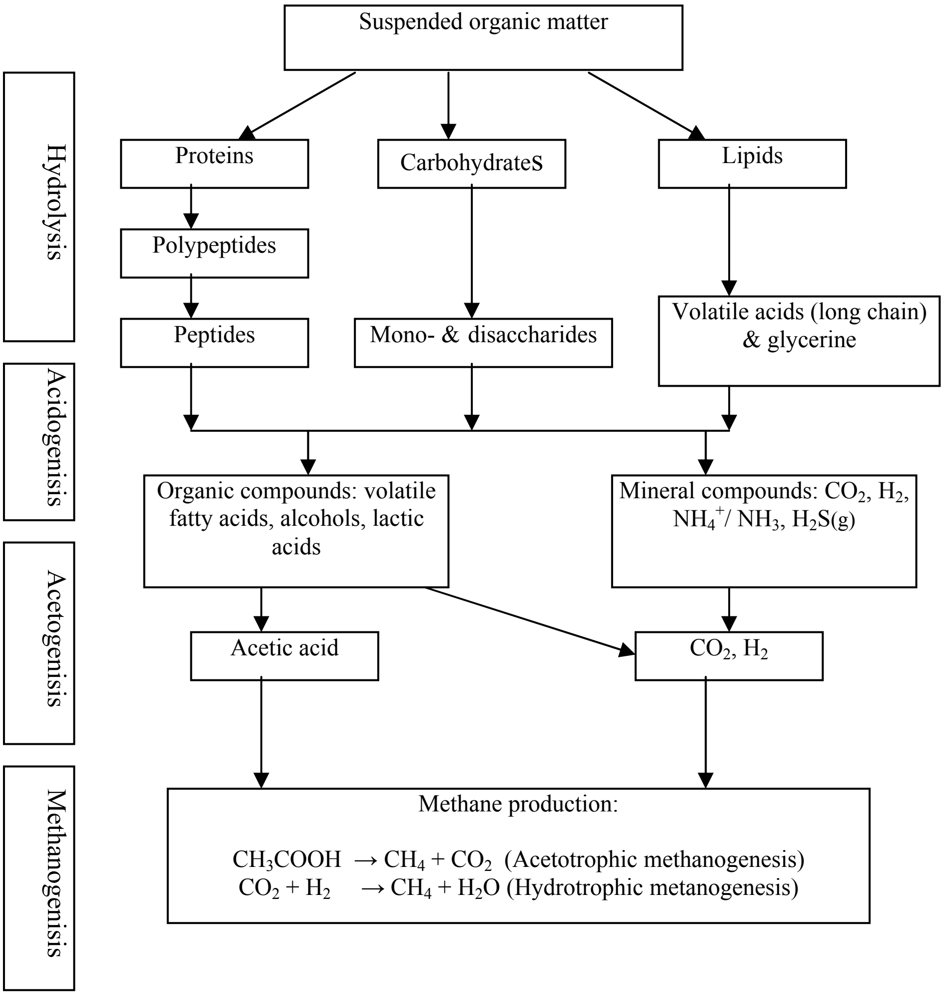


Figura-1.6. *Complex molecules reaction sequence during anaerobic digestion (van Haandel and Lettinga, 1994).*

The common use of anaerobic digestion for sludge stabilization was done because their several advantages: solids reduction of up to 60%, production of renewable energy (methane) from sewage sludge and improvement of dewatering and handling properties of digested sludge (Monteiro, 1997). However, some operating problems (poor process stability) caused by the slow growth of metahnogenic bacteria and loss of efficiency due to foaming, have been also cited as ones of the most common problems in anaerobic digestion (Pitt and Jenkins, 1990; Pagilla et al., 1996).

On other hand, agricultural use of sewage sludge is the more prioritized destination option by the EU legislation due to its positive effect on nutrients recycling and organic material reconstitution in the soil. However, sludge reuse in agricultural soil must satisfy healthiness conditions in order to avoid detest agents and harmful elements transference to the receptor medium.

Table 1.5. *Sludge classes and Pathogen reduction*

	US EPA 40 CFR 503		**Working document on sludge (CEC, 2000)**	
	Class A	Class B	Conventional treatment	Advanced treatment
Salmonella spp (MPN/4g TS)	< 3	-	-	No presence in 50g 4 log reduction
E. coli (CFU/ g TS)	-	-	2 log reduction $< 10^3$	6 log reduction $< 5 \cdot 10^2$
Cl. perfrigens (MPN/ gTS)	-	-	$< 3 \cdot 10^3$	-
F. Coliform (MPN/ g TS)	$<10^3$	$< 2 \cdot 10^6$	-	-
Enterovirus (MPN/4g TS)	<1	-	-	-
Helminth eggs (Ova/4 g TS)	<1	-	-	-

E. coli: Escherichia coli; Cl- perfringens: Clostridium perfringens; F. coliforms: fecal coliforms (human).

In that sense, the current legislation related to sludge application to land (EU Directive 86/278) is under revision (Working Document on sludge, 3rd draft, 2000) in order to ensure the long-term beneficial use of sludge. In the mentioned document, different types of treatment (conventional and advanced) are proposed as well as the limit values for several hazardous substances (Heavy metals, Pathogens and organic compounds). Besides, the conditions for sludge use on land are established.

Moreover, thermophilic anaerobic digestion was reported as an advanced treatment that can be reduce the pathogen load for land use without restriction (Carrington, 2001), which cannot be achieved with mesophilic anaerobic digestion treatment (see Table 1.5).

Since the important role of the temperature in the anaerobic treatment in determining the rate of digestion, particularly rates of hydrolysis and methane formation, was proved. The design operating temperature establishes the minimum SRT (or HRT) required to achieve a given amount of volatile solids destruction (Kiyohara et al., 2000).

Most digesters, of whatever configuration, tend to be operated in the mesophilic temperature range (30–35 °C). However, because of the increased demands on sewage sludge treatment (hygienization, dewatering, storage and sludge reduction), the mesophilic process is being supplemented or complemented with a thermophilic treatment.

The mesophilic digestion usually requires over 20-d retention time and it is not very efficient in the reduction of volatile solids and the deactivation of pathogenic organisms, thus producing Class B sludge, which cannot be reused without site and application restriction. To overcome these limitations, interest in thermophilic digestion has increased (Fang and Chung, 1999; Zabranská et al., 2000).

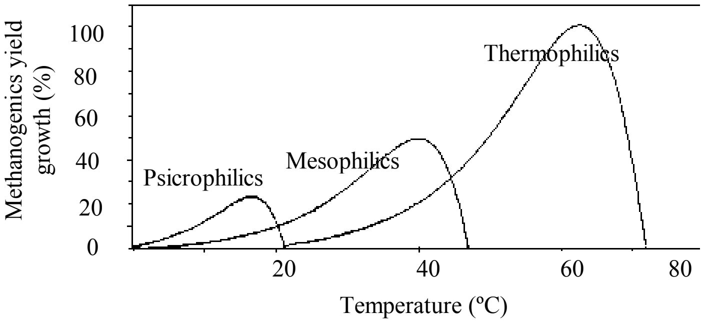


Figure 1.7. *Methanogenics growth temperature dependence (van Lier et al., 1997).*

The thermophilic AD brings an acceleration of the biochemical reactions, a greater degree of hygienization and higher efficiency in the degradation of organic matter in comparison with the mesophilic processes. Many mesophilic bacteria have their thermophilic homologues, but they may not be always present in the thermophilic sludge (Uemura and Harada, 1993). Such bacteria need a sufficient adaptation period after temperature change to transform enzymes, proteins, nucleic acids, lipids and other cell components to thermophilic states. The growth rates of thermophilic bacteria are 2-3 times higher than those of mesophilic bacteria (see Figure 1.7) and a gradual increase of methanogenic activity corresponds with increasing temperature from mesophilic to the thermophilic range (van Lier et al., 1997). However, the biomass yield of thermophilic bacteria is substantially lower, which may be attributed to the higher maintenance energy demands (Zinder, 1986).

The better performance of thermophilic digestion in reduction of volatile solids and deactivation of pathogenic organisms leads to Class A sludge, which can be used without any restrictions and represents the highest quality product in terms of pathogen content and vector attraction. In contrast, the effluent quality is poor with high offensive odours, due to high ammoniac and volatile fatty acids (VFA) concentrations (Ghosh et al., 1995; Nielsen and Petersen, 2000), and high-energy requirement (Kim et al., 2002). Furthermore, the thermophilic digestion is a little more sensitive to operational conditions, such as temperature, the organic loading rate and the characteristics of the influent.

Table 1.6. *Operational conditions and yields of mesophilic and thermophilic digesters treating sewage sludge.*

	Influent	Reduction (%)		SGP (m^3/kg-VS)			Reference
	VS (g/L)	VS	COD	Biogas	Methane	% CH$_4$	
Mesophilic	34	31.3	-	0.32[a] (1.00[b])		65	Rimkus et al., 1982
	19	27	-	0.30[a]	-	62	Cecchi et al., 1992
	12	44 - 56	36	0.81 - 0.91[b]	0.49 - 0.55	61	Tapana and Pagilla, 2000
	-	54 - 62	-	0.74 - 0.93[b]	-	-	Pagilla et al., 1997
	17	50 - 54	-	-	0.40 - 0.70[b]	-	De la Rubia et al., 2002
	11 - 16	53 - 54	-	-	-	66	Zabranská et al., 2000
	-	32 - 47	-	-	0.50 [b]	65 - 72	Han et al., 1997
	12 - 57	48	-	0.39[a]	-	-	Oles et al., 1997
	-	51 - 57	-	-	0.28 - 0.33[a]	-	Fujishima et al., 2000
	-	50	-	-	-	-	Killilea et al., 2000
	-	41	-	0.96[b]	0.60 [b]	63	Malina, 1961
	-	45	-	1.14[b]	0.71 [b]	62	Toya, 1984
	-	42	45	-	0.60 - 0.80 [b]	-	Govin et al., 1991
	14 - 19	35 - 52	-	-	0.41 - 0.50 [b]	62 - 67	Song et al., 2004
	-	-	-	0.54[a]	-	66 - 67	Dohanyos et al., 2004
	8 - 11	50 - 63	-	0.5 - 0.58	0.30 - 0.35	61 - 63	Watanabe et al., 1997
Thermophilic	34	34	-	0.40[a] (1.20[b])		65	Rimkus et al., 1982
	37	-	-	0.19[a]	-	65	Cecchi et al., 1992
	14 - 16	54 - 56	-	-	-	66	Zabranská et al., 2000
	-	44	-	0.81[b]	0.52 [b]	63	Malina, 1961
	-	50	-	1.05[b]	0.65 [b]	62	Toya, 1984
	14 - 17	41 - 52	-	-	0.35 - 0.48 [b]	62 - 65	Song et al., 2004
	-	-	-	0.71[a]	-	66 – 67	Dohanyos et al., 2004
	10 - 17	22 - 53	-	0.13-0.53	0.07 - 0.33	53 - 62	Watanabe et al., 1997

SGP: Specific Gas Production; [a]Expressed per kg VS fed; [b]Expressed per kg VS destroyed.

Concerning the ability to dewater the residual sludge, there is no common trend in literature; while some authors state that the thermophilic process cause an improvement of the dewatering properties of digested sludge (Buhr and Andrews, 1977; Garber, 1982), other indicate the opposite (Nielsen and Petersen, 2000; Kim et al., 2002)

Although each process has its unique advantages, depending on the digestion environment, microorganisms and process configuration, all these features of the thermophilic process are of great technological importance, because they enable to operate the digestion with a higher loading rate or use a smaller volume of digester.

Therefore, the change of temperature from mesophilic to thermophilic conditions leads to a better utilization of the existing facilities and consequently avoids the overloading of the digesters, the higher degradation efficiency is connected with higher biogas production and a lower content of volatile solids in the stabilized sludge (see Table 1.6).

The enhancement of AD efficiency and advanced hygienisation achieved under thermophilic digestion, as reported by several researchers (Nielsen y Petersen 2000; Watanabe et al., 1997; Zábranská et al., 2000b), induced to think that thermophilic AD can be to promote the removal of some recalcitrant organic micropollutants (Christensen et al., 2004; Feitkenhauer and Märk, 2003, Feitkenhauer et al., 2003; Holliger and Zehnder, 1996; Trably et al., 2003;).

1.2. References

Barceló D., Petrovic M. (**2004**). Los microcontaminantes orgánicos: Problemática y situación en Cataluña bajo la perspectiva da le Directiva Europea**.** Jornada sobre gestión y tratamiento de lodos en estaciones depuradoras de aguas residuales. Barcelona (Spain), December. (In spanish).

BOE nº 166. (**2001**). Plan Nacional de Lodos de Depuradoras de Aguas Residuales 2001-2006. July 12th. (In Spanish).

Buhr H.O., Andrews J.F. (**1977**). Review paper. The thermophilic. anaerobic digestion process. Water Res., 11, 129-143.

Campbell H.W. (2000). Sludge management – Future issues and treands. Wat. Sci Technol., 41(8), 1-8.

Carballa M. (**2005**). Fate of pharmaceutical and personal care products (PPCPs) in sewage treatment plant focusing on the anaerobic digestion of sludge. PhD Thesis, University of Santiago de Compostela.

Carrington E.G. (**2001**). Evaluation of sludge treatments for pathogen reduction. European Commission DG Environment, Final report, sept 2001, Office for official publications of the European communities, Luxembourg.

CEC (**2000**). Working Document on Sludge (**3rd Draft**), Commission of the European Communities Directorate-General Environment, ENV.E.3/LM, 27 April 2000, Brussels.

Cecchi F., Pavan P., Musacco A., Mata-Álvarez J., Sans C. (**1992**). Comparison between thermophilic and mesophilic anaerobic digestion of sewage sludge coming from urban wastewater treatment plants. Wat. Sci. Technol., 26, 2409-2412.

Christensen N., Batstone D.J., He Z., Angelidaki I. y Shmidt J.E. (**2004**). Removal of polycyclic aromatic hydrocarbons (PAHs) from sewage sludge by anaerobic degradation. Wat. Sci. Tech., 50 (9), 237-244.

De la Rubia R.M.A. 2000. Viabilidad técnica de la implantación de la degradación anaerbia termofílica de lodos de EDAR. PhD Thesis, University of Cádiz. (in Spanish).

De la Rubia M-A., Pérez M., Martínez A., Andrades J.A., Romero L.I., Sales D. (**2002**). Puesta en marcha y operación de un digestor anaerobio mesofilico de lodos. Tecnol. Agua, 220, 53-57.

De la Rubia R.M.A., García P.M., García R. L.I., Márquez S.D. (**2001**). Dgestión anaerobia termofílica versus digestión anaerobia mesofílica de lodos de EDAR. Residuos, 62 (11), 64-68.

Dohanyos M., Zabranská J., Kutil J., Penicek P. (**2004**). Improvement of anaerobic digestion of sludge. Wat. Sci. Technol., 49, 89-96.

Eljarrat E., Barceló D. (**2002**). Congener-specific determination of dioxins and related compounds by gas chromatography coupled to LRMS, HRMS, MS/MS and TOFMS**.** J. Mass Spctrom., 37(11), 1105-1107.

Fang H.H.P., Chung D.W.C. (**1999**). Anaerobic treatment of proteinaceous wastewater under mesophilic and thermophilic conditions. Wat. Sci. Technol., 40, 77-84.

Feitkenhauer H., Märkl H. (**2003**). Biodegradation of aliphatic and aromatic hydrocarbons at high temperatures. Wat. Sci. Tech., 47 (10), 123–130.

Feitkenhauer H., Müller R., Märkl H. (**2003**). Degradation of polycyclic aromatic hydrocarbons and long chain alkanes at 60 70 ºC by *Thermus* and *Bacillus* spp. Biodegradation, 14, 367–372.

Fujishima S., Miyahara T., Naike T. (**2000**). Effect of moisture content on anaerobic digestion of dewatered sludge: ammonia inhibition to carbohydrate removal and methane production. Wat. Sci. Technol., 41, 119-127.

Garber W.F. (**1982**). Operating experience with thermophilic anaerobic digestion. J. Wat. Poll. Fed., 54, 1110-1117.

Ghosh et al., 1995 Ghosh S., Buoy K., Dressel L., Miller T., Wilcox G., Loos D. (**1995**). Pilot- and full-scale two-phase anaerobic digestion of municipal sludge. Wat. Env. Res. 67: 206-214.

Govin R., Flaherty P.A., Dobbs R.A. (**1991**). Fate and effects of semivolatile organic pollutants during anaerobic digestion of sludge. Wat. Res., 25, 547-556.

Han Y., Sung S., Dague R.R. (**1997**). Temperature-phased anaerobic digestion of wastewater sludge. Wat. Sci. Technol., 36, 367-374.

Holliger C., Zehndert A. JB. (**1996**). Anaerobic biodegradation of hydrocarbons. Current Opinion in Biotech., 7, 326-330.

Killilea J.E., Colleran E., Sachill C. (**2000**). Establishing procedures for design, operation and maintenance of sewage sludge anaerobic treatment plants. Wat. Sci. Technol., 41, 305-312.

Kim M., Ahn Y-H., Speece R.E. (**2002**). Comparative process stability and efficiency of anaerobic digestion; mesophilic vs. Thermophilic. Wat. Res., 36, 4369-4385.

Kiyohara Y., Miyahara T., Mizuni O., Noike T., Ono K. (**2000**). A comparative study of thermophilic and mesophilic sludge digestion. The Journal of the Chartered Institution of Water and Environmental Management, 14, 150-154..

Magoarou P. (**2000**). Urban wastewater in Europe- what about the sludge? Proc. EU workshop on problems around sludge. 28-29 November, Stresa, Italy: 9-16.

Malina J.F. (**1961**). The effect of temperature on high-rate digestion of AS. In proceedings of the 16th Industrial Waste conference. Perdue University, Lafayette, Indiana, 232-250.

Metcalf & Eddy, Inc. revised by George Tchobanoglous, Franklin L.B. (**1991**). Wastewater engineering treatment, disposal, and reuse. 3rd ed. New York [etc.] McGraw-Hill corp.

Monteiro P.S. (**1997**). The influence of the anaerobic digestión process on the sewage sludges rheological behaviour. Wat. Sci. Technol., 36, 61-67.

Nielsen B., Petersen G. (**2000**). Thermophilic anaerobic digestion and pasteurisation. Practical experience from Danish wastewater treatment plants. Wat. Sci. Technol., 42, 65-72.

Oles J., Dishtl N., Niehoff H. (**1997**). Full scale experience of two stage thermophilic/mesophilic sludge digestion. Wat. Sci. Technol., 36, 449-456.

Ortiz, S. (**2004**). La gestión de biosólidos en la agència catalana de l´aigua. Jornada sobre gestión y tratamiento de lodos en estaciones depuradoras de aguas residuales. Barcelona (Spain), December. (In spanish)

Pagilla K.R., Craney K.C. and Kido W.H. (**1996**). Aerobic thermophilic pre-treatment of mixed sludge for pathogen reduction and Nocardia control. Wat. Environ. Res., 68, 1093-1098.

Pagilla K.R., Craney K.C., Kido W.H. (**1997**). Causes and effects of foaming in anaerobic sludge digesters. Wat. Sci. Technol., 36, 463-470.

Pavlostathis S.G., Jagalal K. (**1991**). Desorption behavior of trichloroethylene in contaminated soil. Environ. Sci. Technol., 25,274.

Pavlostthis S.G., Giraldo-Gómez E. (**1991**). Kinetics of anaerobic treatment. Acritical Reviews in Environmental Control, 22(5-6), 411-490.

Pitt P.A., Jenkins D. (1990). Causes and control of Nocardia in activated sludge. J. Water Pollut. Control Fed., 62, 143-150.

Ray B.T., Lin T.G. and Rajan R.V. (1990). Low level alkaline solubilisation for enhanced anaerobic digestion. Res. J. Water Pollut. Control Fed., 62, 81-87.

Rimkus R., Ryan J., Cook E. (**1982**). Full scale thermophilic digestion at the west-southetwest sewage treatment works, Chicago, Illinois. Journal WPFC, 54 (11), 1447-1457.

Rulkens, W.H. (**2004**). Sustainable sludge – what are the challenges for the future?. Wat. Sci Technol., 49 (10), 11-19.

Song Y.C., Kwon S.J., Woo J.H. (**2004**). Mesophilic and thermophilic temperature co-phase digestion compared with single-stage mesophilic and thermophilic digestion of sewage sludge. Wat. Res., 38, 1653-1662.

Spinoza, L. (**2001**). Evolution of sewage sludge regulation in Europe. Wat. Sci Technol., 44(10), 1-8.

Subramaniam K., Stepp C., Pignatello J.J., Smets B., Grasso1D. (**2004**). Enhancement of polynuclear aromatic hydrocarbon. Desorption by complexing agents in Weathered Soil. Environ. Eng. Sci., 21(4), 515-523.

Tapana C., Pagilla K.R. (**2000**). Anaerobic thermophilic/mesophilic dual-stage sludge treatment. J. Environ. Eng., 126, 796-801.

Toya Y. (**1984**). Comparison of sludge digestion between mesophilic and thermophilic temperatures. In proceedings of the 9th Conference on Wastewater Treatment Technology in the Republic of China. Tainan, Taiwan, 29-40.

Trably E., Patureau D., Delgenes J.P. (**2003**). Enhancement of polycyclic aromatic hydrocarbons removal during anaerobic treatment of urban sludge. Wat. Sci. Tech., 48 (4), 53–60.

Uemura S., Harada H. (**1993**). Microbial characteristics of methanogenic sludge consortia developed in thermophilic UASB reactors. Appl. Microbiol. Biotechnol., 39, 654-660.

USEPA. (**1999**). (*40 CFR 503 Rule*) Environmental Regulations and Technology. Control of Pathogens and Vector Attraction in Sewage Sludge. (Including Domestic Septage) Under 40 CFR Part 503. United States Environmental Protection Agency Office of Research and Development.

Washington, DC 20460 EPA/625/R-921013 Revised October 1999. www.epa.gov/ORD/NRMRL.

Van Haandel A., Lettinga G. (1994). Anaerobic sewage treatment : a practical guide for regions with a hot climate . editor: Wiley , New York, USA.

Van Lier J.B., Rebac S., Letting G. (**1997**). High rate anaerobic wastewater treatment under psychrophilic and thermphilic conditions. Wat. Sci. Technol., 35, 199-206.

Watanabe H., Kitamura T., Ochi S., Ozaki M. (**1997**). Inactivation of Pathogenic Bacteria under Mesophilic and Thermophilic Conditions. Wat. Sci. Tech., 36(6-7), 25-32.

Zábranská, J., Stepová J., Wachtl R., Jenícek P., Dohányos M. (**2000**). The activity of anaerobic biomass in thermophilic and mesophilic digesters at different loading rates. Wat. Sci. Tech., 42, 49-56.

Zinder S.H. (**1986**). Thermophlic waste treatment systems. Brock T.D. (e.d.). thermophiles: general, molecular and applied biology. Wiley-Interscience, New York, 257-277.

Chapter 2

Objectives

Chapter 2

2. Objectives

The main objective of the present thesis is to study the fate of some organic micropollutants during raw sewage sludge (RSS) anaerobic digestion and the effect of thermophilic temperature conditions on treatment efficiency, when compared with mesophilic anaerobic digestion. To this purpose, the influence of a mesophilic and thermophilic anaerobic digestion and Hydraulic Retention Time (HRT) reduction on the biodegradability/biotransformation of some selected organic micropollutants, in accordance with the 3rd draft of Directive presented to the European Commission (CEC, 2000) is studied. Furthermore, the effect of a complementary treatment, such as the ultrasonic pretreatment, on the biodegradability of the organic feed and the behaviour of the subsequent anaerobic digestion is also examined, focussing on the micropollutants reduction.

Particularly, the specific objectives of this study are:

- To study the start-up of the anaerobic digesters using Waste Activated Sludge (WAS) as the inoculum in comparison with other procedures (Chapter 4).
- To determine the minimum HRT reachable in the thermophilic digestion of sewage sludge and to evaluate the evolution of the most relevant yield parameters (Chapter 4).
- To determine the fate of two very recalcitrant compounds, Polycyclic Aromatic Hydrocarbons (PAH) and Di-2-(Ethyl-Hexyl)-Phthalate (DEHP), which worldwide use could not be substituted yet (Chapter 5).
- To monitor the evolution of halogenated compounds, expressed as Adsorbed Organic Halogenated compounds (AOX) and the specific family PolyChlorinated Biphenyls (PCB) in the sludge when it is treated by means of an anaerobic digestion process (Chapter 6).

- To examine the content of anionic and non-ionic surfactants, namely Linear AlkylBenzene Sulphonates (LAS) and Nonyphenols (NPE), when thermophilic and mesophlic anaerobic digestion is applied (Chapter 7).

- To assess the effect of HRT on the aforementioned micropollutants removal (Chapters 5, 6 and 7)

- To assess the influence of ultrasonic pre-treatment of RSS on its biodegradability and to evaluate the consequent enhancement of both naphthalene and pyrene removal efficiencies after anaerobic treatment (Chapter 8).

All these microcontaminants have been selected because they are pointed out in the 3rd draft of European Directive as frequent xenobiotics, which can be found in sludge and their content elimination and/or reduction below the limit values should be carried out for their agricultural use.

Since anaerobic digestion is considered as the main sludge stabilization treatment, it is very important to verify their performance regarding the actual and possible future restrictions to guaranty the safety and healthness conditions and the environmental friendly use of stabilized sludge. Moreover, the introduction of new facilities and processes to enhances anaerobic digestion performance and effluent quality states, up till now, under study or at limit industrial application. Hence, the advantages of the pretreatment incorporation in anaerobic sewage sludge stabilization process for quality enhancement should be assessed.

Chapter 3

Materials and Methods

3. Materials and Methods

3.1. Experimental set-up

Lab-scale digesters

Two completely mixed and jacketed anaerobic digesters (5 L) were used in this study. Both digesters were equipped with pH probe (Crison pH28) and were connected to feed tank and biogas collection-measuring system (Figure 3.1). Two peristaltic pumps (Multiflex) performed the fill and draw of the reactor. A Siemens programmable logic controller (PLC Logo230RC-DM8 230R) was used to control the system. The operating temperature was controlled by means of two heating systems (Haake DC 40) at 55°C and 35ºC for the thermophilic and mesophilic reactors, respectively.

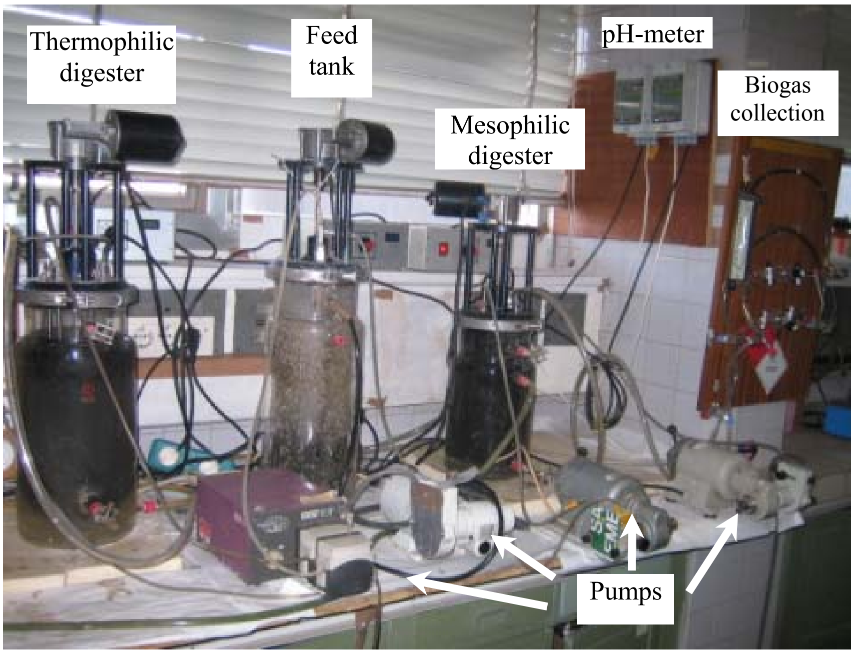


Figure 3.1. *lab-scale anaerobic digestion installation.*

Biogas production meter

The measuring system, designed by Mata-Álvarez et al. (1986), is based on a simple displacement device through the two identical glass columns whose are connected at the lower ends and medium zones for acidified Black Eriochromo T (BET) solution. The BET solution rise/decrease, in the second column, activates photocell-electromechanical pulse counter system and records half/one counter unit (Figure 3.2). The equipment was equilibrated to measure 60-70 mL per counter unit.

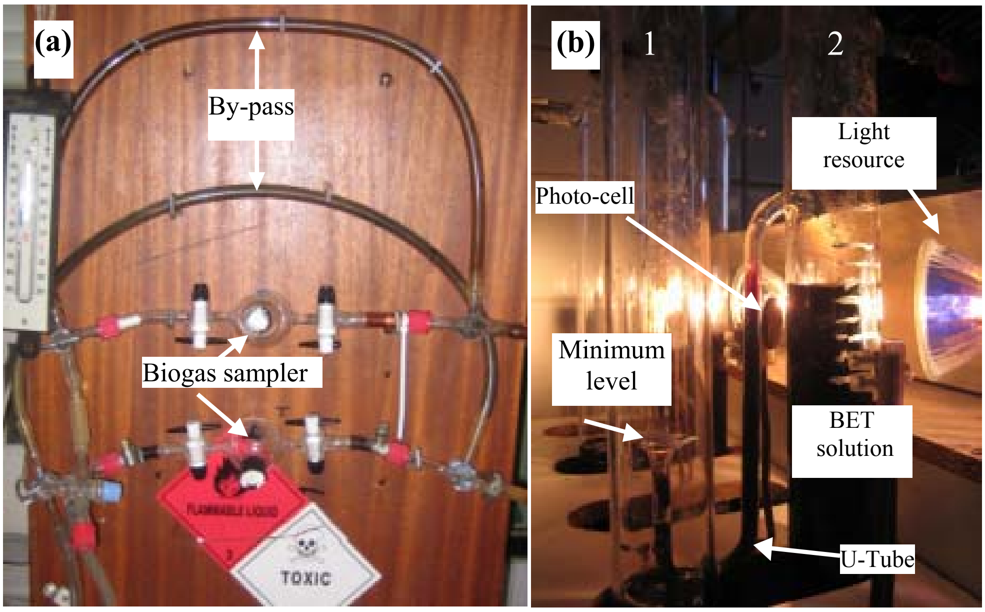


Figure 3.2. *Biogas recollection (a) and biogas measuring device (b).*

3.2. Analytical methods

In this section, the methods used for the determination of the conventional parameters of wastewater and sludge are described. For soluble fraction analysis, the samples were previously centrifuged at 10,000 rpm for 30 minutes.

3.2.1. Conventional parameters

Solids (TS, TSS, VS, VSS)

Total solids (TS), Total suspended solids (TSS), Volatile Solids (VS) and Volatile Suspended Solids (VSS) are determined following the Standard Methods (APHA, 1992).

TS are determined weighing about 5 mL well-mixed sample in a previously clean dish (heated to 105 ºC) after being evaporated at 105 ºC until constant weight in a drying oven (AFORA PM-9952). The increase in weight over that of the empty dish represents the total solids in a correspondent initial sample amount or volume (see Equations 3.1 and 3.2).

$$Total\ Solids\,(\%) = TS(\%) = \frac{Weight_{105\,°C} - Weight_{dish}}{Weight_{sample}} \times 100 \qquad (3.1)$$

$$TS(g/L) = \frac{Weight_{105\,°C} - Weight_{dish}}{Volume_{sample}} \times 1{,}000 \tag{3.2}$$

For the determination of TSS a well-mixed sample volume is filtered through a weighed glass-fiber filter (0.7 μm pore size) and the residue retained on the filter is dried to a constant weight at 105 ºC. the increase in weight of the filter represents the total suspended solids.

To determine the VS or VSS, the residue from TS or TSS is ignited to constant weight at 550 ºC in a muffle furnace (Herotec JB20). The weight loss on ignition corresponds to the volatile solids (See Equations 3.3 and 3.4).

$$VolatileSolids(\%) = VS(\%) = \frac{Weight_{105°C} - Weight_{550°C}}{Weight_{105°C} - Weight_{dish}} \times 100 \tag{3.3}$$

$$VS\ (g/L) = \frac{Weight\ _{550°C} - Weight\ _{105\,°C}}{volume_{sample}} \times 1000 \tag{3.4}$$

Chemical Oxygen demand (COD)

The Chemical Oxygen Demand is an indirect organic matter amount mesure. It is defined as the amount of oxygen required to oxidize the matter present in the sample using a strong chemical oxidant (potassium dichromate) in an acid environment. The tube-test, based on a closed reflux-colorimetric standard method (APHA, 1992), was followed to COD determination. Velp-ECO25 thermo-reactor and Shimadzu-UV1203 spectrophotometer were used.

Volatile Fatty Acids (VFA)

Several methods, based on different techniques, were cited for VFAs determination, volumetric (Di Lallo and Albertson, 1961), colorimetric (Stafford et al., 1982), chromatographic (Alen et al., 1985) and extractive (Spiller et al., 1980).

In this study, VFA concentration and composition analysis were performed on Hewelett-Packard-5890A chromatograph equipped with Flame Ionisation Detector

(FID) and using N_2 as gas carrier (Llabrès and Mata-Álvarez, 1988). The operating conditions were as follow:

COLUMN: Nukol 15m. 0.53 mm, 0.5μm film
SUPELCO 25325 column # 16379-02c

COLUMN CONDITIONS :	Initial temperature: 85 ºC Initial time: 1.5 min
	1st Ramp: 8 ºC/min 1st Final Temperature: 120 ºC 1st Final Time: 0.00 min
	2nd Ramp: 10 ºC/ min 2nd Final Temperature: 145 ºC 2nd Final Time: 0.00
	3rd Ramp: 20 ºC/min 3rd FinalTemperature: 175 ºC 3rd Final Time: 2.00 min
INJECTOR CONDITIONS:	280 ºC
FID CONDITIONS:	300 ºC
CARRIER GAS:	HELIUM: 33 mL/min 11 psi
AUXILIAR GAS:	AIR: 400 mL/min H_2: 3 mL/ min
SPLIT	Inlet vent: 56 mL/ min Septum purge: 3.5 mL/ min

Table 3.1. *typical retention time and factors calibration of Acid standards mixture.*

Compound	Retention time (min.)	1/Area (s·mV)	Concentration (ppm)
Acetic Acid	1.795	$8.39147\ 10^{-4}$	499.10
Propionic Acid	2.482	$5.74605\ 10^{-4}$	609.60
Isobutyric Acid	2.686	$4.51378\ 10^{-4}$	776.26
Butyric Acid	3.361	$5.01221\ 10^{-4}$	885.28
Isovaleric Acid	3.762	$5.34219\ 10^{-4}$	887.04
Valeric Acid	4.576	$5.37766\ 10^{-4}$	1052.64
Isocaproic Acid	5.307	$6.71813\ 10^{-4}$	1052.64
Caproic Acid	5.814	6.4458710^{-4}	1160.00
Heptonic Acid	7.005	$7.99958\ 10^{-4}$	1163.48

Previously, calibration curve was made by acid standards mixture purchased from Supelco. The typical retention times and calibration factors are shown in Table 3.1.

Alkalinity

Alkalinity of sludge or water is the acid-neutralizing (or medium-buffering) capacity, which represents the sum of all medium existing bases. It is mainly function of carbonate (CO_3^{2-}), bicarbonate (HCO_3^-) and hydroxide (OH^-) content.

Alkalinity measurements are used in the interpretation and control of anaerobic processes, since the buffering capacity of the system should be enough to avoid the system destabilization by the possible accumulation of acid compounds, such as VFA, which would lead to a pH drop and, consequently, the microorganisms activity inhibition. Usually, the anaerobic digesters present a typical supernatant alkalinity of 2000-4000 mg $CaCO_3$/L depending basically on solids content.

In fact, alkalinity/acidity equilibrium maintenance is considered as the principal key for efficient anaerobic systems (Rimkus et al., 1982). Hence, it has been suggested Total Acidity/ Alkalinity ratio (TA/Alk) to be used as control parameter in anaerobic digesters, recommending a value between 0.1 and 0.3 depending on operating conditions (temperature and organic load) (Rimkus et al., 1982; USEPA, 1976).

Some researchers proposed the addition of buffering elements, normally $CaCO_3$, if the anaerobic digesters destabilization was stated (le Roux et al., 1979).

The alkalinity was measured following the standard methods (APHA, 1992) using automatic titration instrument (TitroLine Alpha plus TA20 SCHOTT) equipped with Hamilton electrode (Bonaduz AG). The method consists of a titration of a centrifuged supernatant sample volume (20 mL) at room temperature with standard acid (HCl 0.1 N) to desired end point (pH 4).

The alkalinity, expressed as mg $CaCO_3$/L, is then calculated from the following equation.

$$Alkalinity\ (mgCaCO_3/L) = \frac{mL_{HCL} \cdot 0.1N \cdot 50{,}000\ (mg/N)}{mL_{sample}} \tag{3.5}$$

Total Acidity

Sludge or water acidity is the quantitative capacity for their reaction with a strong base until a designed pH end point. This alkalinity value indicates the promoted acidity by the present acids (strong minerals acids, carbonic acids). Their determination procedure is similar to the alkalinity measurement, changing the titrant agent by NaOH (0.1 N) and the end point titration to pH 8.3. Then, the total acidity, expressed as mg acetic acid (HAC)/L, is calculated from the following equation.

$$Acidity\ (mgHAc/L) = \frac{mL_{NaOH} \cdot 0.1N \cdot 60{,}000\ (mg/N)}{mL_{sample}} \qquad (3.6)$$

pH

pH is one of the important parameters used in wastewater and sludge monitoring, since its bearing in the adequate range is a required condition for the biological activity in the treatment process.

Digesters pH measurement was performed with an electrode (pH Crison-52:21) connected to a measurement device pH/mV (CRISON pH28). The electrode calibration was carried out by two buffer solution of pH 7.2 and 4.0 or 9.0. However, the pH sewage sludge (Feed) was measured with a portable pHmeter (Crison 507).

Ammonia-Nitrogen

In water and wastewaters, the main forms of nitrogen are nitrate, nitrite, ammonia and organic nitrogen. All these forms, as well as nitrogen gas (N_2), are biochemically interconvertible and they are the components of the nitrogen cycle.

In anaerobic digestion, ammonia nitrogen is distinguished as the most available nitrogen form for microorganisms. Insufficiency or excessive amount can cause process destabilization (Berruela and Castrillón, 1997). For this, anaerobic digesters ammonia concentration control is considered fundamental.

The ammonia nitrogen determination was performed using ammonia-selective electrode method, specified for high ammonia concentration (APHA, 1992; Martínez-Viturtia, 1989; Torres, 1992). It is based on the conversion of dissolved ammonia ($NH_{3(aq)}$ and NH_4^+) to $NH_{3(aq)}$ by raising sample pH to above 11 with a strong base (10N NaOH) and a subsequent $NH_{3(aq)}$ diffusion through a gas-permeable membrane. Then, the voltage (mV) of the recorded potential (CRISON MicropH2002) is converted to the corresponding amount (Equation 3.7).

$$\text{Log (ppm } NH_4^+\text{-N)} = \alpha \cdot (\text{mV}) + \beta \qquad (3.7)$$

Previously, the calibration curve is done with 8 points (5-100 mg $N\text{-}NH_4^+$/L) using NH_4Cl as standard solution. The optimum slope value (α) of the calibration curve is estimated to be around -0.017 (APHA, 1992)

Biogas Composition

The biogas composition is an important parameter to determine the methanization potential of the anaerobic biomass. Besides, it is a good indicator of reactor efficiency, since an accumulation of the acids in the digesters would lead to an increase a content of other gas as CO_2 (produced during the neutralization of the acids by the bicarbonate).

The gas chromatography equipped with Thermal Conductivity Detector (TCD) is considered as the best and reliable determination method of biogas composition (Martínez-Viturtia, 1989; Torres, 1992).

Hence, The biogas composition analysis is determined by a Shimadzu GC-9 A chromatograph with TCD using two identical stainless steel columns (Porpack Q 80/100) with 3 m of length and 1/8" as external diameter. One of the columns is used as reference whereas the biogas sample (0.5 mL) is injected in the second. Helium is used as carrier gas with a flow of 30 mL/min. The analysis conditions were as follow:

Injector Temperature: 45ºC
Oven Temperature: 37ºC
Detector Temperature: 100ºC
Sensibility: 100 mA
Time: 8 min

3.2.2. Organic Micropollutants analysis

Soxhlet extraction and Solid phase extraction

The first common step for all micropollutant analysis in dry sludge samples was the extraction. The Soxhlet extraction (Figure 3.3a) is considered the most strong and efficient extraction method (Amellal et al., 2001; Düring and Gäth, 2000; Eijsackers et al., 2001; Jiries et al.,2000; Pryor et al., 2002; Shu et al., 2003). However, analysis of organic micropollutant, in environmental samples at the low level, requires a pre-concentration of these elements prior to their quantitative determination. The Solid Phase Extraction (SPE) is a widely method used. This technique has replaced many classical methods, such as liquid-liquid extraction. In general, during SPE enrichment the analytes are sorbed when the liquid sample (extracted sample) is passed through the solid phase material and desorbed by elution with an organic solvent (see Figure 3.3b). The adsorption mode of the analytes onto SPE material depends on the characteristics of the applied materials and can be based on various interactions such as dispersion-, dipole/dipole-, ion/dipole-, hydrophobic interactions and ion exchange (Carballa, 2005)

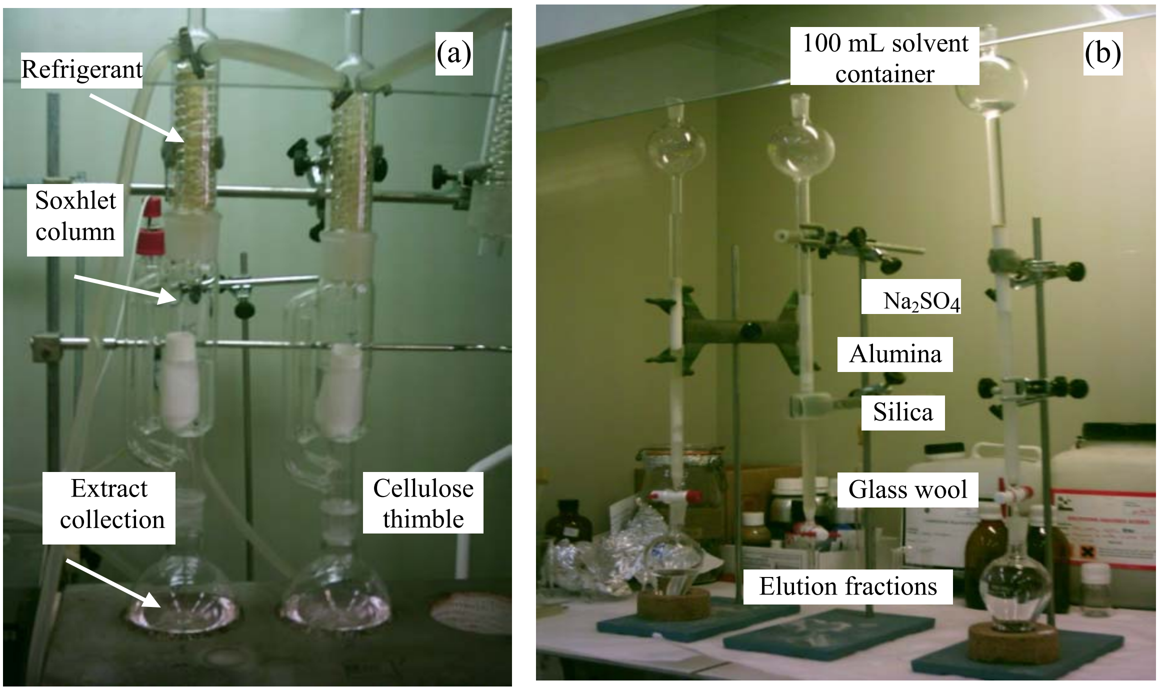


Figure 3.3. *(a) Soxhlet extraction and (b) Solid phase extraction.*

Chromatography

Chromatography techniques are dynamic processes wherein a mobile phase transports the sample mixture across or through stationary-phase medium. As the sample comes in contact with stationary phase, interactions between the sample and the stationary phase molecules occur. A partitioning or separation of the components in the mixture results from the different affinity of each component of the stationary phase. As the separated components emerge or elute, a detector responds with a signal change that is plotted against time, thus producing a chromatogram.

Two main types of chromatography are widely used, namely Gas (GC) and High Performance Liquid (HPLC) Chromatography.

In gas chromatography, the mobile phase is an inert carrier gas (He, Ar, N_2 depending on analysed sample characteristics) and the stationary phase is often a high molecular weight compound, which is deposited on the finely divided particles or on the wall of the long capillary tubing. The GC column is coupled with a temperature controlled injection port and sample extracts are injected into the carrier gas stream at a temperature sufficient to insure vaporization of all components. The vaporized sample is transported through the column by the flow of the inert mobile phase to detector. The main parameters that can be influenced by the analysis method are: temperature, gas flow, type and thickness of stationary phase, column length and diameter.

High performance liquid methods are used to separate dissolved substance. Compounds are separated by injecting a plug of the sample mixture onto column. The components in the mixture pass through the column at different retention time, due to difference in their partitioning behaviour between the mobile liquid phase and stationary solid phase. Two conditions can be used in HPLC determination: normal phase, which implies a very polar stationary phase and an unpolar mobile phase, and reverse phase, which is just the opposite. The latter technique is frequently applied in the trace analysis of the organic pollutants.

Mass Spectrometry and UV-Fluorescence detection

Chromatography techniques are very powerful for analytes separation, but they cannot identify them. Mass spectrometry provides detailed structural information and high selectivity in the quantification of the compounds. This makes both techniques very compatible.

A mass spectrum is the plot of the relative abundance of the molecule ions and its fragments versus their mass-charge-ratio (m/z). Therefore, MS comprises three separate processes: ionization, mass separation and recording of the ions formed. The ionization techniques depend on the chromatography used before. For GC, Electon Ionization (EI) and Chemical Ionization (CI) are the most common; however, Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI) are applied in HPLC. Mass spectrometers are classified according to the principle for separation of the ionic masses. Among the different detection systems available, ion trap and quadruple mass spectrometers have achieved the widest use, due to their relative easy handling, maintenance and their reasonable price.

HPLC detectors use several detection methods. Ultraviolet (UV) detectors measure the ability of a sample to absorb light at one or more wavelengths. Light scattering detectors nebulize the effluent, vaporize the solvent, and then detect droplets in a light scattering cell. Others detect the fluorescence that occurs when compounds are excited (lighted) by shorter wavelength energy and emit higher wavelength radiation.

PAH, DEHP, PCB, NPE analysis procedure

In this study, PAH, DEHP, PCB and NPE were analysed concomitantly using solvents and chemicals of analytical grade (Merck, Barcelona).

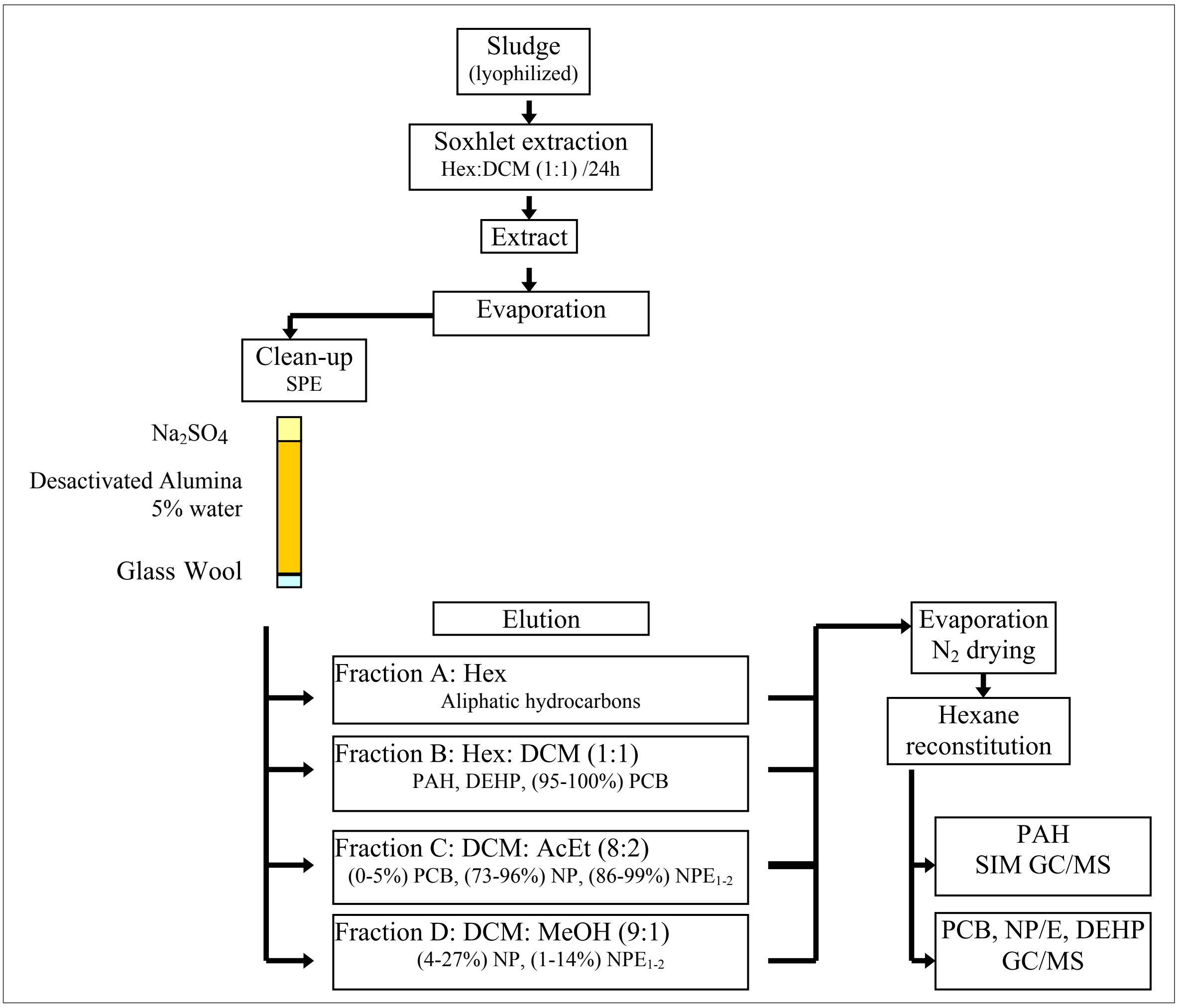


Figure 3.4. *Scheme of PAH, DEHP, PCB and NPE analysis procedure.*

16 PAH compounds mixture (including EU-PAH: Sum of Low Molecular Weighted (LMW) PAH (acenapthene, phenanthrene, fluorene) and High Molecular Weighted (HMW) PAH (fluoranthene, pyrene, benzo(b+j+k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, indeno(1, 2, 3-c,d)pyrene)) were supplied by Supelco (Barcelona). DEHP (95% purity) and Anthracene d_{10} were supplied by Fluka (Barcelona) and Sigma-

]

Aldrich (Barcelona), respectively. 6 PCB congeners mixture (including Sum of lightly chlorinated biphenyls “LCB” nº 28 y 52 which corresponds to tri- and tetra-chloro biphenyls and High chlorinated biphenyls “HCB” PCB nº 52, 101, 138, 180 which corresponds to penta-, hexa-, hexa- and hepta-chlorobiphenyls were supplied by Dr. Ehrenstorfer Gmbh-Sharlau S.A.(Barcelona). NP isomers mixture and NP1EO and NP2EO mixture (75:25) were supplied by Dr. Ehrenstorfer Gmbh-Sharlau S.A.(Barcelona).

Sample analysis were carried out as follows (see Figure 3.4): 10 g of lyophilized samples, initially spiked with anthracene d_{10}, an equivalent amount of 0.5 mg PCB/kg dm, an equivalent amount of 50 mg DEHP/kg dw and an equivalent amount of 100 mg NP/kg dw (PCB, DEHP and NP doping was for influent sub-sample only), were reflushed in a Soxhlet apparatus filled with a mixture of Dichloromethane: n-Hexane “DCM: Hex” (1:1) during 24 hours. The extract was purified and dried on alumina-sodium sulphate column by means of several elution solvents with different polarities, namely, (*i*) Hexane, (*ii*) Hex: DCM (1:1), (*iii*) DCM: AcEt “ethyl acetate” (8:2) and (*iv*) DCM:MeOH “methanol” (9:1). Then, eluates were concentrated under a gentle N_2 stream and were reconstituted in 1 mL Hex, before their injection into GC/MS. Both PAH and DEHP compounds were recovered in the second fraction (ii) of the elution solvents used (DCM: Hex). The PCB congeners were recovered in the second fraction (Hex:DCM) at more the 95% and in the third fraction (DCM:AcEt). However, NP, NP1EO and NP2EO compounds were recovered in the third and fourth fractions (iii and iv) of elution solvents used (DCM: AcEt and DCM:MeOH).

For PAH analysis, the samples were injected split-less by an automatic sampler (Shimadzu AOC-20i+S) into a gas-chromatograph (Shimadzu QP2010) equipped with an HP-5MS column (30m×0.25mm×0.25µm: length× inner diameter× film thickness). The injector temperature was 280ºC. and the flow 1mL/min. The oven was set to an initial temperature of 60 ºC for 1 min followed by an increasing temperature (10 ºC/min) up to 320 ºC, which was kept for 5 min. Helium was used as gas carrier. The MS was run in SIM mode, which differenced two groups, the first from 128 to 188 m/z and the second from 202 to 278 m/z. For DEHP analysis, the flow was set at 1.01 mL/min and the injector temperature was 280 ºC. The oven temperature programme had an initial temperature of 60 ºC for 3 min, followed by an increase (15 ºC/min) to 200 ºC; subsequently, the increasing rate was changed to 3 ºC/min until 300 ºC that was kept for 5 min. The last increasing rate was 5 ºC/min, until 320 ºC were reached. At this temperature, the oven was kept in isothermal conditions during 3 min. The SCAN mode was employed for identifying the DEHP corresponding picks (primary ion 149 m/z and the secondary ions 167 and 279 m/z).

For PCB analysis, the eluated samples were injected split-less mode. The injector temperature was 280ºC. The flow was 1mL/min. The oven was set to an initial temperature of 40 ºC for 1 min followed by an increasing temperature (20 ºC/min) up to 150ºC, then by (6 ºC/min) up to 300 ºC, which was kept for 5 min. Helium was used as gas carrier.
The MS detection was performed in SIM mode.

For NPE analysis, the eluated samples were injected split-less mode. The injector temperature was 280ºC and the flow was 1.01 mL/min. The oven was set to an initial temperature of 60 ºC for 3 min, followed by an increasing temperature (15 ºC/ min) up to 200ºC, then by (3 ºC /min) up to 300 ºC which was kept for 5 min and, finally, with (5 ºC /min) up to 320 ºC which was kept too for 3min. Helium was used as gas carrier.

The SCAN mode was employed for identifying the NPE, corresponding to picks in the ion range 50-550 m/z, correspondent to retention time range from about 5 min to 57.7 min.

LAS Analysis procedure

For LAS analysis (see Figure 3.5), 2.5 g of sludge, previously lyophilized and homogenized, were mixed with sodium hydroxide (5:1) and extracted three times by sonication for 20 min with 20 mL methanol. The final extracts were separated from the sediment by centrifugation and filtration, then vacuum evaporated until 1 mL at 35 ºC and dried under a gentle nitrogen stream (Comellas et al., 1993; Riu et al., 2001). 50 mL of methanol-water solution (30:70) were added and the pH was adjusted to 1.0 with concentrated HCl. Before the clean-up step, the treated extracts were sonicated for at least 1 min to dissolve sodium chloride. Octadecylsilica (C_{18}) cartridge was conditioned with 2 mL of methanol and 3 mL of 0.1 *M* HCl before its use. The entire sonicated solution was percolated through the C_{18} cartridge at 5 mL/ min. The column was washed with 2 mL of 0.1 *M* HCl. The target compounds were eluted twice with methanol (2×5mL) at 5mL/min with a waiting time of 5 min between the two elution steps. The methanol eluate could be injected directly into HPLC system (Comellas et al., 1993).

For LAS aqueous phase determination, supernatant samples (200 mL) were filtered and passed through C_{18} cartridge, previously conditioned with 7 mL of methanol and 3 mL of water (pH 3), at 1 mL/min. The cartridge was cleaned with 1 mL of water (1mL/min, pH 7) and dried for approximately 30 min under vacuum conditions (100 kPa negative pressure). The target compounds were eluted with methanol (2×5mL) at 5mL/min with a waiting time of 5 min between the two elution steps. The eluate was evaporated under a gentle nitrogen stream at 25 ºC before their reconstitution in 1 mL of water (Riu et al., 2001).

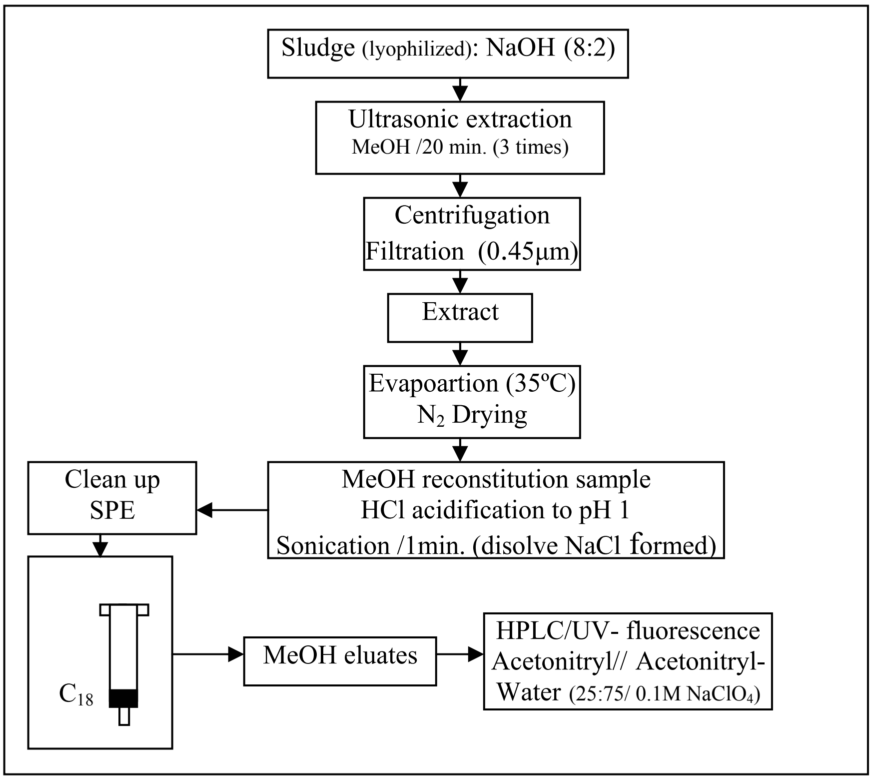


Figure 3.5. *Scheme of LAS sludge analysis procedure.*

The HPLC analysis was performed using Lichrosorb RP-18 250×4mm 10-µm column from TRACER ANALITICA. The chromatography system consisted of Waters 600 pump and Waters 700 Satellite WISP injector. Detection was performed by UV-Fluorescence spectrofluorimeter (KONTRON INSTRUMENTS SFM25) at 225 for UV detection and 225/295 as excitation/emission wavelengths for fluorescence detection (Comellas et al., 1993; Marcomini and Giger, 1988). UV detection provides a linear response between 30 and 2000 mg/L and a detection limit of 30 mg/L. Fluorescence detection reduces the detection limit to 40 µg/L (Comellas et al., 1993).

LAS determination for both solid and aqueous sample, was carried out by HPLC/UV-Fluorescence system using Acetonitrile-water (25:75) containing 0.1 M $NaClO_4$ (phase A) and acetonitrile as gradient eluants (phase B), at a flow rate of 1mL/min. A linear gradient elution was performed as described in Table 3.2 (Comellas et al. 1993).

Table 3.2. *LAS homologues separation conditions in liquid chromatography.*

Time (min)	Flow (mL/min)	%A	%B	Time (min)	Flow (mL/min)	%A	%B
Initial	1.00	85	15	25	1.00	30	70
1	1.00	85	15	35	1.00	30	70
20	1.00	60	40	37	1.00	85	15
22	1.00	60	40	45	1.00	85	15

LAS standard patrons were prepared with commercial LAS (Petresul® 550) purchased from PETRESA S.A. (Cádiz, Spain) in aqueous solution of water-NaLAS (10:1). The proportional weight composition of different homologues was: C10 (14.2%), C11 (35.6%), C12 (29.3%), C13 (20.9%) and average lineal alkyl chain carbon number and molecular weight were 11.57 and 318.97, respectively.

In order to estimate the recovery level index, additional feed samples were spiked with an amount corresponding to 2 mg LAS/L in supernatant and 400 mg LAS/kg dw in lyophilised sludge. The results obtained in the analytical procedure recorded a recovery index, for sum homologues LAS amount, between 74% and 108%. These results have a relative standard deviation in the range of 3%-35%.

AOX determination methodology [DIN 38414-S18]

For AOX analysis, the method applied was as described by DIN 38414 Part 18 established by Deutshe norm. Its principle is based on removal inorganic halogen from sludge by halide-free nitric acid solution (of pH 0.5) before activated carbon addition. Then, the mixture was pyrolised to convert the organically bound halogens (chloride and bromide) to hydrogen halide, which quantified by coulometric method as mg of chlorine. This procedure was performed using Euroglass AOX-Analyser, model ECS 2000.

The modification in this method consists in sludge-nitrate mixture solution filtration because the unavailability of auxiliary material for filter-paste pyrolisis and to avoid filter saturation with suspended particles

For AOX analysis, the 10 mL of nitrate wash solution (HNO_3-$NaNO_3$ solution) were added to 3 mg of dry sludge and filtered through 0.45 μm pore size filter before 20 mg activated carbon addition (100-200 mesh). After suspended carbon solution shaking (for not less than one hour), the mixture passed through 0.4 μm pore size polycarbonate membrane filter (Trallero & Schlee. S.L). Then, the washed, moist filter cake along with polycarbonate membrane filter was ignited in the pyrolisis apparatus (at not less than 950 ºC) and with adequate oxygen stream, previously passed through an absorber filled with concentrated sulphuric acid.

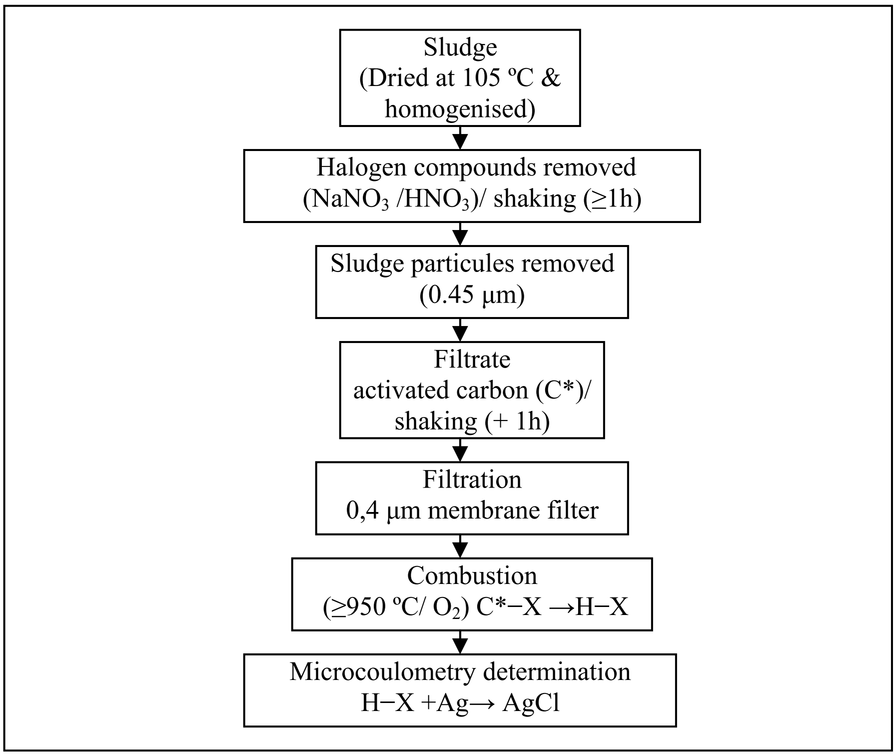


Figure 3.6. *Scheme of AOX analysis [DIN 38414-S18 (modified)].*

For checking combustion apparatus and microcoulometer detection, standard patrons (in the range 0.01-0.2 mg Cl/L) were prepared from 1mg Cl/L p-chlorophenol solution. However, for method recovery index, the sub-samples (feed dry sludge) were spiked with an equivalent amount of 250 mgCl/kg dm. The typical recovery index oscillated between 79% and 104%.

3.3. References

Alen R., Jannari P., Sjostrom E. (**1985**). Gas-liquide chromatographic determination of lactic acid and C_1-C_6. volatile fatty acids as their benzyl esters on a fused-silica capillary column. Finnish Chemical Letter, 5, 190-192.

Amellal N., Portal J-M., Vogel T., Berthelin J. (**2001**). Distribution and location of polycyclic aromatic hydrocarbons (PAHs) and PAH-degrading bacteria within polluted soil aggregates. Biodegradation 12, 49–57.

APHA. (**1992**). Standard Methods for the Examination of Water and Wastewater. American Public Health Association. 18ª ed., Washingthon.

Berruela J., Castrillón L. (**1997**). Efectos del N-NH_4^+ sobre el tratamiento de lixiviados de vertedero. Ingeniería Química, Junio, 121-125.

Carballa M. (**2005**). Fate of pharmaceutical and personal care products (PPCPs) in sewage treatment plant focusing on the anaerobic digestion of sludge. PhD Thesis, University of Santiago de Compostela.

Comellas L., Portillo J.L., Vaquero M.T. (**1993**). Development of analytical procedure to study linear alkylbenzenesulphonate (LAS) degradation in sewage sludge-amended soil. J. Chromatogr. A. 637, 35-31.

Di Lallo R., Albertson O.E. (**1961**). Volatile acids by direct titration. Journal of WPCF, 33, 356-365.

DIN38414-18. (**1989**). Sludge and sediments (Group S). Determination of adsorbed organically bound halogens (AOX) (S18). Deutsche Norm, November.

Düring R-A., Gäth St. (**2000**). Microwave assisted methodology for the determination of organic pollutants in organic municipal wastes and soils: extraction of polychlorinated biphenyls using heat transformer disks. Fresenius J. Anal. Chem., 368, 684–688.

Eijsackers H.,Van Gestel C. A. M., De Jonge S., Muijs B., Slijkerman D. (**2001**). Polycyclic Aromatic Hydrocarbon-Polluted Dredged Peat Sediments and Earthworms: A Mutual Interference. Ecotoxicology, 10, 35-50.

Jiries A., Hussain H., Lintelmann J. (**2000**). Determination of polycyclic aromatic hydrocarbons in wastewater, sediments, sludge and plants in Karak Province, Jordan. Wat. Air Soil Pollu., 121, 217–228.

Le Roux N.W., Wakerley D.S., Simpson M.N. (**1979**). The microbial production of methane from household wastes. Consevation and Recycling, 3, 164-174.

Llabrés P., Mata-Alvarez J. (**1988**). The hydrolytic step in a dry digestion system. Biological Wastes, 23(1), 25-37.

Marcomini A., Giger W. (**1988**). Behaviour of LAS in sewage treatment, Tenside Surf. Det., 25, 226-229.

Martínez-Viturtia A. (**1989**). Estudio de la digestión anaerobia en dos fases de residuos de frutas y verduras. PhD Thesis, Chemical Engineering department (University of Barcelona).

Mata-Álvarez J., Martínez-Viturtia A, Torres R. (**1986**). A simple device to measure biogas production in laboratory scale digesters. Biotechnology Letters, 8(10), 719-720.

Pryor S.W., Hay A.G., Walker L.P. (**2002**). Nonylphenol in anaerobically digested sewage sludge from New York State. Environ. Sci. Technol., 36, 3678-3682.

Rimkus R., Ryan J., Cook E. (**1982**). Full scale thermophilic digestion at the west-southetwest sewage treatment works, Chicago, Illinois. Journal WPFC, 54 (11), 1447-1457.

Riu J., Martínez E., Barceló D., Ginebreda A., Tirapu L.L. (**2001**). LC-MS determination of linear alkylbenzene sulfonates and their carboxylic degradation products in influent and effluent water samples and sludges from sewage-treatment plants Fresenius J. Anal. Chem., 371 (4),448-455.

Shu Y.Y., Wang S.S., Tardif M., Huang Y., (**2003**). Analysis of polychlorinated biphenyls in aqueous samples by microwave-assisted headspace solid-phase microextraction. Journal of Chromatography A, 1008(1), 1–12.

Spiller G.A., Chernoff M.C., Hill R.A., Gates J., Nasser J.J., Shipley E.A. (**1980**). Effect of purified cellulose pectin and a low-residue diet on fecal volatile fatty acids, transit time and fecal weight in humans. American Journal of Clinical Nutrition, 33(4), 754-759.

Stafford D.A. (**1982**). The effect of mixing and volatile fatty acids concentrations on anaerobic digester performance. Biomass, 2, 43-45.

Torres R. (**1992**). Estudi de la digestió anaeróbica en una i dues fases dels residus ramaders. Estudi en planta pilot d´un sistema en dues fases. PhD Thesis, Chemical Engineering department (University of Barcelona).

USEPA, (**1976**). Anaerobic sludge digestion. Operations manual.Office of water program operations (WH-547), Washington DC 20460. EPA 430/9-76-001, February, MO-11.

Chapter 4

Start-up and HRT influence in thermophilic and mesophilic anaerobic digesters seeded with waste activated sludge

Chapter 4

4. Start-up and HRT influence in thermophilic and mesophilic anaerobic digesters seeded with waste activated sludge[*]

Summary

Since thermophilic anaerobic digestion represents an efficient alternative to mesophilic anaerobic digestion, multiple studies have been developed to compare their performance and viability. One of the problems related to thermophilic anaerobic digestion is the availability of an adequate seed to start-up the process.

In this chapter, the possibility of using waste activated sludge (WAS) as a seed for start-up of both mesophilic (35 ºC) and thermophilic (55 ºC) anaerobic digesters, fed with a real sludge waste (primary and secondary sludge mixture) was evaluated.

Based on the gradual substitution of synthetic substrate by real feed, both anaerobic digesters were rapidly stabilized within 60 and 85 days at a hydraulic retention time (HRT) of 35 and 30 days with 450 and 520 mL biogas/g COD added and 65% and 72% as methane content under mesophilic and thermophilic conditions respectively.

Moreover, HRT was progressively reduced in order to assess the maximum organic load that can be treated in the thermophilic reactor. The minimum HRT reached was 8 days with a VS removal efficiency of 50.32 % and a biogas yield index of 440 mL biogas/g VS added (54% as methane content).

---

[*] **Benabdallah el Hadj T., Dosta J., Mata-Álvarez J**. (2006). Start-up and HRT influence in thermophilic and mesophilic anaerobic digesters seeded with waste activated sludge. CHEMICAL AND BIOCHEMICAL ENGINEERING QUARTERLY (accepted).

4.1. Introduction

The increasing amount of sludge from wastewater treatment plants (WWTP) and the restrictive legislations on their management and final destination invite to enhance the actual treatment processes and/or to find a reliable alternative. In Europe, since new legislations have been approved, the sludge produced has increased more than 50% from 1992 to 2005 (CEC, 2000). Nowadays, the main destinations of this product are landfilling, incineration and soil fertilization. This last destination is the more prioritized option by the EU legislation due to its positive effect on nutrients recycling and organic material reconstitution in the soil. However, sludge reuse in agricultural soil must satisfy healthiness conditions in order to avoid detest agents and harmful elements transference to the receptor medium. The most common processes used for sludge treatment are composting, lime stabilization and anaerobic digestion. This last treatment, also known as biomethanization, is the most widely used process for reduction large WWTP sludge stabilisation because it is addressed to both energy recovery and environmental protection.

4.1.1. ***Anaerobic digesters start-up*** *(thermophilic inoculum availability)*

The anaerobic thermophilic digestion (55ºC) seems to be a feasible alternative to anaerobic mesophilic digestion (35ºC) in order to improve the sludge quality, to reduce vector attraction and to minimize the pathogenic load. Alatiqi et al. (1998) reported that thermophilic anaerobic digestion was a good alternative to the mesophilic process, especially for the treatment of raw sewage sludge (RSS) with a high organic load (54 g COD /L) in warm climates.

However, one of the main problems of thermophilic digesters start-up is the availability of an appropriate seed, since a limited number of WWTP operate in this range of temperature. Then, several researchers have studied the procedure of the thermophilic start up in terms of how to increase the temperature from the mesophilic to the thermophilic range and which seed source can be used. They have considered mesophilic anaerobic sludge to be a good inoculum for thermophilic anaerobic sludge, because it is grown in a similar anaerobic environment. Van Lier et al. (1992) reported that fast start-up of thermophilic UASB reactors was achieved at process temperatures of 46 ºC, 55 ºC, and 64 ºC using mesophilic granular sludge as inoculum and fatty acid mixtures as feed. The start up was brought about by increasing the temperature of mesophilic UASB reactors in a single step, which initially led to a sharp drop in the methane production rate. Thereafter, stable thermophilic methanogenesis was achieved within a period of 1–2 weeks, depending on the temperature of operation. However, other researchers have reported a long time (43 weeks) mesophilic inoculum aclimatation to thermophilic conditions for Completely Stirred Tank Reactor (CSTR) start-up (Rimkus et al., 1982). Hence, some researchers have also shown the same or better activity from other sources. Wiegant et al. (1985) showed the results from a start-up experiment with four inocula. Cow manure, mesophilic granular sludge, digested sewage sludge, and a mixture of these materials, were used to identify which bacterial source would be appropriate to start-up a thermophilic anaerobic digestion system. This experiment revealed that any bacterial source exerting a reasonable mesophilic methanogenic activity manifested thermophilic methane production, indicating that these materials would suffice for the start-up of a thermophilic digestion process. Van Lier et al. (1993a) also used another source, digested organic fraction of municipal solid waste (OFMSW), for their study of temperature effect on the methanogenic and VFA degrading activity. The mesophilic methanogenic activity was not reduced at thermophilic temperature. Such a thermo-tolerant property was not observed in anaerobic digesters which were inoculated with mesophilic granular sludge (MGS) from a full-scale UASB reactor using temperature changes, 55 ºC → 35 ºC → 55 ºC, and 35 ºC → 55 ºC → 35 ºC. Several researchers have studied methanogens in aerobic activated sludge and its use as a seed source of anaerobic digestion. Lens et al. (1995) showed that acetotrophic and hydrogenotrophic methane producing bacteria as well as lactate, acetate, and propionate oxidizing sulfate reducing bacteria were present in all their aerated reactor types investigated, except in an activated sludge reactor aerated with pure oxygen. Besides, Wu et al. (1987) reported that waste aerobic activated sludge (WAS), for several reasons, is a good alternative to anaerobic digested sewage sludge. First, a considerable amount of methanogenic bacteria is found in aerobic activated sludge. Second, it is easy to obtain large amounts of the sludge from aerobic activated sludge plants. Finally, the aerobic activated sludge usually contains little sand and soil and is mainly composed of biomass. In their UASB reactors, the characteristics of granulation, using WAS as seed, were substantially similar to those obtained previously by using anaerobic digested sewage sludge as the seed. Noyola and Moreno (1994) also studied anaerobic granulation with flocculants aerobic sludge obtained from WAS. They concluded that flocculent sludge obtained from WAS can be converted to active anaerobic granular sludge in a short time (<8 h). The granules obtained with the described methodology maintained the granulation once seeded in a UASB reactor, and evolved to a good quality granular sludge. Kim and Speece (2002) studied the possibility of converting WAS to an anaerobic inoculum using synthetic substrate (acetate and propionate) with an acceptable methanogenic activity without a previous acclimation to the temperature range. Besides, it is recorded the better efficiency of the thermophilic digester seeded with WAS, compared to mesophilic conditions and Anaeobic Digested Sludges (ADS) seeded digesters. Therefore, it appears that WAS is a good candidate for a start-up seed source of anaerobic digestion.

The aim of this research is to evaluate the possibility of using WAS as a seed for mesophilic (35 ºC) and thermophilic (55 ºC) anaerobic digesters fed with a real raw sewage sludge (RSS) from the Barcelona Metropolitan Area (mixture of primary and secondary sludge) and to compare their efficiency at different hydraulic retention time (HRT) conditions.

4.2. Materials and Methods

4.2.1. Experimental set-up

Two completely mixed and jacketed anaerobic digesters (5 L) were used in this study (see Chapter 3). Each one was seeded with 3.5 L of waste activated sludge (WAS) from the municipal Gavá WWTP treating residual effluent of Barcelona Metropolitan Area. The effective volume was selected as recommended by EPA (USEPA, 1976). It is stated 20-30% of the total digester volume as the optimal digester biogas space.

4.2.2. Analytical methods

Total chemical oxygen demand (CODt), soluble chemical oxygen demand (CODs), total solids (TS), volatile solids (VS), pH, alkalinity and ammonium concentration (NH_4^+-N) Volatile fatty acids (VFA) and gas composition were analysed as described in Chapter 3.

4.2.3. Substrate and inoculum

The WAS used as seed for both reactors was pre-concentrated to achieve a concentration up to 14.6 g VS/ L. Table 4.1 shows the main characteristics of WAS and the real raw sewage sludge (RSS) used in the experiments. This RSS was composed by a mixture of primary (75 % on TS basis) and secondary (25%) sludge from a WWTP of the Barcelona Metropolitan Area (for full data see Annex I, Tables I.1 and I.2). The influent solid concentration was maintained by dilution/concentration around 40.28 g /L or 51 g COD/L as recommended by Alatiqui et al. (1998).

Table 4.1. *Waste activated sludge (WAS) and raw sewage sludge (RSS) characterisation.*

	TS (g/L)	VS (g/L)	VSS (%)	CODt (g /L)	CODs (g/L)
Pre-concentrated WAS	29.75 ± 1.49	14.60 ± 0.70	66 ± 3.3	17.12 ± 0.86	4.90 ± 0.29
RSS	40.28 ± 1.26	29.95 ± 0.89	74 ± 5.7	51.00 ± 3.13	15.00 ± 1.8

4.2.4. Start-up procedure

The start-up procedure was divided into two steps. In the first step, acetate was used as sole substrate to promote anaerobic action of the WAS in both digesters as reported by Ahn et al. (2000). Once anaerobic activity was developed, acetate was substituted by glucose, since this carbon source is more complicated and it can incite other microbial groups activation and proliferation (Farhan et al., 1997). In the second step, the synthetic feed was changed gradually substituting the amount of glucose by an equivalent amount of CODs from the RSS substrate (Lepistö and Rintala, 1997; Merkel et al., 1999).

Table 4.2. *Synthetic and real RSS feed mixture composition in the second step of the start-up*

	Thermophilic				**Mesophilic**			
	A	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Total CODs (g CODs L_r^{-1})	0.535	0.535	0.535	0.535	0.437	0.437	0.437	0.437
Glucose (% of CODs)	75	50	25	0	75	50	25	0
RSS added (g CODt $(L_r\ d)^{-1}$)	0.450 (6.2)	0.890 (4.51)	1.334 (3.57)	1.700 (4.28)	0.328 (7.2)	0.728 (6.3)	1.093 (2.3)	1.457 (1.48)
Glucose + RSS added (g CODt $(L_r\ d)^{-1}$)	0.850 (8.1)	1.156 (4.9)	1.468 (5.7)	1.700 (6.1)	0.691 (3.14)	0.947 (4.5)	1.202 (4.2)	1.457 (7.2)

Values between brackets refer to RSD (%)

As stated in Table 4.2, this step was divided in four periods. The first period (A) corresponded to the feeding with a mixture of 75% of CODs from glucose and 25% of CODs from RSS. Subsequently, periods B, C and D (corresponding to feeding with 50%:50%, 25%:75% and 0%:100% of CODs from glucose: CODs from RSS, respectively) were run.

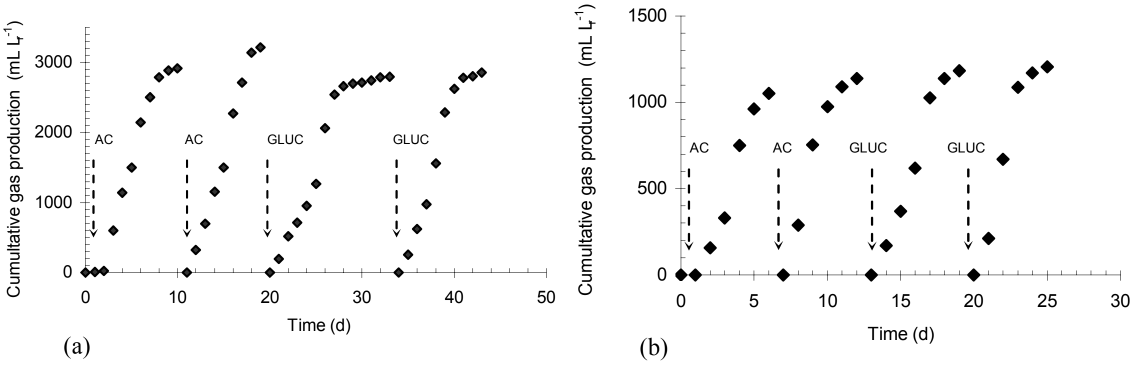
4.2.5. *HRT reduction procedure*

After the seed acclimation to anaerobic conditions fed with a mixture of primary and secondary sludge (start-up procedure), the steady HRTs reached were 35 and 30 days in the mesophilic and thermophilic digester, respectively. The procedure selected for HRT reduction for both digesters was the successive increasing of the organic load by ~14% of the initial VS reactor content (Rimkus et al., 1982). Steady state conditions for every studied HRT were achieved approximately after a time equivalent to three times HRT.

4.3. Results and discussion

4.3.1. *Start-up experience*

The performance of both digesters was examined by means of biogas production, methane content in the biogas, pH, CODt, CODs and VFA levels at each step. COD/VS values, which reported by Kim and Speece (2002), were considered in order to assess the maximum initial concentration of synthetic feed (acetate and glucose) for both mesophilic and thermophilic reactors (see Annex I, Tables I.3 and I.4).



(a) (b)

Figure 4.1. *Biogas production within synthetic substrate feeding step in (a) thermophilic and (b) mesophilic digester. (AC: acetate feeding; GLUC: glucose feeding).*

First step. Acetate was initially added to develop the methanogenic activity of WAS and subsequently it was substituted by glucose. When acetate was changed by glucose as substrate, neither major alteration in biogas production nor in digester stability was observed. For the mesophilic digester, the maximum initial concentration of organic substrate was the same for both synthetic feeds (2.187 g COD/L). However, for the thermophilic digester, the maximum initial concentration was 5.425 g COD/L for acetate and 4.800 g COD/L for glucose.

Figure 4.1 illustrates the accumulated biogas production in the thermophilic and mesophilic reactor. As observed in this Figure, the biogas production capacity of the thermophilic reactor was clearly higher for both synthetic substrates (see Annex I, Tables I.3 and I.4). The biogas production profile was almost the same, but the high amount of feed loaded to the thermophilic digester had a noticeable impact on the amount of biogas produced. At the fourth feeding with synthetic substrate, a biogas production of 595±95 and 551±73 mL biogas/g CODt was measured with a methane content of 64.0% and 61.2% for the thermophilic and mesophilic digester, respectively. These results indicate that WAS has a significant capacity to degrade acetate and glucose under anaerobic conditions at an acceptable removal rate and methane content in the biogas produced.

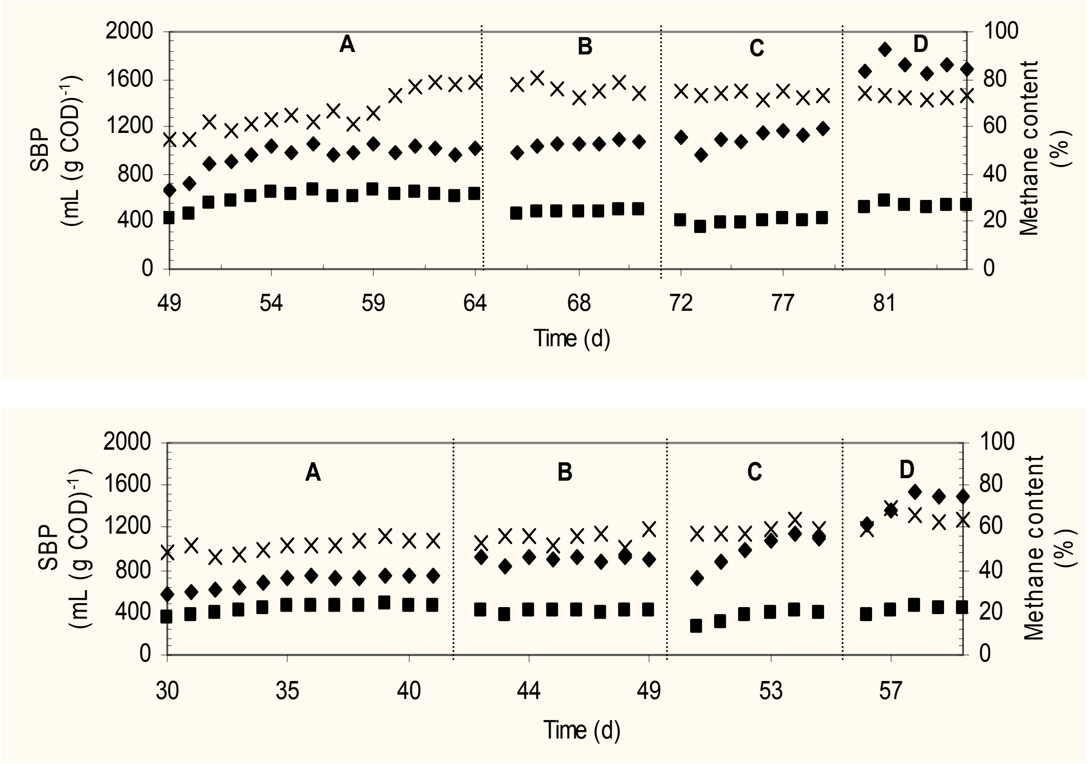


Figure 4.2. Biogas production per unit organic matter and methane composition in the (a) thermophilic and (b) mesophilic digester within RSS substitution feed step
(■ mL biogas/g CODt, ♦ mL biogas/g CODs, × methane content (%))

Second step. Table 4.2 shows the operational conditions of this second period, where the feed was changed gradually substituting the quantity of glucose added by an equivalent amount of CODs from the RSS substrate. In Figure 4.2 it can be appreciated the evolution of biogas production per unit of COD added and its methane content during this treatment. At the end of period A, it was observed a sensitive increase in biogas production that exceeded 630±65 and 470±36 mL biogas/g CODt in the thermophilic and mesophilic digester, respectively. Therefore, the biogas production efficiency was highly improved under thermophilic conditions. These results indicate that WAS has a high initial yield of biogas production when it is used as an anaerobic digestion seed, especially in the thermophilic temperature range, and can biodegrade a complex substrate such as RSS.

At period B, the thermophilic and the mesophilic digester produced, approximately, 490±53 mL biogas/g CODt and 410±37 mL biogas/g CODt, respectively. This clearly indicates that WAS, at both temperatures, was able to biodegrade a complex feed in absence of an important amount of easily biodegradable organic matter. On the other hand, the biogas production per unit of soluble COD added was higher than in the previous stage, since the substitution of synthetic substrate by RSS was done on soluble COD basis and, consequently, the total COD added to the system was increased.

During period C, the biogas production per unit of total COD added in the mesophilic and thermophilic digester were 400±35 mL biogas/g CODt and 430±41 mL biogas/g CODt, respectively. When the digesters were fed exclusively with RSS (period D), they were rapidly stabilized (5-7 days). The average biogas production was 480±38 mL biogas/g CODt with 65% of CH_4 and 525±59 mL/g CODt with 72% CH_4 under mesophilic and thermophilic conditions, respectively. The difference between both reactors, in terms of the aforementioned values, demonstrates the higher efficiency of the thermophilic digester inoculated with WAS in relation to the mesophilic digester when a real RSS is fed.

Therefore, it is concluded that WAS represents an appropriate seed to develop the anaerobic digestion process for the treatment of a real RSS and, taking into account the short period of time necessary to start-up the process and the efficiencies reached, it represents a very good alternative to other studied inoculums (Rimkus et al., 1982; Lepistö and Rintala, 1997; Alatiqi et al., 1998; de la Rubia et al., 2002).

4.3.2. HRT reduction

A gradual reduction of the HRT for both anaerobic digesters was carried out following the procedure described by Rimkus et al. (1982). Figure 4.3 shows the specific biogas production (SBP) profile for both systems at every studied HRT (see Tables IV.6 and I.8 of annex I). As it can be appreciated, for every HRT tested, the SBP was enhanced under thermophilic conditions with respect to mesophilic conditions, which is in concordance with van Lier et al. (1993b, 1997), who experienced an improvement of the anaerobic activity at high temperatures. On the other hand, the SBP slightly decreased with HRT reduction and it was maintained within the range of 400-450 mL biogas/g VS_f (thermophilic digestion) and 300-350 L/g VS_f (mesophilic digestion). The assessed biogas yield values were similar or higher than other reported values (Rimkus et al., 1982; Speece, 1988; Cecchi and Traverso, 1986). Similar results were obtained when SBP is calculated considering organic matter expressed as COD (see Annex I, Figure I.1).

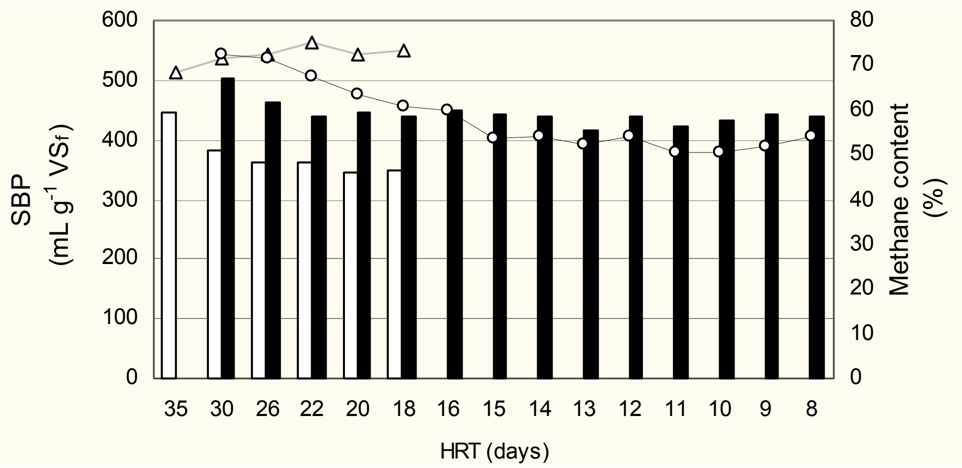


Figure 4.3. *Biogas production per organic matter unit added (VS_f) and methane content in both digesters at HRT reduction period.*
(☐ mesophilic specific biogas production; ■ thermophilic specific biogas production; Δ Methane biogas content in the mesophilic digester; ○ methane biogas content in the thermophilic digester).

Mesophilic digester was stopped after several tested HRTs (from 35 to 18d), since thermophilic conditions clearly improved the anaerobic digestion efficiency. Therefore, thermophilic digestion performance was evaluated until achieving the minimum feasible HRT.

Figure 4.4 shows the VS removal (VS_r) efficiency for all the tested HRTs, where it is stated that thermophilic conditions clearly enhanced the VS_r yield. Besides, VS_r efficiency decreased sensibly at short HRTs in the mesophilic digester (in Table I.6 of annex I, the detailed results are present). However, at thermophilic conditions, HRT decrease did not clearly influence VS_r efficiency (see Table I.8 of annex I). Only the first HRT reduction (from 30 to 26 d) provided a significant decrease of VS removal.

Moreover, it was observed that in both mesophilic and thermophilic digesters the VS reduction was carried out at the same efficiency range values (50-55 %). Same results were obtained monitoring COD parameter (see Annex I, Figure I.2).

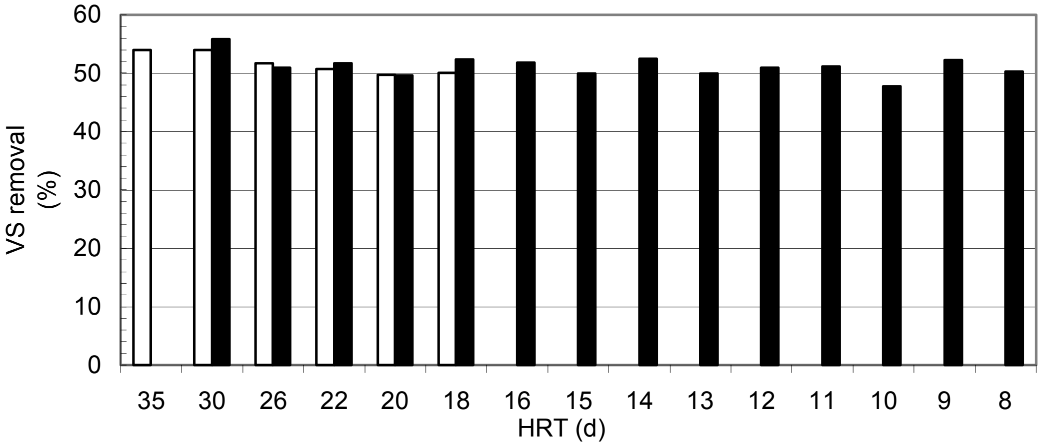


Figure 4.4. *Evolution of volatile solids removal (%) at each HRT.*
(□Mesophilic digester; ■ thermophilic digester).

On the other hand, the biogas methane content oscillated between 70 and 75% at the HRTs studied in the mesophilic digester (see Figure 4.3). In this case, the methane content increase could be explained by the fact that the methanogenic consortium adaptation was enhanced during the lag time. However, the thermophilic biogas methane content was lower than that obtained under mesophilic conditions and decreased with HRT reduction. This descent can be divided into two phases: in the first one (30-15 d HRT), the methane biogas content decreased progressively and in the second phase (15-8 d HRT) it slightly fluctuated between 50-54 % CH_4. From these results, it seems that HRT reduction in the thermophilic digester affected drastically the methanogenic activity, and at low HRT (<15 d), the thermophilic methanogenesis was adapted to the organic load increasing regime. This experimental data is in concordance with the volatile fatty acids (VFA) concentration profile during the HRT reduction experience (shown in Figure 4.5) (for mesophilic and thermophilic digester VFA full data see Annex I, Table I.9). As it is shown in this Figure, the VFA accumulation inside the reactor increased until the limit inhibitory concentration was reached.

At HRT 7 d, the biogas production reached 149±15 mL/g VS_f with 23% as methane content, due to an inhibition of the methanogenic activity caused by a pH decrease and a high total VFA (sum of C_2, C_3, C_4, C_5, C_6 and C_7) concentration, particularly acetic acid as reported by Dugan and Takahashi (1985), Ahring et al. (1995) and Baraza (2002).

From these results, it can be concluded that a concentration of 1250 mg VFA/L represents the inhibitory limit for the studied thermophilic digestion. However, this reactor failure was overcome after the external addition of alkalinity and returning to a HRT of 8 d (see Figure 4.5).

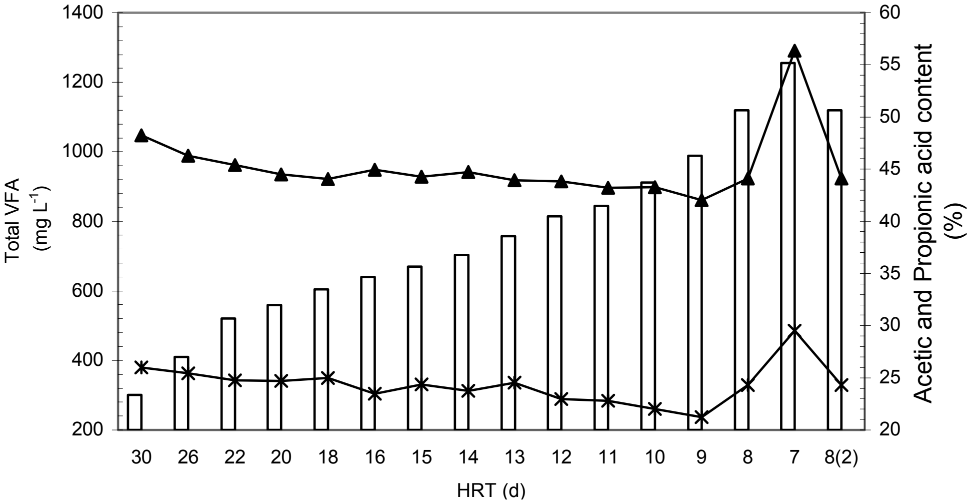


Figure 4.5. *Total VFAs amount and Acetic and Propionic acids content in the thermophilic digester effluent. (□Total VFA; ▲ Acetic Acid; ж Propionic Acid).*

The pH value remained in the ranges 6.95-7.11 and 7.18-7.48 in the mesophilc and the thermophilic digester, respectively, at all applied HRTs (see Figure 4.6). These values are within the indicated interval reported by de la Rubia et al., 2001, 2002; Torres and Mata-Álvarez, 1987 for both temperature conditions. Several researchers explain pH increase at thermophilic temperature by high free ammonia concentration, which leads to carbon dioxide (CO_2) solubility reduction and, then, alkalinity system decrease (Gallert and Winter, 1997).

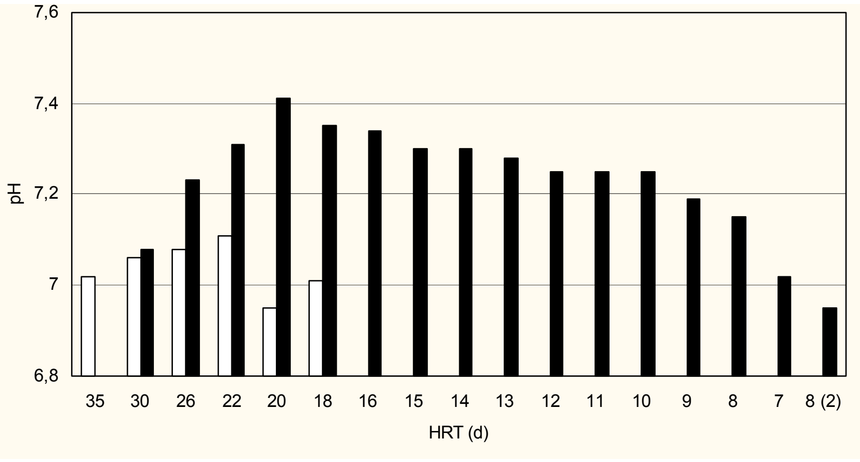


Figure 4.6. *pH evolution in the mesophilic (□) and the thermophilic (■) digester at HRT reduction period*

Several studies insist on ammonia role in anaerobic digestion performance (Braun et al., 1981; Koster and Lettinga, 1983, 1988). Inhibitory concentration for bacterial methanogenic group in mesophilic anaerobic digestion is reported to be around 1400 mg/L (Berruela and Castrillón, 1997; Robbins et al., 1989 and van Veelsen, 1979).

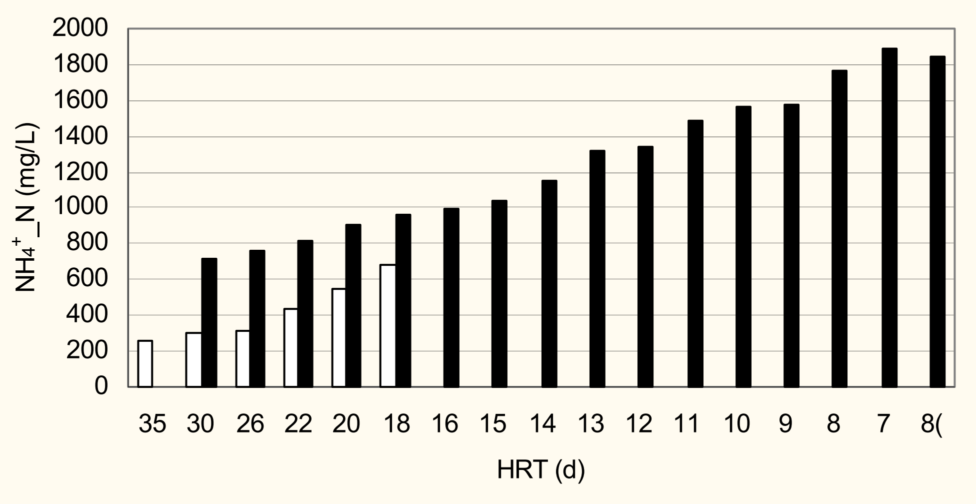


Figure 4.7. Ammonia nitrogen evolution in the mesophilic (□) and the thermophilic (■) digester at HRT reduction period

In this study, the ammonia nitrogen (NH_4^+_N) concentration values experimented a sustainable increase (from ~257 to ~759 mg/L) at HRT reduction period in the mesophilic digester (see Figure 4.7). However, under thermophilic conditions the ammonia nitrogen concentration reached 1885 mg/L at HRT of 7 days. This concentration is below the inhibitory concentration for acetotrophic (3.5g/L) and hydrotrophic methanogenics (7 g/L) in thermophilic anaerobic digestion at pH between 7.2 and 7.3 as reported by Angelidaki and Ahring (1993, 1994) and Borja et al. (1996a, 1996b). Hence, it seems that ammonia concentration was not the failure thermophilic digester cause. From the VFAs accumulation as aforementioned above, it appears that the high organic load could be the destabilization digester promoter.

Methanization yield and energetic excess were considered as comparative parameters to complete this study.

Anaerobic digestion, so-called methanogenic digestion, releases methane as the principal product generated. On the basis of COD calculations and assuming that 64 g O_2 is the COD value equivalent to 1 mol of methane, the methanization yield (M%) can be defined as the fraction of organic matter fed what converting to methane during anaerobic digestion (Baraza, 2002).

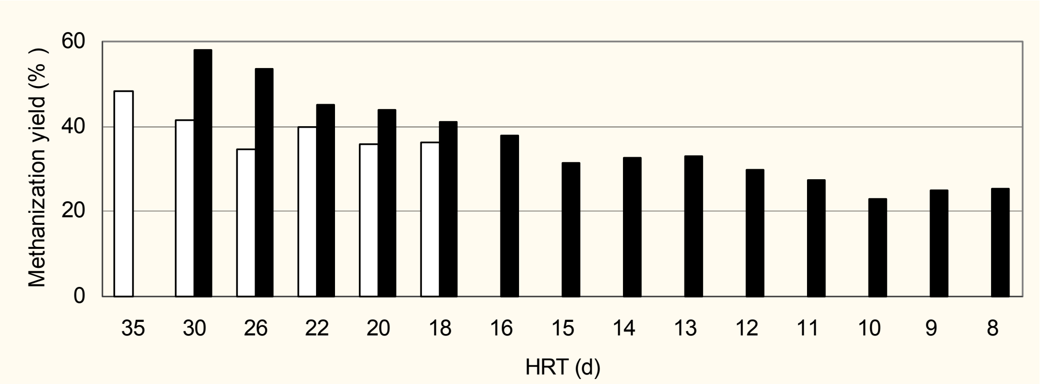


Figure 4.8. *Methanazation yield (M%) in the mesophilic (□) and the thermophilic (■) digester at HRT reduction period.*

Figure 4.8 shows the methane yield along the HRT reduction period. It can be observed that the minimum of the methanization yield corresponds to 26 d (34.8%) and 10 d (22.95%) as HRT in the mesophilic and thermophilic digester, respectively. However, the same profile is observed for both digesters, the methanization yield values decrease with HRT shorting. Then, the maximum methanization yield values correspond to 35 d and 30 d as HRT in the mesophilic and thermophilic digester, respectively. Besides, at the same HRTs the thermophilic digester has better methanization yield than the mesophilic one. This superiority decreases also with HRT reduction.

Due to higher anaerobic digestion energy requirement, the energetic balance is another factor of consider. Their evaluation is based on energetic excess (or deficiency) computation. Assuming calorific conversion of methane is total (100%), the both process are developed under isothermal conditions (energy loses are null). Other assumptions are, temperature of feed 20 ºC, calorific capacity (Cp) and density of the sludge equal to the water (Cp_{H20} =1cal/g =4.183J/g; d_{H2O} = 1). The energetic excess (EE) per feed litre can be determined as follow:

$$\boxed{\text{EE (KJ /L.d)} = \frac{\dfrac{Cp_{CH4} \times V_{CH4} \times 16}{22400} - \dfrac{Cp_{H2O} \times 1000 \times (T-20)}{HRT}}{1000}} \qquad (4.1)$$

where:

Cp_{CH4}: Heat of combustion of methane (50400 J/g of methane)
V_{CH4}: Daily methane volume production (mL/d)
T: Final temperature required (ºC) for mesophilic range (35 ºC) and thermophilic range (55 ºC)
HRT: Hydraulic retention time (d).

Figure 4.9 illustrates the EE profile in both digesters. For mesophilic digester, it can be observed the coincidence of the minimum EE and the minimum M% at the same HRT (26d). However, the maximum EE (10.73 kJ/L) is released at the minimum applied HRT (18d).

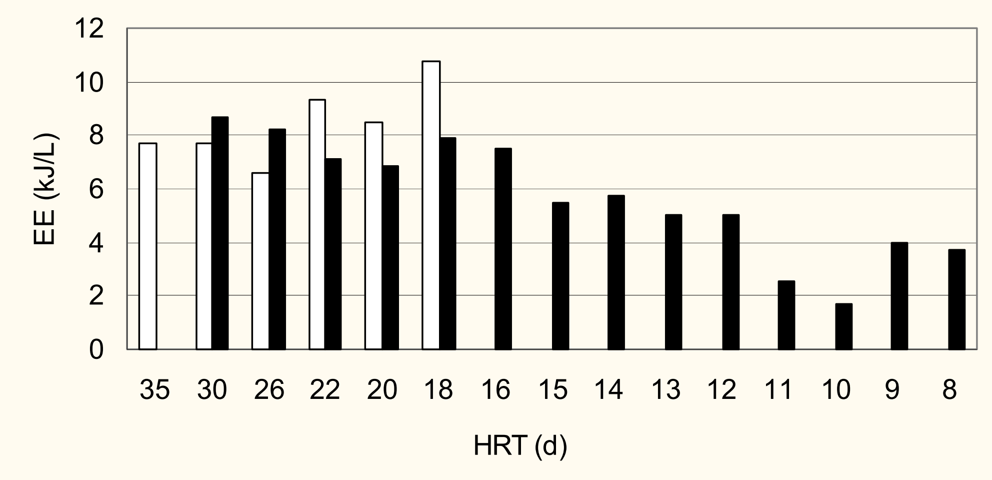


Figure 4.9. *Energetic excess (EE) in the mesophilic (□) and the thermophilic (■) digester at HRT reduction period*

In the thermophilic digester, several fringes are distinguished. The first one (from 30 d to 16 d of HRT) has an average EE around 7.73 kJ/L with the maximum of 8.69 kJ/L (at 30 d-HRT) and the minimum of 6.87 kJ/L (at 20 d-HRT). In the second one (from 15d to 12d-HRT) has an average EE of 5.31 kJ/L. In the third, after the minimums recorded at 11d and 10 d (2.55 and 1.7 kJ/L, respectively), the EE recovered high relative values (3.84 kJ/L). Then, depending on HRT it can be distinguished high, median and low EE bands. Besides, thermophilic digester decline is detected from 11d HRT and not from 10d HRT, as illustrated in the M% profile.

Although applied the short HRT in the thermophilic digester, the EE in anaerobic digester at mesophilic conditions remains higher due to the relatively major energetic requirements in the thermophilic digester (more than 20 ºC)

4.4. Conclusions

The results obtained in this study show that WAS can be used as inoculum to the anaerobic digestion at both mesophilic and thermophilic conditions. Furthermore, the start-up of the anaerobic digestion based on the gradual substitution of synthetic substrate by real substrate in anaerobic reactors seeded with WAS is very fast compared with other seeds commonly used to develop this biological process.

The anaerobic digestion of real raw sewage sludge (mixture of primary and secondary sewage sludge) provided good removal efficiencies for both mesophilic and thermophilic digesters (around 50%). However, the treatment was highly improved under thermophilic conditions.

Moreover, the HRT effect on thermophilic anaerobic efficiency was studied in order to assess the maximum organic load that can be treated in the thermophilic reactor. A minimum HRT of 8 days was found, since volatile fatty acids accumulation and pH decrease inhibited the process when working at a lower HRT.

4.5. References

Ahn Y., Lee Y.J., Kim H-S., Park S. (**2000**). Monitoring of specific methanogenic activity of granular sludge by confocal laser scanning microscopy during start-up of thermophilic upflow anaerobic sludge blanket reactor. *Biotechnology Letters,* 22, 1591-1596.

Ahring B. K., Sandberg M., Angelidaki I. (**1995**). Volatile fatty acids as indicadors of process imbalance in anaerobic digestion. Applied Micorbiology and Biotechnology, 43(3), 559-565.

Alatiqi M., Hamoda .M F., Dadkhdakh A. (**1998**). Kinetic analysis of thermophilic digestion of wastewater sludge. *W. Air. and Soil Pollu.* 107, 393-407.

Angelidaki I., Ahring B.K. (**1993**). Thermophilic anaerobic digestion of livestock waste: the effect of ammonia. Appl. Microbiol. Biotechnol., 38, 560-564.

Angelidaki I., Ahring B.K. (**1994**). Thermophilic anaerobic digestion of manure at different ammonia loads : effect of temperature. Wat. Res., 28, 727-731.

Baraza J. (**2002**). Contribución al estudio del tratamiento integrado de residuos municipales en un vertedero. PhD Thesis, University of Barcelona. Department of Chemical Engineering. (In Spanish).

Berruela J., Castrillón L. (**1997**). Efectos del $N\text{-}NH_4^+$ sobre el tratamiento de lixiviados de vertedero. Ingeniería Química, Junio, 121-125.

Borja R., Sánchez E., Durán. M.M. (**1996a**).Effect of the clay mineral zeolite on ammonia inhibition of anaerobic thermophilic reactors treating cattle manure. Journal of Environmental Science and Health A31, 479-500.

Borja R., Sánchez E., Weiland P. (**1996b**). Influence of ammonia concentration on thermophilic anaerobic digestion of cattle manure in upflow anaerobic sludge blanket (UASB) reactors. Process Biochemistry 31, 477-483.

Braun R., Huber P., Meyrath J. (**1981**). Ammonia Toxicity in Liquid Manure Digestion. Biotechn. Letters, 3, 159-164.

CEC (**2000**). Working Document on Sludge (3rd Draft), Commission of the European Communities Directorate-General Environment, ENV.E.3/LM, 27 April 2000, Brussels.

Cecchi F., Traverso P.G. (**1986**). Biogas from the organic fraction of the municipal solid waste and primary sludge. Part II. *Chim. Ind. Quad. Ing. Chim. Ital.*, 22, 7-9, 9-13.

De la Rubia M.A., Pérez M., Romero L.I., Sales D. (**2002**). Anaerobic Mesophilic and thermophilic municipal sludge digestion. *Chem. Biochem. Eng. Q.*, 16 (3), 119-124.

De la Rubia R.M.A., García P.M., García R. L.I., Márquez S.D. (**2001**). Dgestión anaerobia termofílica versus digestión anaerobia mesofílica de lodos de EDAR. Residuos, 62 (11), 64-68.

Dugan G.L, Takahashi P.K. (**1985**). Integrated energy and food production and waste treatment in Hawaii. IX Energy Biomass Waste, New Jersey (USA).

Farhan M.H., Chin-Hong P.H., Keenan J.D., Shieh W.K. (**1997**). Performance of anaerobic reactors during pseudo-steady-state operation. *J. Chem. Tech. Biotechnol.*, 69, 45-57.

Gallert C., Winter J. (**1997**). Mesophlic and Thermophilic anaerobic digestion of source-sorted organic waste: effect of ammonia on glucose degradation and methane production. Appl. Microbiol. Biotechnol., 48, 405-410.

Kim M., Speece R. (**2002**) Aerobic waste activated sludge (WAS) for start-up seed of mesophilic and thermophilic anaerobic digestion *Wat. Res.,* 36, 3860-3866.

Koster I.W., Lettinga G. (**1983**). The influence of ammonium-nitrogen on the specific activity of pelletized methanogenic sludge. Agricultural Wastes, 9(3), 205-216.

Koster I.W., Lettinga G. (**1988**). Anaerobic digestion at extreme ammonia concentration. Biological Wastes, 25, 51-59.

Lens P.N., De Poorter M-P., Cronenberg C.C., Verstraete W.E. (1995). Sulfate reducing and methane producing bacteria in aerobic wastewater treatment systems. Water Res., 29, 871-880.

Lepistö S.S., Rintala J.A. (**1997**). Start-up and operation of laboratory-scale thermophilic upflow anaerobic sludge blanket Reactors treating vegetable processing wastewaters. *J. Chem. Tech. Biotechnol.*, 68, 331-339.

Llabrés P., Mata-Alvarez J. (**1988**). The hydrolytic step in a dry digestion system. *Biological Wastes*, 23(1), 25-37.

Martínez-Viturtia A. (**1989**). Estudio de la digestión anaerobia en dos fases de residuos de frutas y verduras. PhD Thesis, University of Barcelona. Department of Chemical Engineering. (In Spanish).

Mata-Álvarez J., Martínez-Viturtia A, Torres R. (**1986**). A simple device to measure biogas production in laboratory scale digesters. *Biotechnology Letters*, 8(10), 719-720.

Merkel W., Manz W., Szewzyk U., Krauth K. (**1999**). Population dynamics in anaerobic wastewater reactors: Modelling and in situ characterization. *Wat. Res.*, 33(10), 2392-2402.

Noyola A., Moreno G. (**1994**). Granule production from raw waste activated sludge. Water Sci. Technol., 30, 339-346.

Rimkus R., Rayan R.,Cook E. (**1982**). Full-scale thermophilic digestion at the west-southwest sewage treatment works, Chicago, Illinois. *Journal WPCF.*, 54(11), 1447-1457.

Robbins J. E., Gerhardt S.A., Kappel T.J. (**1989**). Effects of total ammonia on anaerobic digestion and an example of digestor performance from cattel manure-protein mixture. Biological Wastes, 27, 1-4.

Speece R.E. (**1988**). A survey of municipal anaerobic sludge digesters and diagnostic activity assay. Wat. Res., 22(3), 65-372.

Standard Methods for the Examination of Water and Wastewater (**1992**). 18th ed, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.

Torres R., Mata-Álvarez J. (**1987**). Two-phases anaerobic digestion of a mixture of cow manure and bedding straw. IV Congress of Mideterranean Chemical engineering . Barcelona, pp 311.

Van Lier J.B., Brummeler E., Lettinga G. (**1993a**). Thermo-tolerant anaerobic degradation of VFA by digested organic fraction of municipal solid waste. J. Ferment. Bioeng., 76, 140-144.

Van Lier J.B., Grolle K.C.F., Stams A.J.M., de Macario E.C., Lettinga G. (**1992**). Start-up of a thermophilic UASB reactor with mesophilic granular sludge. Appl. Microbiol. Biotechnol., 37,130-135.

Van Lier J.B., Hulsbeek J., Stams A.J., Lettinga G. (**1993b**). Temperature susceptibility of thermophilic methanogenic sludge: Implication for reactor start-up and operation. *Bioresource Technology*, 43, 227-235.

Van Lier J.B., Rebac S., Letting G. (**1997**). High rate anaerobic wastewater treatment under psychrophilic and thermphilic conditions. Wat. Sci. Technol., 35, 199-206.

Van Veelsen A.F.M. (**1979**). Adaptation of methanogenic sludge to high ammonia-nitrogen concentrations. Wat. Res., 3 (10), 995-999.

Wiegant M., Lettinga G. (**1985**). Thermophilic anaerobic digestion of sugars in UASB reactors. Biotechnol. Bioeng., 27,1603-1607.

Wu W, Hu J, Gu X, Zhao Y, Zhang H, Gu G (**1987**). Cultivation of anaerobic granular sludge in UASB reactors with aerobic activated sludge as seed. Wat. Res. 21(7): 789-799.

Chapter 5

Biodegradation of PAH and DEHP micro-pollutants in mesophilic and thermophilic anaerobic sewage sludge digestion

Chapter 5

5. Biodegradation of PAH and DEHP micro-pollutants in mesophilic and thermophilic anaerobic sewage sludge digestion[*]

Summary

Anaerobic digestion for the treatment of sludge in wastewater treatment plants has been reported to produce a low organic loaded effluent with an acceptable economic cost. But in the last years, new regulations and the increasing sludge production invite to find an alternative and/or to improve the process efficiency. Moreover, the use of the effluent as fertilizer in agriculture imposes more restrictions on digestion process product and its micropollutant contents to protect the environment.

In this chapter, the performance of the anaerobic digestion under mesophilic and thermophilic conditions at different hydraulic retention times (HRT) is assessed and the removal efficiencies of two important family compounds (Polycyclic Aromatic Hydrocarbons, PAH, and Di-2-(Ethyl-Hexyl)-Phthalate, DEHP) are evaluated. A positive effect of thermophilic temperature was observed on both micropollutants biodegradation. However, HRT effect had also an important role for DEHP and low molecular weighted PAH removal.

---

[*] **Benabdallah el Hadj T., Dosta J., Mata-Álvarez J**. (2006). Biodegradation of PAH and DEHP micro-pollutants in mesophilic and thermophilic anaerobic sewage sludge digestion. Water Science and Technology, 53 (8), 99-107.

5.1. Introduction

The increasing amount of sludge from wastewater treatment plants (WWTPs) and the restrictive legislations on their management and final destination invite to enhance the actual treatment processes and/or to find a reliable alternative. In Europe, since new legislations have been approved, it was estimated that the sludge produced has increased more than a 50% from 1992 to 2005 (CEC, 2000). Nowadays, the main destinations of this product are landfilling, incineration and soil organic fertilization. This last destination is the more pryorized option by the EU legislation due to a positive effect on nutrients recycling and organic material reconstitution in the soil.

However, sludge reuse in agricultural soil would satisfy healthiness conditions in order to avoid detest agents and harmful elements transference to the receptor medium. Within these elements, organic micropollutants are distinguished. In the 3rd draft presented to EU commission (CEC, 2000) some compounds and family compounds were selected for limiting their content in the dry sludge. Polycyclic Aromatic Hydrocarbons (PAH) family, Di(2-Ethyl-Hexyl)-Phthalate (DEHP), Adsorbed Organic Halogen compounds (AOX), PolyChlorinated Biphenyls (PCB), Anionic (as linear alkylbenzensulphonate) and nonionic (as nonylphenol ethoxylates) were within the selected organic compounds (see Chapter 1.1.3).

5.1.1. Polycyclic Aromatic Hydrocarbons (PAH)

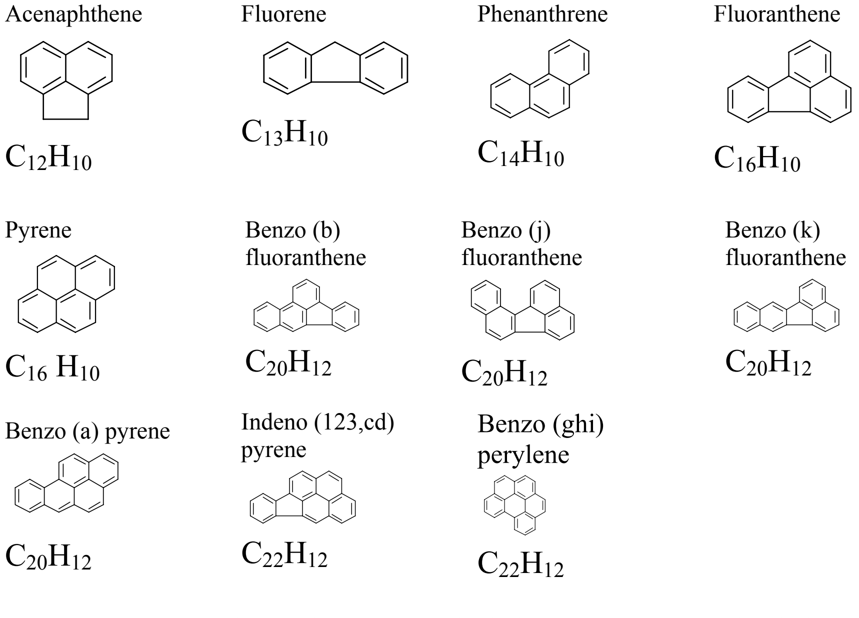


Figure 5.1. *PAHs chemical structures which their sum content in the sludge proposed to be regulate in EU, EU-PAH (CEC, 2000).*

The PAHs are chemicals composed of hydrogen and carbon only (see Figure 5.1). These compounds are the product of incomplete combustion of organic substances such as fossil fuel, wood and mineral oil (Angelidaki et al., 2000). Due to the PAHs hydrophobic character and their high tendency to be adsorbed on the enriched organic solid matter, these compounds are accumulated on biosolids, particularly on the sludge generated in WWTPs (Pavlostathis and Jaglal, 1991; Subramaniam et al., 2004). Typical concentration of them in sludge lies around 1-10 mg/kg dw (CEC, 2000; Wild and Jones, 1989). However, PAH content can reach up to 80 mg/kg dw in Catalonian sludges as reported by Barceló and Petrovic (2004).

Table 5.1. *PAH degradation rates (mg/kg.d) under various reduction conditions (Chang et al., 2003).*

Treatment	Acenaphthene		Fluorene		Phenanthrene		Anthracene		Pyrene	
	Pc	Mp	Pc	Mp	Pc	Mp	Pc	Mp	Pc	Mp
Inoculated control	0.091	0.024	0.077	0.037	0.045	0.091	0.040	0.059	0.029	0.067
Nitrate-reducing condicions	0.083	0.009	0.073	0.012	0.035	0.040	0.015	0.018	0.05	0.013
Sulphate-reducing condicions	0.583	0.055	0.489	0.173	0.313	0.643	0.242	0.321	0.140	0.234
Methanogenic Condicions	0.483	0.049	0.373	0.106	0.240	0.438	0.125	0.121	0.021	0.213

Pc: Petrochemic sludge; Mp: Municipal sludge.

Anaerobic stabilization is a common treatment of sludge in WWTPs. Hence, to reduce some organic pollutant content, some studies showed doubts on their efficiency (Kelcka et al., 1990; Volkering et al., 1993; Ghoshal et al., 1996). In contrast, other researchers (Coates et al., 1996; Rockne and Strand, 1998) observed an acceptable PAH degradation rate, especially for Low Molecular Weighted (LMW) PAH, such as naphthalene, acenafthene and fenanthrene. Chang et al. (2003) reported 3.5 d as the half-life of some PAH compounds under methanogenic conditions, but without clear preference for LMW PAH (see Table 5.1). In a recent study carried out by Trably et al.(2003), it was observed a biodegradation of 13 PAH compounds including High Molecular Weighted (HMW) PAHs.

Temperature effect on PAH degradation was studied by some authors (Feitkenhauer and Märk, 2003; Feitkenhauer et al., 2003) who stated an increase of naphthalene solubility (approximately 10%) when the temperature was increased from 20 to 75 ºC under aerobic conditions. Besides, Trably et al. (2003) observed an increase from 46% to 53% in the elimination rate of 13 PAH when temperature was increased from 35 ºC to 55 ºC.

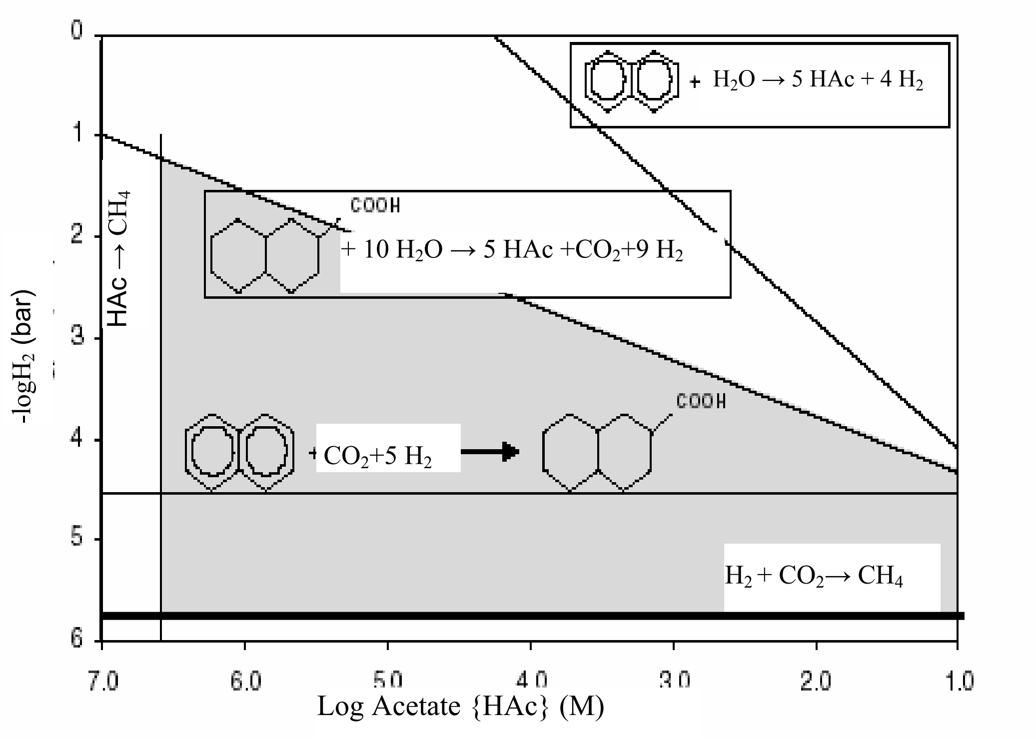


Figure 5.2. *Thermodynamics of naphthalene degradation in anaerobic digester at 25 ºC, with naphthalene at saturation concentration and 1 mM* CO_2*. Shared regions show simultaneous decalate (*C_{10}*) oxidation, and hydrogen conversion are possible (Christensen et al., 2004).*

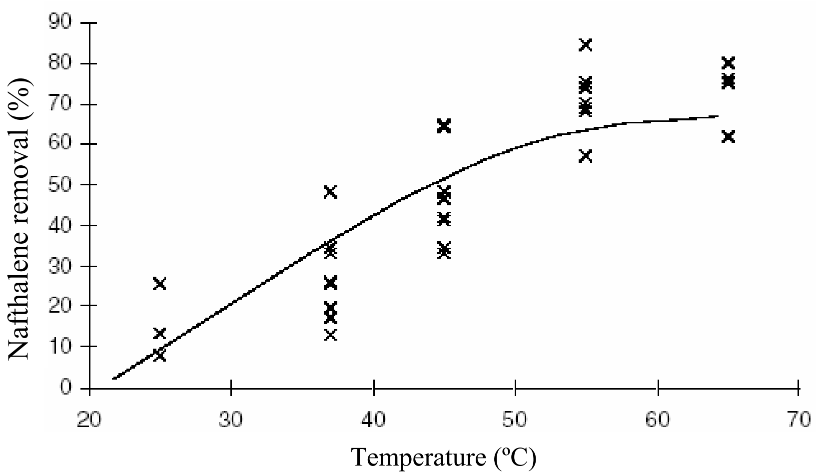


Figure 5.3. *Naphthalene removal as a function of temperature in enriched samples under anaerobic digester (Christensen et al., 2004).*

Christensen et al. (2004) reported an enhancement from 10-50% to 60-85% in naphthalene biodegradation rate when temperature range was changed from mesophilic to thermophilic conditions (see Figure 5.3). This improvement was related to compound transference to aqueous medium, which is intensified with increasing temperatures. However, this assumption must be verified since temperature effect under anaerobic conditions on diffusion and sorption phenomena is still not well defined (Holliger and Zehnder, 1996).

On the other hand, from sterilized reactors, it was observed an important abiotic removal rate, especially for fluorescence PAH (phenanthrene, fluorene and anthracene). It was estimated more than 50%, 40% and 25 % at 55, 45 and 35 ºC, respectively (Trably et al., 2003). Similar results were reported by Christensen et al. (2004) for naphthalene.

5.1.2. Di-2-(Ethyl-Hexyl)-Phthalate (DEHP)

The Di-2-(Ethyl-Hexyl)-Phthalate (DEHP), as a distinguished Phthalate Acid Ester (PAE), represent the 90% of the annual total phthalate amount production (4.2 millions Mt) and, generally, has an industrial use, mainly as plastizer (Ejlertsson et al., 1997; Staples et al., 1997).

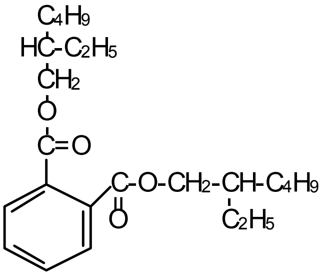


Figure 5.4. *DEHP structure formula*

Since DEHP is only physically bound to its matrices, it may leach out from products during contact with water and end up in municipal sewage (Cheng et al., 2000). Nowadays, it is considered an ubiquitous pollutant in many aquatic and terrestrial compartments with approximately 23,000 tons per year as permanent amount release to environment (Madsen et al., 1999).

Table 5.2. *DEHP phyisico-chemical data (CEC, 2000).*

Abreviation	Formula	Vapour pressure (Pa)	Solubility (mg/L)	log K_{ow}	K_{oc} (cm^3/g)	Henry constant
DEHP	$C_{24}H_{38}O_4$	0.6E-05	0.23E-04	4.88	35,567	0.53E-05

K_{ow}: octanol/water coefficient; K_{oc}: adsorption coefficient.

The main source of DEHP is sewage treatment plants, where DEHP is often found in elevated concentrations in the dewatered sewage sludge. Hence, due to the hydrophobic-lipophilic character of DEHP (see Table 5.2), this compound tends to be accumulated in the solid matter. Then, the concentration of DEHP may exceed a few hundreds mg per kg dry weight of sludge (Table. 5.3). However, It is reported that 10-100 mg/kg dw as typical DEHP content in the sludge (CEC, 2000).

Table 5.3. *DEHP content in the WWTP sludge (mg/kg dm)*

Country	Range	Median	References
Norway	1-140	58	CEC, 2000
Sweden (1989-91)	25-661	170	NSEPB (1992)
Denmark (1995)	3.9-170	-	DEPA (1995)
Germany	170	-	Schnaak et al., 1997
USA	136-578	-	Staples et al., 1997
Canada	11-959	-	CEC, 2000
Taiwan (China)	105-153	-	Cheng et al., 2000

DEHP removal from sludge before its use as a soil conditioner, is an important preventive action, since the aged xenobiotic in soil is more recalcitrant (Bollag et al., 1992; Alexander, 1995; Hatzinger and Alexander, 1995, Madsen et al., 1999). DEHP recalcitrance is due to their high hydrophobicity index and their tri-dimensional structure, which obstructs the enzymatic hydrolysis (Ejlertsson et al., 1997).

DEHP aerobic removal was verified by different studies. Marttinen et al. (2004) reported 4%, 33-41% and 50-62% as elimination percentage at a retention time of 1, 7 and 28 d, respectively. Likewise, Banat et al. (1999) observed a 30-40% of DEHP elimination in an activated sludge system at 20 ºC. However, from various studies carried out on DEHP monitoring in anaerobic systems, anaerobic DEHP removal was not confirmed (Horowitz et al., 1982; Shelton et al., 1984; O'Conner et al., 1989; Ziogou et al., 1989; Ejlertsson et al, 1996; Ejlertsson and Svensson, 1996). Ejlertsson et al. (1997) observed a removal efficiency within a range of 87-91% for some phthalates with high solubility water index (11.2-50 mg/L) at incubation time ranging from 35 to 100d, but no removal index was detected for DEHP, which solubility coefficient was estimated as 3μg/L. On the other hand, Reinhart and Pohland (1991) experienced the total disappearance of DEHP from municipal solid waste incubated in a methanogenic lysimeter after 4 years. Madsen et al. (1999) estimated a mineralization portion of 32% of the initial DEHP content (1.6 mg/kg dw) in sludge-amended soil after 1 year of incubation period under anaerobic conditions at 20 ºC.

The anaerobic degradation of DEHP was reported to depend on the inoculum used. The use of landfill leachate was verified as an efficient seed for phthalates biodegradation including DEHP (Angelidaki et al., 2000). Gavala et al., (2003), estimated DEHP anaerobic degradation rate constant, adsorbed in primary sludge, between 0.0035 and 0.0099 d^{-1} with half–life time in the range of 198-70 d after anaerobic mesophilic digestion.

Recently, the temperature effect on DEHP biodegradation was investigated by Banat et al. (1999), who observed an increase of DEHP biodegradation from 22% to 31% and 44% with temperature increase from 20 ºC to 52 ºC and 62 ºC, respectively, in activated sludge aerobic treatment. On the other hand, Marttinen et al., (2004), recorded DEHP elimination rates in the range of 30-60% in composting process for primary, activated and anaerobic sludge treatment with initial content between 57-77 mg/kg dw.

The use of hyperthermophilic processes (68 ºC and 5d as HRT) for treating thermophilic anaerobic digester effluent enhanced DEHP elimination by an increase from 9.6% to 34-53% (Hartmann and Ahring, 2003).

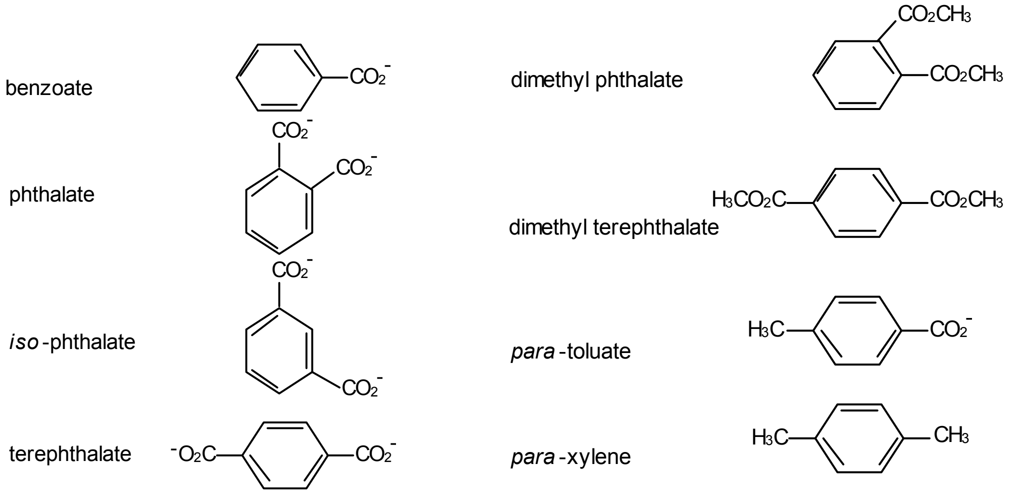


Figure 5.5. *Structural formula of some compounds related with anaerobic phthalate biodegradation (Kleerebezem et al., 1999)*

As aforementioned, in both aerobic and anaerobic environments several phthalic acid esters can be metabolised. The initial step in both the aerobic and anaerobic mineralization of phthalic acid esters is hydrolysis of the ester side chains, resulting in formation of monoalkyl phthalate and phthalate. The most common pathway for aerobic degradation of phthalate is through the protocatechuate pathway, followed by ring cleavage and complete mineralization to carbon dioxide and water. Mineralization of phthalate under anaerobic conditions has only been described for denitrifying cultures. These cultures decarboxylate phthalate under formation of benzoate (Kleerebezem et al., 1999). Benzoate is anaerobically reduced to carboxy-cyclohexene level, followed by ring cleavage (see Figure 5.5) (Schink et al. 1992). Complete or partial methanogenic biodegradation has been demonstrated for phthalate, dimethyl phthalate, diethyl phthalate, dibutyl phthalate and butyl benzyl phthalate. No detailed information concerning the mineralization of dioctyl phthalate and DEHP. For these last both compounds, the solubilization of the ester side chains is identified as the principal limiting factor to understood biodegradation mechanism (Kleerebezem et al.,1999). One time it is overcame; it is possible to predict the biodegradation mechanism which can be, probably, similar to the proposed dimethyl terephthalate biodegradation pathways (see Figure 5.6) as suggested by Kleerebezem et al. (1999) and Qiu et al. (2006).

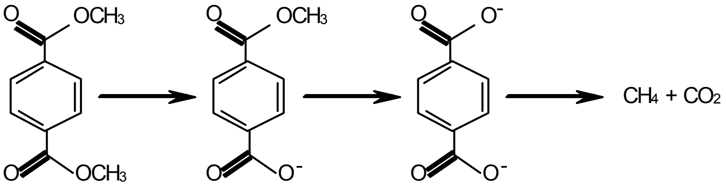


Figure 5.6. *Proposed pathways for anaerobic biodegradation of dimethylterephthalate*

The aim of this Chapter is to compare the efficiency of mesophilic and thermophilic conditions on PAH and DEHP micro-pollutants biodegradation at different hydraulic retention times (HRT), during anaerobic sewage sludge digestion.

5.2. Materials and methods

Experimental set-up

As described in Chapters 3 and 4.2., the anaerobic digestion was carried out in two 5L reactor under mesophlic and thermophilic temperature conditions. The inlet and outlet of the digesters were collected during two times HRT period after a lag time equal to one time HRT period, at the selected HRTs (26, 22, 18, 12 and 8 days). Due to high hydrophobic character of DEHP and PAH (see Chapter 1), the control of both micropollutants content was carried out only on dried sludge.

Anlytical methods *(see Chapter 3)*

The analysis of PAH and DEHP was done as described in Chapter 3. Both PAH and DEHP compounds were recovered in the second fraction (ii) of elution solvent used (DCM: Hex) at 78-96% and 100 %, respectively (see Table 5.4).

Table 5.4. *Ratio of PAHs recovery in the second fraction (%)*

HRT (d)	26	22	18	12	8
Feed	83.22 ± 3	**96.44** ± 9	88.05 ± 7	85.64 ± 5	91.23 ± 2
Mesophilic	82.00 ± 5	90.51 ± 6	93.05 ± 6	-	-
Thermophilic	**77.65** ± 8	89.87 ± 4	87.76 ± 2	84.60 ± 4	91.70 ± 6

For PAH identification and quantification, the MS was run in SIM mode, differenced two groups, the first from 128 to 188 m/z corresponds to LMWPAH, namely acenapthene, phenanthrene and fluorene, and the second from 202 to 278 m/z corresponds to HMWPAH, namely fluoranthene, pyrene, benzo(b+j+k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene and indeno(1, 2, 3-cd)pyren (see Table 5.5 and Annex II, Figure II.1). However, the SCAN mode was employed for identifying the DEHP corresponding picks (primary ion 149 m/z) as showed in Annex II, Figure II.2.

Two calibration curves were prepared for PAH calibration, the first below 100 µg/L (ppb) and the second from 100 to 1000 ppb (see Annex II, Figure II.3). For concentrations around 100 ppb (± 10%), the mean average values calculated from both curves were considered, except for Benzo (ghi) perylene, whose curve tendency could not be determinated for low concentrations. For DEHP quantification, the curve calibration was prepared in the range 100-2000 mg/L.

Table 5.5. *PAHs identification (typical retention time)*

Compound	**m/z**	**RT (min.)**	**Compound/s**	**m/z**	**RT (min.)**
Naphthalene	128	8.50	Benzo (a) anthracene	228	22.70
Acenaphthylene	152	12.82	Crysene	228	22.75
Acenaphthene*	154	12.77	Benzo (b+j+k) fluoranthene*	252	25.20
Fluorene*	166	14.02	Benzo (a) pyrene*	252	25.80
Phenanthrene*	178	16.34	Indeno (1,2,3,cd) pyrene*	276	28.00
Anthracene	178	16.47	Benzo (ghi) perylene*	276	28.59
Fluoranthene*	202	19.25	diBenzo (ah) anthracene	278	28.20
Pyrene*	202	19.50	Anthracene D_{10}	188	16.47

*: EU-PAHs list

Table 5.6. *Recovery indexes and RSD for PAH mixture and DEHP* analysis (%)

	Feed					Thermophilic					Mesophilic		
HRT(d) / Compound	26	22	18	12	8	26	22	18	12	8	26	22	18
Anthracene d_{10}	73.5 ± 6	79.8 ± 3	113.1 ± 4	97.8 ± 4	76.3 ± 8	71.2 ± 5	89.7 ± 8	91.9 ± 3	75.6 ± 4	102.8 ± 9	93.0 ± 4	74.2 ± 3	82.7 ± 5
RSD (PAH)	5.53	6.31	7.6	9.0	5.8	3.8	12.8	3.0	3.6	2.8	6.2	3.9	14.7
DEHP	85.2 ± 3	92.1 ± 4	95.7 ± 4	92.6 ± 7	89.3 ± 6	-	-	-	-	-	-	-	-
RSD (DEHP)	2.3	5.0	8.2	13.4	11.5	6.9	3.5	16.1	8.0	3.6	11.5	13.8	6.0

RSD: relative standard deviation

To estimate method recovering index (Table 5.6), PAHs samples analysis was spiked with Anthracene d_{10}. However, for DEHP sample analysis another sample from feed sludge was spiked with the corresponding patrons with an amount equivalent to 50 mg/kg dw of DEHP (see Chapter 3).

5.3. Results and discussion

5.3.1. PAH biodegradation

Table 5.7 shows the PAH removal results obtained during anaerobic digestion at different conditions. It can observed that the PAH content in fresh sludge was higher than in the treated sludge. Apparently the PAH sludge treated content was more influenced by the initial amount than the HRT digester decrease. As can be seen that all values of total PAH cited en 3rd draft (CEC, 2000) were below the limit content proposed (6 mg/kg dw) in the thermophilic sludge for every HRT tested. However, the mesophilic digested sludge PAH content was higher than the limit value at 26 d HRT.

Table 5.7. *Inlet and outlet sludge PAH content at different conditions (mg/kg dw)*

HRT (d)	26	22	18	12	8
Feed	11.44	8.5	5.26	11.57	5.64
Thermophilic effluent	6.50	5.39	3.07	-	-
Mesophlic effluent	3.95	3.82	1.88	4.73	2.76

Figure 5.7a illustrates the total PAH elimination percentage in the mesophilic and the thermophilic anaerobic digesters (Table II.1 in Annex II presents the detailed content values of all PAH analysed in sludges). Under thermophilic conditions, this percentage oscillated between 51.01% and 65.46% without a clear tendency with the HRT. In the mesophilic digester, the total PAH elimination rate was lower than in the thermophilic digester, where 36.56-43.20% was registered as the interval values of PAH removal efficiency. These results were coherent with the fact that high temperatures can enhance organic micropollutants biodegradation, concordantly with the results reported by Christensen et al. (2004) and Trably et al. (2003). Similarly to the thermophilic digester, the mesophilic removal efficiencies had not a clear tendency with HRT reduction.

On the other hand, it was observed high removal percentages of LMW PAH in both digesters (see Figure 5.7c). LMW PAH elimination efficiency was in the range of 59.82-81.72% and 52.60-57.07% in the thermophilic and the mesophilic digester, respectively, with an HRT dependency effect observation. At higher HRT, the elimination efficiency of

LMW PAH was more important than at lower HRT.

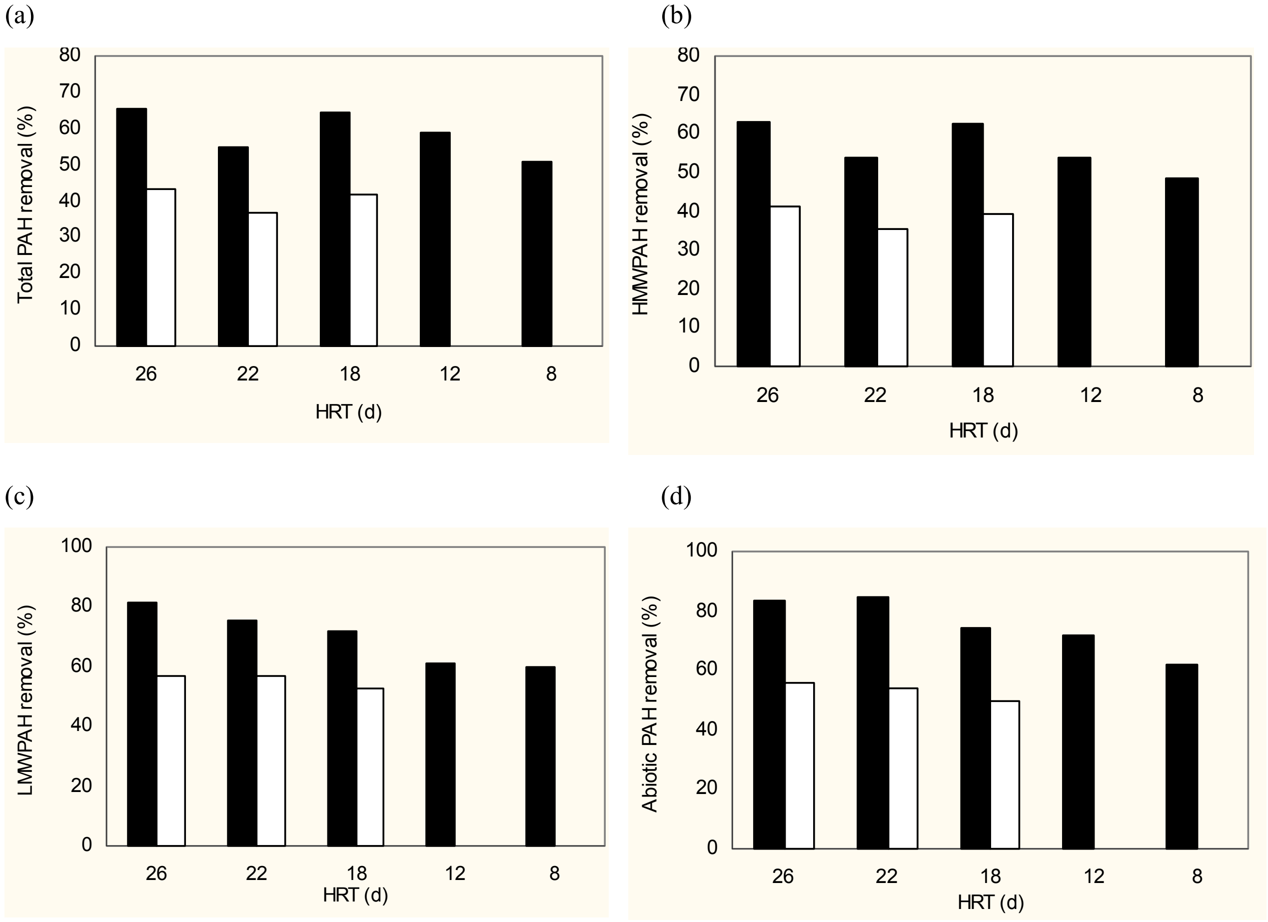


Figure 5.7. *Anaerobic removal efficiencies of (a) total PAH, (b) HMWPAH, (c) LMW PAH and (c) PAH with abiotic characters (phenanthrene and fluorene) under thermophilic (■) and mesophlic (□) conditions at the tested HRTs (Tables II.4 and II.5 in annex II present the detailed results)*

Furthermore, LMW PAH removal percentage was more pronounced than the removal efficiency of total PAH. As an example, at HRT 22d, LMW PAH removal efficiency was achieved with 20% more than the total PAH removal efficiency value in both digesters (see Figure 5.7). These observations can correlate with the fact that the PAH with HMW have a higher coefficient sorption (K_{ow}) than LMW PAH (see Annex II, Table II.2), which is correlated with the diffusion and bioavailability phenomena (Coates et al., 1996; Rockne and Strand, 1998). However, these results (see Annex II, Table II.3) are discrepant with Chang et al. (2003) observations, where another removal order was reported without molecular weight influence [fenanthrene(178) > pyrene(202) > anthracene(178) > fluorene(166) > acenaphthene (154)], probably because of the seed used and the implemented adaptation mode (adapted to phenanthrene).

In Table 5.8 the percentage of LMW PAH with respect to the total PAH of the sludge for every studied case is presented. The effect of LMW PAH high elimination efficiency on the total PAH elimination efficiency was limited because of their low percentage with respect to the total PAH, except at HRT 12 days.

Table 5.8. *LMW PAH content in the total PAH content in sludge (%).*

HRT (d)	26	22	18	12	8
Feed	12.76 ± 1.0	6.12 ± 0.6	18.54 ± 1.3	72.92 ± 3	22.08 ± 2
Thermophilic effluent	6.75 ± 0.5	3.31 ± 0.4	14.49 ± 1.0	69.58 ± 5	18.11 ± 0.9
Mesophilic effluent	9.64 ± 0.3	4.17 ± 1.2	15.06 ± 0.9	-	-

For some compounds (such as phenanthrene and fluorene), the removal percentage values were higher than the other PAH including the remaining LMW PAH (see Figure 5.7c). A higher performance of the elimination rate of these compounds was probably due to the abiotic elimination phenomena cited by Trably et al. (2003), who reported values greater than 50% as abiotic degradation contribution, especially when high temperature, agitation or pH modification were adjusted at the optimum conditions.

Table 5.9. *Meatabolites resulting from anaerobic degradation of PAH (Meckenstock et al., 2004)*

Putative precursor compounds	PAH metabolites
2-Methynaphthalene	Naphthyl-2-methylsuccinic acid
1-Methylnaphthalene	1-Naphthoic acid
Naphthalene, 2-methylnaphthalene	2-Naphthoic acid
Different methylnaphthalene	Methylnaphthoic acid
	Dimethylnaphthoic acid
	1,2,3,4-Tetrahydro-2-naphthoic acid
Naphthalene, 2-methylnaphthalene	5,6,7,8-Tetrahydro-2-naphthoic acid
Naphthalene, 2-methylnaphthalene	Hexahydro-2-naphthoic acid

On the other hand, few researchers investigated the PAH anaerobic degradation pathways and metabolites fate. Only two polycyclic aromatic hydrocarbons, naphthalene and methylnaphthalene, have been studied in detail (Meckenstock et al., 2004). Closer investigation showed that the degradation pathways of naphthalene and 2-methylnaphthalene converge at the level of 2-naphthoic acid (see Table 5.9 and Figures 5.8 and 5.9). Then, the 2-naphthoic acid ring cleavage in the anaerobic degradation of polycyclic aromatic hydrocarbons occur (Figure 5.10), which leads to 2-carboxycyclohexylacetic acid (Meckenstock et al., 2004). This last compound was identified as available to methanogenic consortium in the mineralization step (Boll et al., 2002).

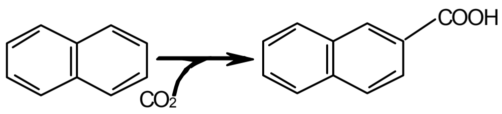


Figure 5.8. *Proposed activation reaction for naphthalene by addition of CO_2 to generate 2-naphthoic acid.*

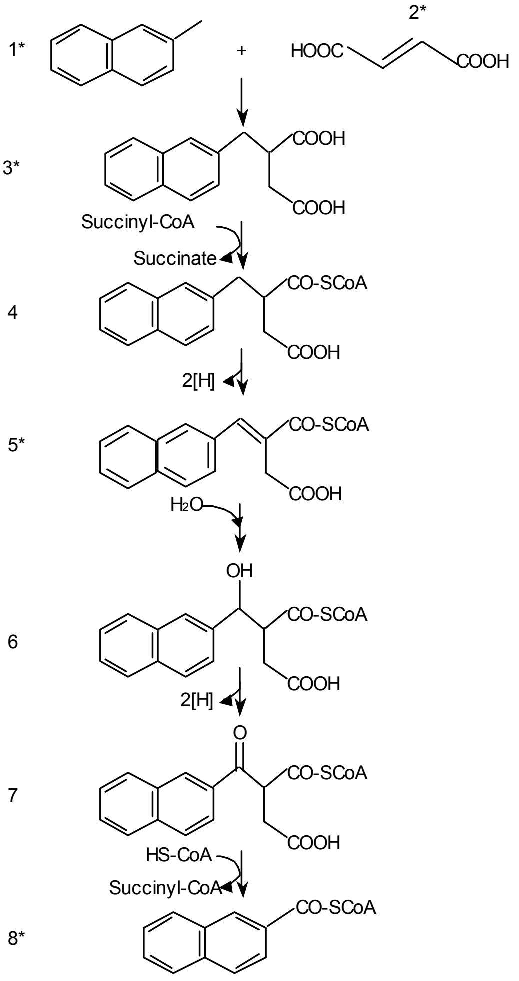


Figure 5.9. *Proposed scheme of the upper pathway of anaerobic 2-methylnaphthalene degradation (1*) to the central intermediate 2-naphthoic acid (8*), (2*) fumaric acid, (3*) naphthyl-2-methyl-succinic acid, (4) naphthyl-2-methyl-succinyl-CoA, (5*) naphthyl-2-methylene-succinyl-CoA, (6) naphthyl-2-hydroxymethyl-succinyl CoA, (7) naphthyl-2-oxomethyl-succinyl-CoA. Compounds marked with (*) have beenidentified as pure substance or free acids.*

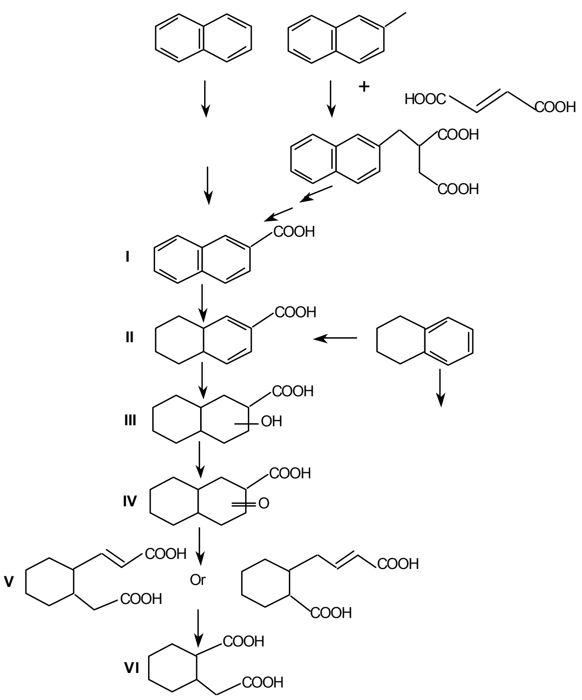


Figure 5.10. *Proposed reductive 2-naphthoic acid pathway of anaerobic PAH degradation. (I) 2-naphthoic acid, (II) 5,6,7,8-tetrahydro- 2-naphthoic acid, (III) hydroxydecahydro-2-naphthoic acid, (IV) b-oxo-decahydro-2-naphthoic acid, (V) $C_{11}H_{16}O_4$-diacid, (VI) 2- carboxycyclohexylacetic acid*

Phenathrene degradation pathways showed also the same naphthalene and methylnaphthalene biodegradation mechanism (Zhang and Young, 1997). However, there is no information available for other PAHs compounds, nor on their metabolites biodegradability and toxicity.

5.3.2. DEHP biodegradation

Figure 5.11 shows DEHP content in the feed, mesophilic and thermophilic sludge (detailed values are presented in Annex II, Table II.6). Both thermophilic and mesophilic anaerobic digestions lead to a reduction in the DEHP content of the treated sludge. Moreover, the decrease in DEHP content was more pronounced under thermophilic conditions. Figure 5.12 presents the DEHP removal efficiency under thermophilic and mesophilic conditions at the tested HRTs (detailed values are presented in Annex II, Table II.6). From this Figure, it is clear that the percentage of removal at thermophilic conditions (31.7%-46.7%) was higher than at mesophilic conditions (21.7%-37.8%). These results are convergent with the conclusions extracted from the studies of Banat et al. (1999) and Fauser et al. (2003).

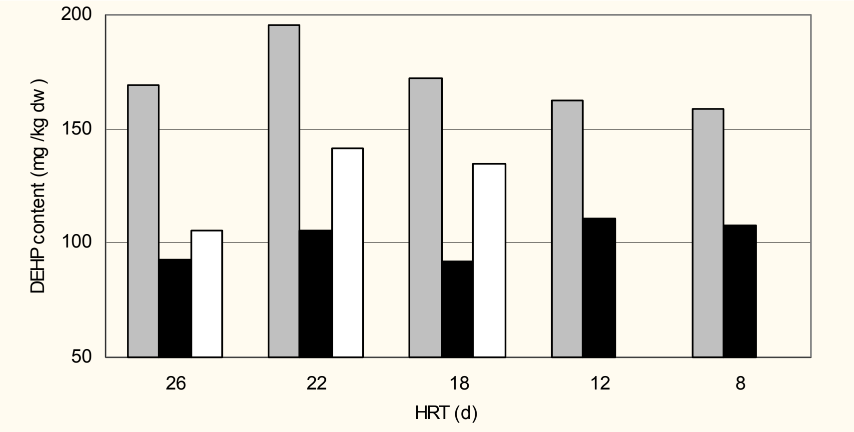


Figure 5.11. *DEHP content in the fresh (▨), thermophilic (■) and mesophilic (□) sludge at the tested HRTs.*

In both digesters, the HRT reduction effect can be appreciated but with different impacts. In the mesophilic digester, the DEHP removal efficiency decreased along with HRT decrease, where elimination percentage deceleration was lower at 22-18 d HRT reduction (it was decreased by 6 % only) than at 26-22 d HRT reduction (10.1%).

In the thermophilic digester, two DEHP removal efficiency values intervals can be distinguished. The first, at higher HRTs (26-18d), where the average removal efficiency value recorded was 46%, and the second interval, at lower HRTs (12-8d), where an average removal value of 32% was registered. It can be observed the dependency of the DEHP elimination on HRT as previously reported by Marttinen et al. (2004). The assessed DEHP removal values in this study were in the range stated by Marttinen et al. (2004) and Angelidiaki et al. (2000), which is 23-61% for an anaerobic system.

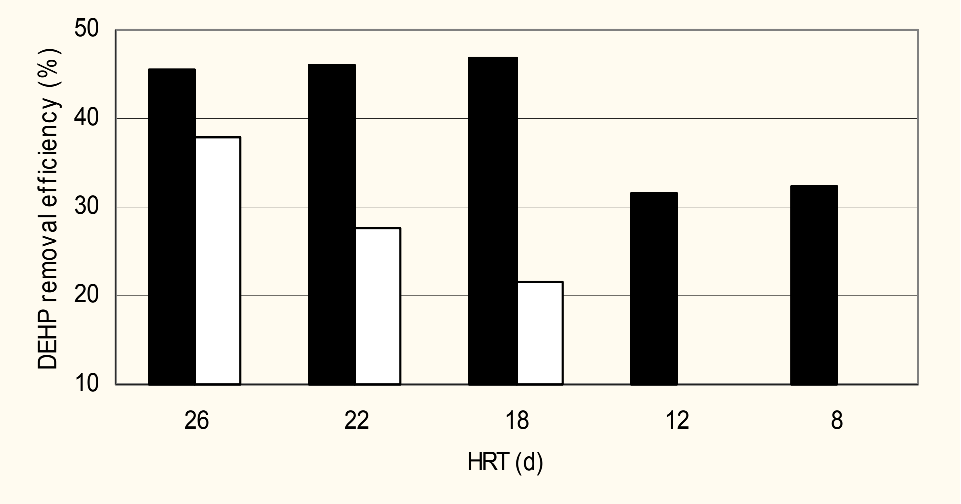


Figure 5.12. *DEHP removal efficiencies at the tested HRTs under thermophilic (■) and mesophilic (□) conditions*

Apparently, the positive effects of high temperature and residence time on DEHP removal observed under aerobic conditions (Knudsen et al., 2000; Fauser et al., 2003), were also stated at anaerobic conditions in this study.

It seems that anaerobic removal of DEHP is confirmed. Hence, as mentioned above (Section 5.1.2), their degradation mechanism stills to be not well clear. Furthermore, the result observed here encourages investigate more to clear DEHP anaerobic biodegradation/biotransformation pathways, monitorizing their possible metabolites and their effect on anaerobic consortia, their toxicity and environmental behaviour.

5.4. Conclusions

An enhancement in Polycyclic Aromatic Hydrocarbons (PAH) removal at the thermophilic temperature range (55 ºC) was observed with respect to the mesophilic temperature range (35 ºC).

The effect of the HRT on the PAH degradation was appreciated only for Low Molecular Weighted PAH (LMW PAH), but the high content of High Molecular Weighted PAH (HMW PAH) and its very recalcitrant characteristics avoided the detection of an HRT effect on the total PAH removal.

Under thermophilic conditions, the Di(2-Ethyl-Hexyl)-Phthalate (DEHP) elimination efficiency was increased by 25% to 50% with respect to mesophilic anaerobic conditions. Furthermore, high HRT had a clear positive effect on DEHP biodegradation.

Additionally, from biogas production and composition results (see Chapter 4), it can be concluded that thermophilic anaerobic digestion at HRT between 12 and 18 d offers the optimum conditions for PAH and DEHP removal from sewage sludge. However, these treatments do not ensure the EU required conditions stated in the 3rd draft (CEC, 2000) for use the sludge in agricultural soil, specailly for DEHP. Hence, an additional treatment, enhanced method actions (pretreatment) or reliable alternative should be carried out if sludge application to soil is required.

Although some studies show the ability of some PAHs compounds mineralization (LMWPAH), but there are not evidences of them for the rest of PAH (HMWPAH). Besides, DEHP metabolites are also not investigated and little information is available on their anaerobic removal mechanism. Hence, it is necessary to verify the fate and toxicity of the PAH and DEHP anaerobic biodegradation metabolites.

5.5. References

Alexander M. (**1995**). How are toxic toxic chemicals in soil. Critical Review. Environ. Sci. Technol.,29, 2713-2717.

Angelidaki I., Mogensen A.S., Ahring B.K. (**2000**). Degradation of organic contaminants found in organic waste. Biodegradation, 11, 377-383.

Banat F. Prechtl S., Bischof F. (**1999**). Experimental assessment of bio-reduction of di-2-ethylhexyl phthalate (DEHP) under aerobic thermophilic conditions. Chemosphere 39, 2097–2106.

Barceló D., Petrovic M. (**2004**). Los microcontaminantes orgánicos: Problemática y situación en Cataluña bajo la perspectiva da le Directiva Europea. Day on mangement and treatment of the WWTPs sludge. Barcelona, December.

Boll M., Fuchs G., Heider J. (**2002**). Anaerobic oxidation of aromatic compounds and hydrocarbons. Curr. Opin. Chemical Biolo., 6, 604-611.

Bollag J.-M. (**1992**). Decontamination soil with enzyms. An in situ method using phenolic and anilinic compounds. Environ. Sci. Technol., 26 (10), 1876-1881.

CEC (**2000**). Working Document on Sludge (3rd Draft), Commission of the European Communities Directorate-General Environment, ENV.E.3/LM, 27 April 2000, Brussels.

Chang B.V., Chang S.W., Yuan S.Y. (**2003**). Anaerobic degradation of polycyclic aromatic hydrocarbons in sludge. Advan. Environ. Res., 7, 623-628.

Cheng H.F., Chen S.Y., Lin J.G. (**2000**). Biodegradation of di-(2-ethylhexyl) phthalate in sewage sludge. Wat. Sci. Technol., 41 (12), 1-6.

Christensen N., Batstone D.J., He Z., Angelidaki I., Shmidt J.E. (**2004**). Removal of polycyclic aromatic hydrocarbons (PAHs) from sewage sludge by anaerobic degradation. Wat. Sci. Tech., 50 (9), 237-244.

Coates J. D., Anderson R. T., Lovley D. L. (**1996**). Oxidation of polycyclic aromatic hydrocarbons under sulphate-reducing conditions. Appl. Environ. Microbiol., 62, 1099-1101.

DEPA, Danish Environmental Protection Agency. (**1995**). Occurrence and effects of environmentally hazardous organic substances in sewage sludge. Working Report Nº 15, prepared by COWI consult.

Ejlertsson J., Svensson B.H. (**1996**). Degradation of bis(2-ethylhexyl) phthalate constituents under methanogenic conditions. Biodegradation 7, 501-506.

Ejlertsson J., Alnervik M., Jonsson S., Svensson B. H. (**1997**). Influence of water solubility, side-chain degradability, and side-chain structure on the degradation of phthalic acid esters under methanogenic conditions. Environ. Sci. Technol., 31, 2761-2764.

Ejlertsson J., Meyerson, U., Svensson B.H. (**1996**). Anaerobic degradation of phthalalic acid esters during digestion of municipal solid waste under landfilling conditions. Biodegradation 7, 345-352.

Fauser P., Vikelsøe J., Sørensen P.B., Carlsen L. (**2003**). Phthalates, nonylphenols and LAS in an alternately operated wastewater treatment plant-fate modelling based on measured concentrations in wastewater and sludge. Wat. Res., 37, 1288-1295.

Feitkenhauer H., Märkl H. (**2003**). Biodegradation of aliphatic and aromatic hydrocarbons at high temperatures. Wat. Sci. Tech., 47 (10), 123-130.

Feitkenhauer H., Müller R., Märkl H. (**2003**). Degradation of polycyclic aromatic hydrocarbons and long chain alkanes at 60 70 ºC by Thermus and Bacillus spp. Biodegradation, 14, 367-372.

Gavala HN, Alatriste-Mondragon F, Iranpour R., Ahring BK. (2003). Biodegradation of phthalate esters during the mesophilic anaerobic digestion of sludge. Chemosphere 52, 673-682.

Ghoshal S., Ramaswami A., Luthy RG. (**1996**). Biodegradation of naphthalene from coal tar and heptamethylnonane in mixed batch systems. Environ. Sci. Technol.,30,1282-1291.

Hartmann H., Ahring B.K. (**2003**). Phthalic acid esters found in municipal organic waste: enhanced anaerobic degradation under hyper-thermophilic conditions. Wat. Sci. Technol., 48(4), 175–183.

Hatzinger P.B., Alexander M.(**1995**). Effect of aging of chemicals in soil on their biodegradability and Extractability. Environ. Sci. Technol., 29, 537-545.

Holliger C.,Zehndert A. JB. (**1996**). Anaerobic biodegradation of hydrocarbons. Current Opinion in Biotech., 7, 326-330.

Horowitz A., Shelton D.R., Cornell C.P., Tiedje J.M. (**1982**). Anaerobic degradation of aromatic compounds in sediments and digested sludge. Dev. Industr. Microbiol., 23, 475-484.

Klecka G.M., Davis J.W., Gray D.R., Madsen S.S. (**1990**).Natural bioremediation of organic contaminants in ground water: Cliffs-Dow superfund site. Ground Water 28, 534-543.

Kleerebezem R., Hulshoff Pol L.W., Lettinga G. (**1999**). Anaerobic biodegradability of phthalic acid isomers and related compounds. Biodegradation 10, 63-73.

Knudsen L., Kristensen G.H., Jørgensen P.E., Jepsen S.-E. (**2000**). Reduction of the content of organic micropollutants in digested sludge by a post-aeration process- a full-scale demonstration. Wat. Sci. Technol., 42 (9), 111-118.

Madsen P.L., Thyme J.B., Henriksen K., Møldrup P., Roslev P. (**1999**). Kinetics of di-(2-ethylhexyl)phthalate mineralization in sludge-amended soil. Environ. Sci. Technol., 33, 2601-2606.

Marttinen S.K., Hänninen K., Rintala J.A. (**2004**). Removal of DEHP in composting and aeration of sewage sludge. Chemosphere, 54, 265–272.

Meckenstock R.U., Safinowski M., Griebler C. (**2004**). MiniReview: Anaerobic degradation of polycyclic aromatic hydrocarbons. FEMS Microbiol. Ecolo., 49, 27-36.

NSEPB, National Swedish Environmental Board (**1992**). Sludge-Content of organic environmentally hazardous materials. Overview and assessment of analytical results. Reports Nº.4085.

O'Conner O. A., Rivera M. D., Young L. Y. (**1989**). Toxicity and biodegradation of phthalic acid esters under methanogenic conditions. Environ. Toxicol. Chem., 8, 569-576.

Pavlostathis S.G., Jagalal K. (**1991**). Desorption behavior of trichloroethylene in contaminated soil. Environ. Sci. Technol., 25,274.

Qiu Y-L., Sekiguchi Y., Hanada S., Imachi H., Tseng I-C., Cheng S-S., Ohashi A., Harada H., Kamagata Y. (**2006**). Pelotomaculum terephthalicum sp. nov. and Pelotomaculum isophthalicum sp. nov.: two anaerobic bacteria that degrade phthalate isomers in syntrophic association with hydrogenotrophic methanogens. Arch. Microbiol.185, 172-182.

Reinhart D. R., Pohland F. G. (**1991**). The assimilation of organic hazardous wastes by MSW in landfills. J. Indust. Microbiol., 8, 193-200.

Rockne K. J., Strand S. E. (**1998**). Biodegradation of bicyclic and polycyclic aromatic hydrocarbons in anaerobic enrichments. Environ. Sci. Technol., 32, 2962-2967.

Schink B, Brune A., Schnell S. (**1992**). Anaerobic degradation of aromatic compounds. In: Winkelmann VCH (Ed) Microbiol Degradation of Natural Products, (pp 219–242). Weinheim–New York–Basel–Cambridge.

Schnaak W.,Kuchler T., Kujawa M., Henschel K.P., Sußenbach D., Donau R. (**1997**). Organic in sewage sludge and their ecotoxicological significance in the agricultural utilization of sewage sludge. Chemosphere, 35, 5-11.

Shelton D. R., Boyd S. A., Tiedje J. M. (**1984**). Anaerobic biodegradation of phthalic acid esters in digested sludge. Environ. Sci. Technol., 18, 93-96.

Staples C.A., Peterson D.R., Parkerton T.F., Adams W.J. (**1997**). The environmental fate of phthalate esters: a literature review. Chemosphere, 35, 667-749.

Subramaniam K., Stepp C., Pignatello J.J., Smets B., Grasso1 D. (**2004**). Enhancement of polynuclear aromatic hydrocarbon. Desorption by complexing agents in Weathered Soil. Environ. Eng. Sci., 21(4), 515-523.

Trably E., Patureau D., Delgenes J.P. (**2003**). Enhancement of polycyclic aromatic hydrocarbons removal during anaerobic treatment of urban sludge. Wat. Sci. Tech., 48 (4), 53-60.

Volkering F., Breure A.M., van Andel J.G. (**1993**). Effect of microorganisms on the bioavailability and biodegradation of crystalline naphthalene. Appl. Microbiol. Biotechnol., 40, 535-540.

Wild, S.R., Jones, K.C. (**1989**). The effect of sludge treatment on the organic contaminant content of sewage sludge. Chemosphere 19 (10), 165–177.

Zhang, X.M., Young, L.Y. (**1997**) Carboxylation as initial reaction in the anaerobic metabolism of naphthalene and phenanthrene by sulfidogenic consortia. Appl. Environ. Microbiol. 63, 4759-4764.

Ziogou K., Kirk P.W.W., Lester J.N. (**1989**). Behavior of phthalic acid esters during batch anaerobic digestion of sludge. Wat. Res., 23, 743-748.

Chapter 6

PCB and AOX removal in mesophilic and thermophilic anaerobic sewage sludge digestion

Chapter 6

6. PCB and AOX removal in mesophilic and thermophilic anaerobic sewage sludge digestion*

Summary

In this study, a comparison of the biodegradation of Adsorbed Organic Halogen compounds (AOX) and PolyChlorinated Biphenyls (PCB) in thermophilic and mesophilic anaerobic digestion (seeded with Waste Activated Sludge) at different Hydraulic Retention Times (HRT) was performed. Results obtained in this work showed an enhancement of both PCB and AOX biodegradation under thermophilic conditions.

The total PCB removal efficiency was in the range of 59.4-83.5 % under thermophilic conditions and 33.0-58.0 % under mesophilic conditions. HRT played an important role in the digester performance since high working HRTs implied more reduction of the total PCB amount in the sludge. The total PCB content in the treated sludge under thermophilic conditions lied below the cut-off limit proposed in the Directive 3rd draft presented to the European Commission (CEC, 2000). Besides, a bioaccumulation of Lightly Chlorinated PCBs was detected in the mesophilic digester, which is in concordance with the theory that the PCBs are anaerobically biodegraded by means of a reductive dechlorination mechanism.

On the other hand, the AOX removal efficiency was in the range of 40.4-50.3% in thermophilic conditions and 30.2-43.2% in mesophilic conditions. The AOX content in the treated sludge of both thermophilic and mesophilic digesters did not exceed the cut-off limit proposed in the mentioned Directive 3rd draft (CEC, 2000). Moreover, high HRTs promoted an improvement of the AOX removal capacity of the anaerobic digestion.

---

* **Benabdallah el Hadj T., Torres R., Dosta J., Mata-Álvarez J**. (2006). PCB and AOX removal in mesophilic and thermophilic anaerobic sewage sludge digestion. Biochem. Eng. J. (submitted)

6.1. Introduction

6.1.1. AOX

The parameter "Adsorbable Organic Halogen compounds (AOX)" was initially introduced in 1976 to denominate those organo-halogen compounds present in the water (chloride, bromide and iodide organic matter) that could be adsorbed on activated carbon surfaces (Müller, 2003). Subsequently, the AOX parameter was extended to AOX-S18 (Adsorbed Organic Halogen compounds) for Sludge and Sediments. In this context, the term Adsorbable was substituted by Adsorbed in order to refer only to some insoluble organo-halogens adsorbed on the solid matter (as Vinyl Chloride, VC, and PolyVinyl Chloride, PVC) that were reported to be potential carcinogenic substances (Salkinoja-Salonen et al., 1995; Auras, 2001). Therefore, AOX as a sum parameter does not represent a specific chemical substance and it is not a direct measure of toxicity.

The major AOX generation sources are paper and pulp industry, which was estimated to be responsible of about 50% of the total organic halogen emissions into the environment in Finland, PVC manufacture and waste incineration (Salkinoja-Salonen et al., 1995; Auras, 2001).

Since the regular screening of prioritized organic pollutants on a day-to-day basis would be complex and uneconomical, it has been suggested that AOX can be used as an indicator of these prioritized substances (Hahn et al., 1999). Although concentrations of AOX in sludge do not really give information about the absence or presence of hazardous substances, this parameter represents a measure of careful soil protection to prevent the input of high amounts of anthropogenic compounds into the soil, some of which may be persistent pollutants (Leschber, 1992).

The average AOX concentration in sewage waste was reported to be around 37 µg/L (Schowanek et al., 1996). In a survey of contamination levels of Danish sewage sludge during 1995, Madsen et al. (1997) found AOX concentrations in the range of 75-890 mg Cl/kg dry weigh (dw) in sludge samples of municipal WWTPs. Low concentration values were reported for Germany's sludge, with 206, 201 and 196 mg/kg dw as the average AOX concentration in 1994, 1995 and 1996, respectively (UMK-AG, 2001).

The anaerobic biodegradation of the AOX present in the sewage sludge of municipal WWTPs has been little studied. The majority of reports were focused on the AOX removal in pulp paper waste. Ali and Sreekrishnan (2000) reported that the AOX initial content (32.2 mg/L) of pulp and paper mill bleach effluent was reduced in a 73% after its anaerobic digestion treatment. Similar results were recorded by Ferguson and Dalentoft (1991) with AOX removals from 40 to 65%. Due to their positive impact, anaerobic digestion was incorporated as a complement of the aerobic treatment system in pulp and paper mill industry to enhance AOX content reduction of a wide effluent range (Ferguson, 1994; Haggblon and Sakinoja-Salonen, 1991; Savant et al., 2006).

The improvement of AOX removal under anaerobic conditions indicates that given a suitable exposure time, methanogenic bacteria are eminently capable not only of withstanding biorecalcitrant and bioinhibitory environments, but also of thriving in them (Ali and Sreekrishnan, 2000). However, the AOX removal mechanism by anaerobic consortium needs to be elucidated since it is still not clearly understood. Some researchers proposed the reductive dehalogenation as the dominant process to remove organo-halogen compounds under anaerobic conditions (Schumacher et al., 1997; van Pee and Unversucht, 2003; Wohlfahrt and Diekert, 1997). An example of this elimination process is the proposed mechanism of tetrachloroethene and trichloroethene dechlorination (see Figure 6.1), that leads to the oxidation of an electron rich compound, such as hydrogen and/or an organic substrate (Magnuson et al., 2000; Rysavy et al., 2005; van de PAS et al., 1999).

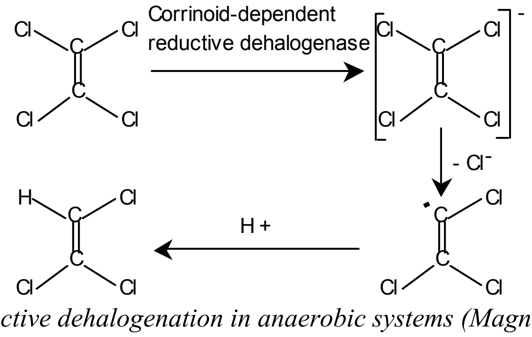


Figure 6.1. *Reductive dehalogenation in anaerobic systems (Magnuson et al., 2000)*

Little information of the temperature effect on halogen organic compounds removal under anaerobic conditions is available. Tripathi and Allen (1999) observed a decrease of the AOX efficiency removal from 70-64% to 60-50% when temperature was increased from 35 to 65 ºC in aerobic sequencing batch reactors treating bleached Kraft pulp mill effluent (AOX content between 4-10 mg Cl/L). These authors also observed an important reduction in the COD removal efficiency when temperature was increased. However, these results cannot be extrapolated to anaerobic conditions because anaerobic digestion at high temperature ranges (e.g. thermophilic conditions) enhances drastically organic matter removal (van Lier et al., 1993, 1997).

6.1.2. PCB

The PCB is a distinguished group of organo-halogen compounds that include 209 congeners that can be synthetized at industrial scale (Borja et al., 2005). They are sold according to their chlorination level under different trade names, such as Aroclor, Phenoclor, Pyralene, Clophen and Kanechlor (Chang et al., 1999). For example, the mixtures of Aroclor are designated by four digits: the first two indicate that the mixture is composed of chlorinated biphenyls and the last two digits indicate the weight percentage of chlorine (Master et al., 2002). Due to their exceptional fire resistance, chemical stability and conductance (Hutzinger et al., 1974), PCBs are used in a wide variety of industrial products, including heat-transfer fluids, hydraulic fluids, solvent extenders, plasticizers, flame retardants, organic diluents and dielectric fluids (Borja et al., 2005). Moreover, PCBs are persistent, lipophilic and strongly hydrophobic substances (see Table 6.1). Therefore, they have a high potential of bioaccumulation and bioconcentration (Alkock and Jones, 1993; Hansen, 1987) and they are ubiquitous contaminants of the environment (Hansen, 1987; Hutzinger and Veer Kamp, 1981).

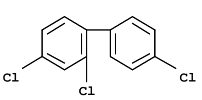
Table 6.1. *Characteristics of some PCB congeners (Gusev et al., 2005)*

PCB congener	Cl atoms number	Molecular weight	Log K_{ow}	log K_{oc}	Vapour pressure (atm)
28	3	257.5	5.67	5.41	$1.43 \cdot 10^{-7}$- $3.31 \cdot 10^{-7}$
52	4	292	6.10	-	$1.28 \cdot 10^{-7}$- $8.90 \cdot 10^{-7}$
101	5	326.4	6.37	-	$5.20 \cdot 10^{-9}$- $3.54 \cdot 10^{-8}$
138	6	360.9	6.65	-	-
153	6	360.9	6.88	6.51	$3.2 \cdot 10^{-10}$- $6.91 \cdot 10^{-8}$
180	7	395.3	7.20	6.97	-

It is estimated that 10 million tons, equivalent to one-third of the total worldwide production of PCBs, had been released into the environment (Savant et al., 2006). PCB contamination still occurs and is of great public concern due to its potential toxicity to humans and wildlife. Exposure to these compounds is undesirable since they are claimed to be carcinogenic, to act as tumour promoters, and suppress the immune and the reproduction system (WHO, 1992).

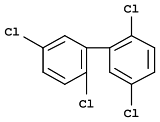
Sewage sludge, which contains traces of many chemicals of natural and synthetic origin, presents a typical PCB content of 1-10 mg/kg with an elevated portion of highly chlorinated PCBs (Alkock and Jones, 1993). In the 3rd draft presented to UE environmental commission (CEC, 2000), the proposed PCB cut-off limit was 0.8 mg/kg dw. This parameter includes the sum of six PCB congeners content (nº: 28, 52, 101, 138, 153, 180). All the six aforementioned PCBs are *ortho* substituted (see Figure 6.2). The selection of these congeners for monitoring PCBs presence is due to their occurrence frequency in sludge and because it was reported that *ortho*-PCBs are the more recalcitrant chlorinated biphenyls (CEC, 2000; Natarajan et al., 1999).

PCB28
$C_{12}H_7Cl_3$



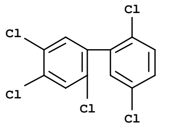
1,1'-Biphenyl, **2**,4,4'-trichloro

PCB52
$C_{12}H_6Cl_4$



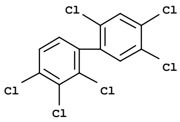
1,1'-Biphenyl, **2,2'**,5,5'-tetrachloro

PCB101
$C_{12}H_5Cl_5$



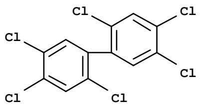
1,1'-Biphenyl, **2,2'**,4,5,5'-pentachloro

PCB138
$C_{12}H_4Cl_6$



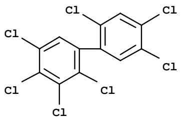
1,1'-Biphenyl, **2,2'**,3,4,4',5'-hexachloro

PCB153
$C_{12}H_4Cl_6$



1,1'-Biphenyl, **2,2'**,4,4',5,5'-hexachloro

PCB180
$C_{12}H_3Cl_7$



1,1'-Biphenyl, **2,2'**,3,4,4',5,5'-heptachloro

(NOTA: numbers in bold type refer to ortho position)

Figure 6.2. *Chemical structure and correspondent nomencalture of PCBs proposed to be regulate their content in sludge destined to agricultural use.*

Aerobic PCB biodegradation and/or biotransformation have been observed in both pure and mixture cultures (Adriaens and Focht, 1990; Chang et al., 1999; Fava and Marchetti, 1991; Furukawa et al., 1987; Pettigrew et al., 1990). However, some researchers observed an acceptable efficiency for lightly chlorinated biphenyls (LCB) biodegradation but not for highly chlorinated biphenyls (HCB) substituted with more the 5 Cl atoms (Nakhla et al., 2002; Rodrigues et al., 2001).

Because a complete mineralization of commercially used PCB mixtures is not achieved by aerobic microorganisms, a sequential anaerobic-aerobic biological treatment system has been proposed (Abramowicz, 1990). The fact that HCBs were susceptible to anaerobic biodegradation to less chlorinated congeners (see Table 6.2), primarily *ortho*- and *para*-substituted, this step permits the availability of the partial or total dechlorinated anaerobic product to aerobic consortium (Maltseva et al., 1999; Megharaj et al., 1997; Rodrigues et al., 2001).

Table 6.2. *Arcolor 1260 biodegradtion in anaerobic-aerobic sequencing treatment (Master et al., 2002)*

	Influent	Anaerobic treatment effluent	Aerobic treatment effluent
PCB concentration (μg/ g)	64.7	58.3	19.6
PCB removal (%)		11	66
Chlorination level (Chlorine atom /PCB molecule)	6.2	5.2	5.5

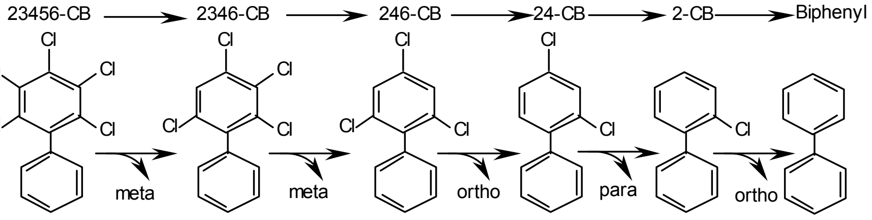


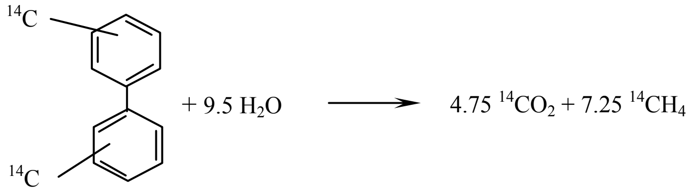
Figure 6.3. *Proposed pathway for anaerobic dechlorination of 2,3,4,5,6-CB by anaerobic microbiological granules. Chlorine removal was observed in the order meta, ortho and parafollowed by ortho dechlorination (Natarajan et al., 1996)*

Reductive dechlorination was reported as the dominant anaerobic biodegradation PCB mechanism (Brown et al., 1984, 1987). Moreover, other researchers (Alder et al., 1993; Bedard et al., 1986; Ye et al., 1992) observed accumulation of *ortho*-LCB after anaerobic action on correspondent *para*- and *meta*-HCB. In a recent study, Nakhla et al. (2002) recorded an anaerobic biodegradation efficiency around 65% and 77% of Aroclor 1260 and Aroclor 1254, respectively. However, the use of anaerobic granulated inoculum to Aroclor 1254 removal from sediments was carried out at 70-75% as efficiency range without neither LCB nor *ortho* PCB accumulation after 24 weeks incubation time (Natarajan et al., 1998) (See Figure 6.3). Since the PCBs removal by other factors can reach 50% within 15 months under anaerobic conditions, Rhee et al.(1993), Hratkamp-Commandeur et al. (1996) and Shiu and Mackay (1986) estimated that ortho-PCB removal can be achieved with high rate than other congeners by evaporation, because they have low solid adsorption tendency, and/or by phototrophic microorganisms (Montgomery and Vogel, 1992).

On the other hand, the total mineralization of PCBs was estimated possible since the viability of LCB biodegradation is recorded (see Table 6.3) and the use of an inoculum adapted to PCBs dehalogenation can degrade biphenyls, supplied as a sole carbon source or cometabolised with glucose and methanol, under anaerobic conditions, as expressed by Reaction 6.1 (Natarajan et al., 1999).

Table 6.3. *Dechlorination rate of sludge under anaerobic conditions (Chang et al., 1999).*

Compound	Lag time (d)	Dechlorination rate (mg/L d)
2,3,4-CB	14	0.063±0.01
2,3,4,5-CB	14	0.050±0.02
2,3,4,5,6-CB	41	0.050±0.01



(6.1)

In this chapter, the study was carried out (i) to compare the removal efficiency of halogenated compounds presented by AOX parameter and the specific chlorinated compounds namely PCBs in anaerobic digester between mesophilic and thermophilic range temperature conditions, (ii) to determine HRT reduction impact on the removal of the aforementioned compounds, and (iii) to corroborate the obtained results with the principles of the proposed dehalogenation mechanisms. This work was carried out due to the little information regarding the organic halogenated compounds anaerobic biodegradation and their fate in sludge digestion. Hence, it is considered as a first study.

6.2. Materials and methods

6.2.1. Experimental set-up

The influent and effluent samples corresponding to selected HRTs (26, 22, 18, 12 and 8 days), were collected in crystal vessels and kept at -10 ºC for micro-pollutant analysis (see Chapter 4).

6.2.2. Analytical methods

Analyses of total chemical oxygen demand (CODt), soluble chemical oxygen demand (CODs), total solids (TS) and volatile solids (VS), pH, bicarbonate alkalinity and ammonium nitrogen (NH_4^+-N); individual volatile fatty acids (VFA) and gas composition were analysed as described in Chapter 3.

Table 6.4. *PCB identification at GC-MS analysis.*

PCB Congener	m/z	Typical Retention Time (min)
PCB 28	256	15.77
PCB 53	292	16.55
PCB 101	326	19.31
PCB 138	360	22.63
PCB 153	360	23.84
PCB 180	394+396+398	26.97

AOX analysis was performed as described in Chapter 3. The recovery method index was in the range (79-104%). It was estimated by the analysis of a supplemental dried influent sludge sample (3 mg) spiked with an equivalent AOX concentration of 250 mg Cl/kg dw (by adding the corresponding volume of a 2 mg Cl/L of p-chlorophenol aqueous solution). Furthermore, the relative standard deviation values registered were between 11% and 23%.

PCB samples were analysed as described in Chapter 3. The GC/MS is used to PCB quantification. For this, the MS was run in SIM mode from 256 to 398 m/z to identify the different PCBs (see Table 6.4 and Figure III.1 in annex III,).

The PCB congeners were recovered in the second elution solvent (Hex:DCM) at more than 95% and the rest of them was eluted in the third fraction (DCM:AcEt) added to the alumina-sodium sulphate column (see Chapter 3). However, the results were corrected from the recuperation grade obtained by means of the spiked samples, which oscillated between 68% and 95%. The maximum relative standard deviation value was 27%.

6.3. Results and Discussion

6.3.1. AOX biodegradation

Figure 6.4 shows the average AOX content in the fresh sludge and in the mesophilic and thermophilic treated sludge for every studied HRT (Annex III, Table III.1 Shows the detailed value). The AOX content in the fresh sludge was in the range of 580-733 mg Cl/ kg dw, which is higher than the cut-off limit (500 mg Cl/ kg dw) proposed 3rd draft (CEC, 2000). Anaerobic treatment at both temperature ranges lead to a reduction in the AOX content, which was below 406 mg Cl/ kg dw in every studied case. For each HRT tested, the thermophilic AOX removal efficiency was higher than the mesophilic one.

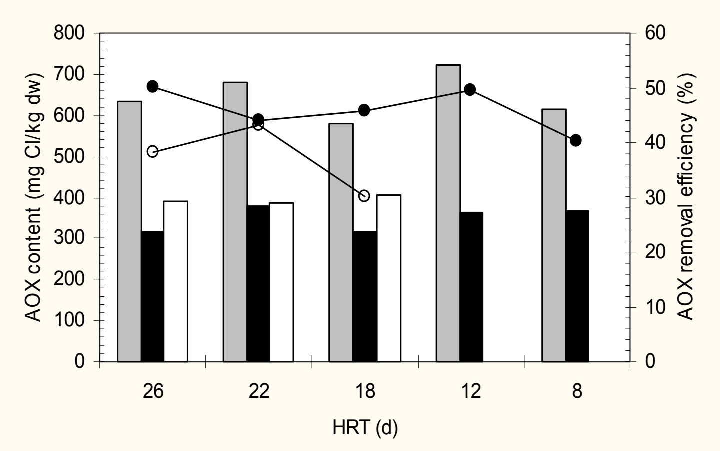


Figure 6.4. *AOX content in the fresh sludge (▨), thermophilic (■) and mesophilic (□) treated sludge and AOX removal efficiency (%) in the thermophilic (-●-) and mesophilic (-○-) digester at the tested HRTs.*

Besides, AOX removal percentage was reduced when shorting the operating HRT for both temperature ranges. In the thermophilic digester, the AOX removal efficiency decreased from 50.3% (at HRT 26 days) to 40.4% (HRT 8 days), while in the mesophilic digester it was reduced from 38.2% (HRT 26 days) to 30.2% (HRT 18 days). Therefore, the observed HRT dependency was more pronounced for mesophilic conditions. Then, the results observed here confirm the organic halogenated compound biodegradation/biotransformation, which is in accordance with the possibility of organo-halogen compounds degradation following reductive dehalogenation pathways (see Figure 6.1).

6.3.2. PCB biodegradation

In Table 6.5, the concentration of different PCB congeners for the fresh and the treated sludge at each HRT tested is presented. The total PCB content in the fresh sludge was in the range of 1-10 mg/ kg dw, which is concordance with the total PCB concentrations reported by Alkock and Jones (1993) for non-treated sludge. Moreover, the total PCB content in the fresh sludge is basically due to Highly Chlorinated PCBs (HCB), which is measured as the sum of PCB 101, PCB 138, PCB 158 and PCB 180.

In the treated sludge, a reduction of the total PCB content was clearly detected. The total PCB thermophilic effluent content remained below the cut-off limit (0.8 mg/kg dw) proposed in the 3rd draft of Directive (CEC, 2000). Under mesophilic conditions, this limit was exceeded for the HRT of 22 and 18 days. The main reasons of this non-fulfilment are the reduced operating HRT and the relatively high amount of PCB in the fresh sludge. On the other hand, HRT played an important role in the digester performance since high working HRTs implied more reduction of the total PCB amount in the sludge.

Table 6.5. *PCB content in the fresh and the treated sludge (mg/kg dw) at the studied HRTs.*

		HRT (d)	26	22	18	12	8
Feed	LCB	PCB 28	0.11 ± 0.01	0.41 ± 0.05	0.13 ± 0.01	0.27 ± 0.04	0.31 ± 0.04
		PCB 52	0.12 ± 0.00	0.29 ± 0.03	0.17 ± 0.01	0.13 ± 0.03	0.21 ± 0.02
	HCB	PCB 101	0.65 ± 0.02	0.83 ± 0.06	1.06 ± 0.10	0.83 ± 0.02	0.55 ± 0.03
		PCB 138	0.33 ± 0.00	0.65 ± 0.06	0.43 ± 0.04	0.52 ± 0.05	0.38 ± 0.03
		PCB 153	0.14 ± 0.00	0.41 ± 0.04	0.27 ± 0.02	0.38 ± 0.03	0.28 ± 0.02
		PCB 180	0.21 ± 0.05	0.32 ± 0.03	0.35 ± 0.06	0.25 ± 0.02	0.16 ± 0.01
		Total PCB	1.56 ± 0.30	2.91 ± 0.40	2.41 ± 0.63	2.38 ± 0.30	1.89 ± 0.50
Thermophilic	LCB	PCB 28	0.10 ± 0.01	0.38 ± 0.00	0.12 ± 0.01	0.25 ± 0.05	0.30 ± 0.04
		PCB 52	0.09 ± 0.01	0.24 ± 0.02	0.14 ± 0.02	0.12 ±0.01	0.18 ± 0.01
	HCB	PCB 101	0.05 ± 0.01	0.03 ± 0.00	0.14 ±0.01	0.18 ± 0.03	0.15 ± 0.01
		PCB 138	0.01 ± 0.00	0.08 ± 0.00	0.05 ± 0.00	0.10 ± 0.01	0.07 ± 0.01
		PCB 153	0.01 ± 0.00	0.04 ± 0.01	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.01
		PCB 180	N.D	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
		Total PCB	0.26 ± 0.03	0.78 ± 0.02	0.50 ± 0.05	0.72 ± 0.12	0.77 ± 0.11
Mesophilic	LCB	PCB 28	0.13 ± 0.01	0.56 ± 0.01	0.22 ± 0.03	-	-
		PCB 52	0.11 ± 0.01	0.26 ± 0.02	0.19 ± 0.02	-	-
	HCB	PCB 101	0.24 ± 0.02	0.38 ± 0.02	0.65 ± 0.04	-	-
		PCB 138	0.09 ± 0.00	0.17 ± 0.00	0.29 ± 0.01	-	-
		PCB 153	0.04 ± 0.00	0.10 ± 0.01	0.16 ± 0.01	-	-
		PCB 180	0.03 ± 0.00	0.07 ± 0.00	0.09 ± 0.00	-	-
		Total PCB	0.65 ± 0.03	1.54 ± 0.06	1.61 ± 0.11	-	-

N.D.: Not Detected

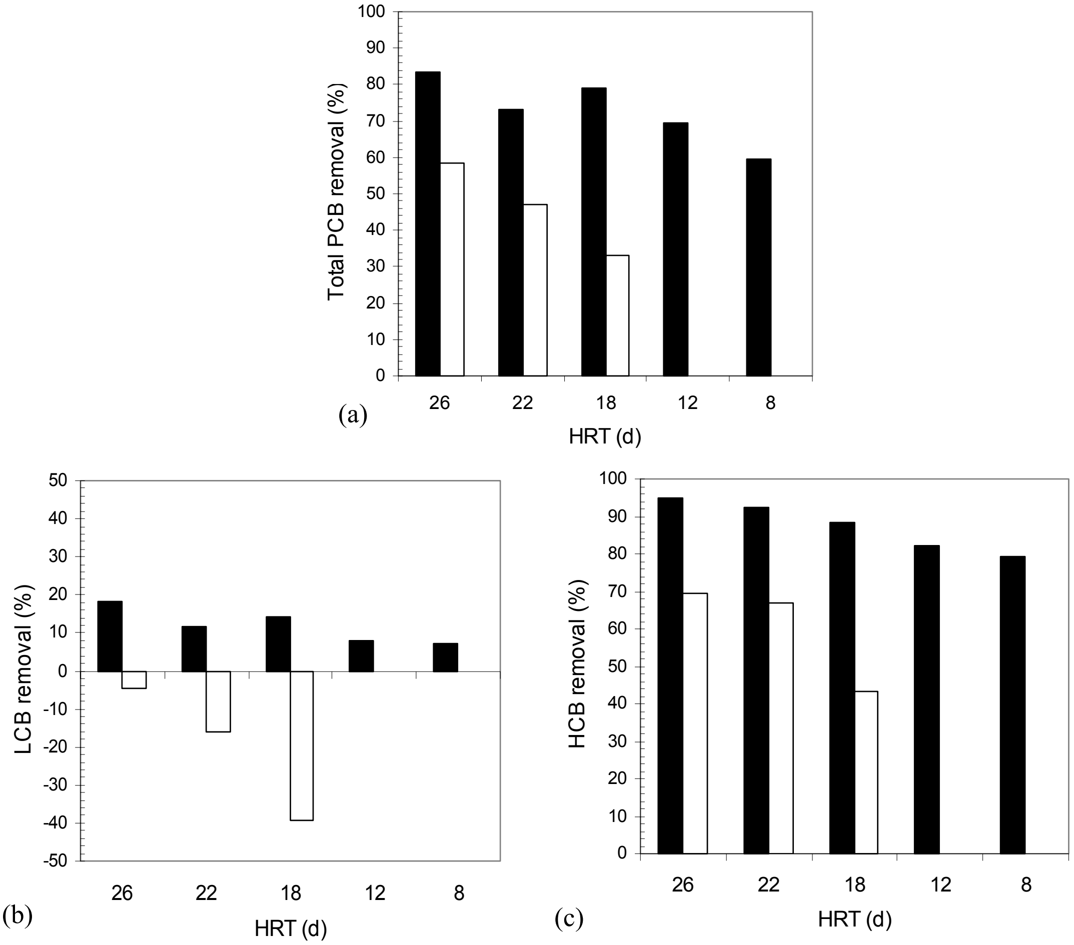


Figure 6.5. *Total PCB (a), LCB (b) and HCB (c) removal efficiencies (%) in the thermophilic(■) and mesophilic (□) digester at the applied HRTs*

In contrast to the fresh sludge, the total PCB content in the treated sludge is mainly related to Lightly Chlorinated PCBs (LCB), namely, PCB 28 and PCB 52. Figure 6.5 shows the reduction of total PCB, LCB and HCB in the mesophilic and thermophilic anaerobic digestion at the tested HRTs. Besides, the percentage of LCB and HCB in fresh and treated sludge is specified in Table 6.6. These results can be explained by the fact that anaerobic digestion degrade the PCBs congeners following the reductive dechlorination process described by Abramowicz (1990) and Borja et al. (2005) (see Figure 6.3).

Table 6.6. *PCB content composition according to chlorination level in fresh and digested anaerobic sludge under thermophilic and mesophilic conditions.*

	HRT (d)	26	22	18	12	8
Feed	LCB (%)	14.7 ± 1.0	24.0 ± 4.5	12.4 ± 1.1	16.8 ± 1.0	27.5 ± 2.1
	HCB (%)	85.3 ± 3.5	75.9 ± 11.5	87.5 ± 4.5	83.2 ± 3.7	72.5 ± 3.3
Thermophilic	LCB (%)	73.1 ± 5.4	78.9 ± 8.9	51.3 ± 4.1	51.1 ± 2.5	62.9 ± 5.1
	HCB (%)	26.9 ± 3.6	21.1 ± 3.5	48.7 ± 3.2	48.9 ± 1.5	37.1 ± 2.1
Mesophilic	LCB (%)	37.1 ± 5.5	52.7 ± 6.5	25.8 ± 2.1	-	-
	HCB (%)	62.9 ± 2.3	47.3 ± 45.1	74.1 ± 3.2	-	-

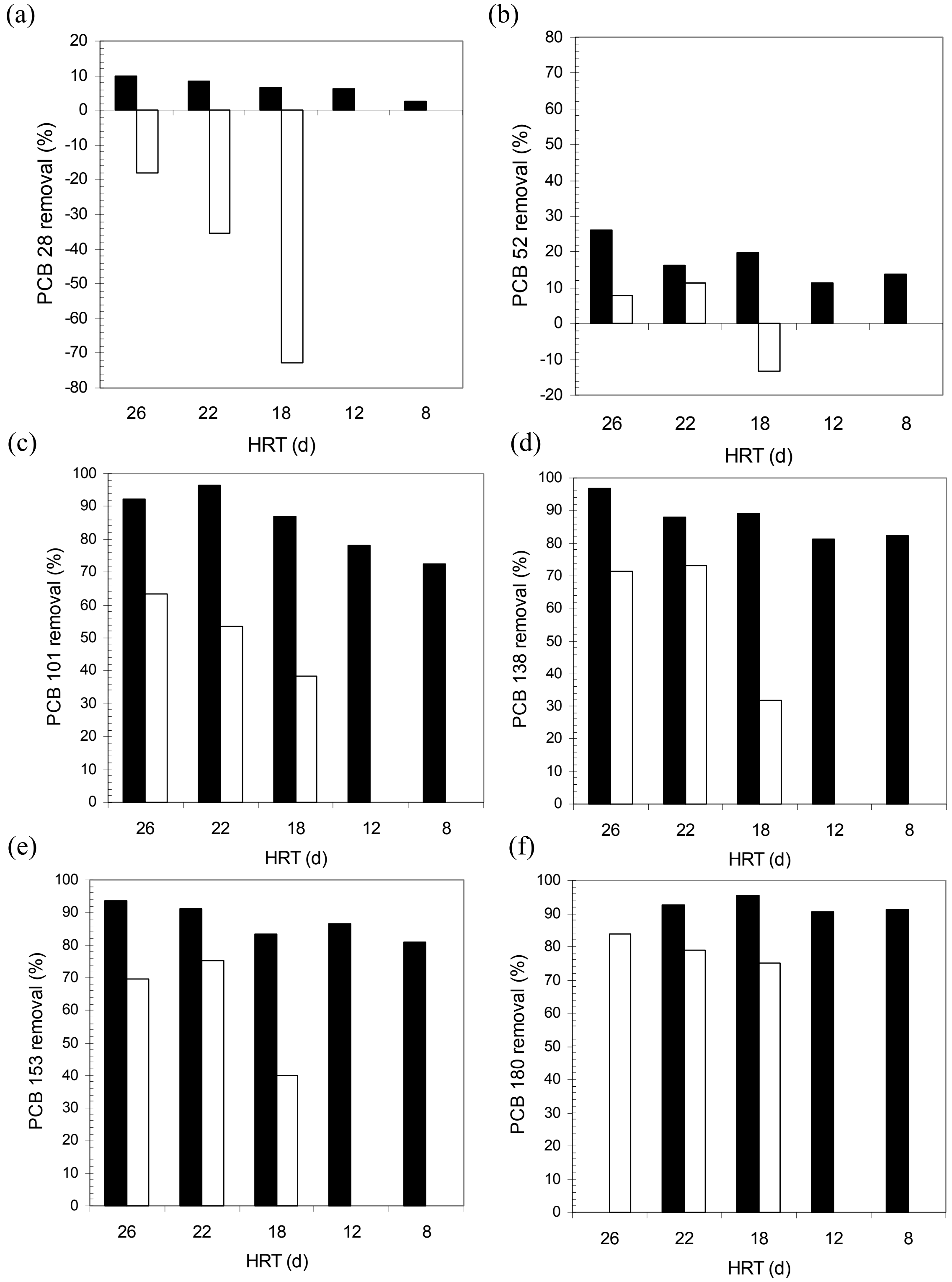


Figure 6.6. *Removal efficiencies (%) of PCB 28 (a), PCB 52 (b), PCB 101 (c), PCB 138 (d), PCB 153 (e) and PCB 180 (f) in the thermophilic (■) and mesophilic(□) digester at the tested HRTs.*

Moreover, LCB are accumulated because the biological conversion of HCB to LCB was faster than the biodegradation of LCB (see Figure 6.3). This bioaccumulation of LCB promote that, at some HRTs tested, the net LCB removal percentage was negative. Figure 6.6 shows the individual PCB congeners biodegradation levels at the corresponding studied HRT. From this figure, it is observed that the apparent elimination percentage is higher with increasing PCB chlorination level. Furthermore, it was observed that the negative effect of organic load increase was more important on LCB elimination than on HCB removal and it was aggravated under mesophilic conditions (see Figure 6.6).

6.4. Conclusions

In this study, the AOX and PCB biodegradation or biotransformation in sewage anaerobic digesters under thermophilic and mesophilic conditions was analysed at different Hydraulic Retention Times (HRT). Results obtained an enhancement of both PCB and AOX biodegradation or biotransformation under thermophilic conditions.

The total PCB removal efficiency was in the range of 59.4-83.5 % and 33.0-58.0 % under thermophilic and mesophilic conditions, respectively. High working HRTs led to a more pronounced reduction of the total PCB amount in the sludge. However, a bioaccumulation of Lightly Chlorinated PCBs was detected in the mesophilic digester, which demonstrated that PCB biodegradation was performed following a reductive dechlorination mechanism.

The AOX removal efficiency was in the range of 40.4-50.3 % for thermophilic conditions and 30.2-43.2% for mesophilic conditions. Moreover, high HRTs promoted an improvement of the AOX removal capacity of the anaerobic digestion.

On the other hand, the metabolites formed during PCBs biodegradation are reported to be able to convert to an available substrate for the anaerobic consortium and their mineralization could occur. However, other halogenated compounds, as some chemicals that include under AOX parameter, their final biodegradation was few investigated and need to be elucidated.

6.5. References

Abramowicz, D. A. **(1990)**. Aerobic and anaerobic biodegradation of PCBs: a review. Crit. Rev. Biotechnol., 10, 241-248.

Adriaens, P., Focht, DD. **(1990)** Continuous coculture degradation of selected polychlorinated biphenyl congeners by *Acinetobacter* spp. in an aerobic reactor system. Environ Sci Technol ; 24: 1042-1049.

Alder, A. C., Haggblom, M. M., Oppenbeimer, S. R., Young, L. Y. **(1993)**. Reductive dechlorination of polychlorinated biphenyls in anaerobic sediments. Environ. sci. Technol., 27(3), 530-538.

Ali, M., Sreekrishnan, T.R. **(2000)**. Anaerobic treatment of agricultural residue based pulp and paper mill effluents for AOX and COD reduction. Process Biochem., 36, 25-29.

Alkock, R.E., Jones, K.C. **(1993)**.Polychlorinated biphenyls in digested UK sewage sludges. Chemosphere, 126, 2199-2207.

AURAS (2001) Ingenieurgesellschaft für aktive Umweltberatung, Risikoanalyse und Sicherheit mbH. Hohe AOX Gehalte im Klärschlamm?.-online: http://auras.de/aox.htm

Bedard, D. L., Unterman, R. D., Bopp, L. H., Brennan, M. J., Haberl, M. L., Johnson, J. **(1986)**. Rapid assay for screening and characterizing microorganisms for the ability to degrade polychlorinated biphenyls. Appl. Environ. Microbiol., 52, 761-768.

Borja, J.; Taleon, D.M.; Auresenia, J., Gallardo, J. (**2005**). Review: Polychlorinated biphenyls and their biodegradation. Process Biochem., 40, 1999-2013.

Brown, J. F., Bedard, D. L., Brennan, M. J., Carnahan, J. C., Feng, H., Wagner, R.E. **(1987)**. Polychlorinated biphenyl dechlorination in aquatic sediments. Science 236, 709-712.

Brown, J.F., Wagner, R.E., Bedard, D.L., Brennan, M.J., Carnahan, J.C., May, R.J., Toemire, T.J. **(1984)**. PCB transformation in upper Hudson sediments. Northeast. Environ. Sci., 3, 167-179.

Bryan, A.M., Stone W.B., Olafsson P.G. **(1987)**. Disposition of toxic PCB congenres in snapping turtle eggs Expressed as toxic equivalents of TCDD. Bull. Environ. Contam. Toxicol., 39, 791-796.

CEC **(2000)**. Working Document on Sludge (3rd Draft), Commission of the European Communities Directorate-General Environment, ENV.E.3/LM, 27 April 2000, Brussels.

Chang B., Chouand S.W., Yuan S. **(1999)**. Microbial dechlorination of plychlorinated biphenyls in anaerobic sewage sludge. Chemosphere, 39 (1), 45-54.

Eisenreich, S.J., **(1987)**. The chemical limnology of nonpolar organic contaminants: polychlorinated biphenyls in Lake Superior. Adv. Chem. Ser., 216, 394-469.

Fava, F, Marchetti, L. **(1991)** Degradation and mineralization of 3-chlorobiphenyl by a mixed aerobic bacterial culture. Appl Microbial Biotechnol ; 36:240-245.

Ferguson J.F. (**1994**) Anaerobic and aerobic treatment for AOX removal. Water Sci. Technol., 29(5–6), 149-162.

Ferguson J.F., Dalentoft E. (**1991**). Investigation of anaerobic removal and degradation of organic chlorine for Kraft bleaching wastewater. Water Sci. Technol., 24(3-4), 241-50.

Furukawa, K., Chakrabarty, A.M. (**1982**). Involvement of plasmids in total degradation of chlorinated biphenyls.Appl. Environ. Microbiol., 44, 619-626.

Furukawa, K., Matsumura, F., Tonomura, K. (**1987**) *Aicaligenes* and *Acinetobacter strains* capable of degrading polychlorinated biphenyls. Agric Biol Chem; 42543-548.

Gusev A., Mantseva E., Shatalov V., Strukov B. (2005). Regional Multicompartment Model MSCE-POP. EMEP/MSC-E Technical Report (June2005).
http://msceast.org/events/review/pop_description.html.

Haggblon M., Sakinoja-Salonen M. (**1991**) Biodegradability of chlorinated organic compounds in plant bleach effluents. Water Sci. Technol., 24(3-4), 161-170.

Hahn, H., Hoffmann, E., Schäfer, M. (**1999**). Managing residuals from wastewater treatment for priority pollutants. European Water Management, 2(1), 49-56.

Hansen, L.G. (**1987**). Environmental toxicology of Polychlorinated biphenyls. *In* : S. Safe (ed.) Polychlorinated Biphenyls (PCBs): Mammalian and Environmental Toxicology. Environmental Toxin Series 1 (Edited by S. Safe and O. Hutzinger) Springer-Verlag, Berlin Heidelberg. p 15-48.

Hartkamp-Commandeur, L., Gerritse, J., Govrs, H., Parsons, J. (**1996**). Reductive dehalogenation of polychlorinated biphebyls by anaerobic microorganisms enriched from dutch sediments. Chemosphere, 32 (7), 1275-1286.

Hutzinger, O., Veer Kamp, W. (**1981**). Xenobiotic chemicals with pollution potential. Microbial degradation of xenobiotic and recalcitrant compounds. T. Leisinger. New York, Academic Press: 3-45.

Hutzinger, O., Safe, S., Zitko, V. (**1974**). The Chemistry of PCBs. CRC Press: Cleveland, Inc., Cleveland, Ohio. ISBN 0-87819-049-X. Chap.1, 1-23.

Leschber, R. (**1992**). Organohalogen Compounds in Sewage Sludges and Their Determination as Cumulative Parameters. Organohalogenverbindungen in Klärschlamm und ihre Bestimmung als kumulative Parameter. -in: Hall, J. E., Sauerbeck, D. R. & P. L'Hermite, 45-53.

Madsen, T., Kristensen, P., Samsö-Petersen, L., Törslöv, J. and Rasmussen, J.O. (**1997**). Application of sludge on farmland - quality objectives, level of contamination and environmental risk assessment.-Specialty conference on management and fate of toxic organics in sludge applied to land. Copenhagen, 30 April - 2 may.

Magnuson, J.K., Romine, M.F., Burris, D.R., Kingsley, M.T. (**2000**). Trichloroethene reductive dehalogenase from *Dehalococcoides ethenogenes*: sequence of *tceA* and substrate range characterization. Appl. Environ. Microbiol., 66, 5141-5147.

Maltseva, O. V., Tsoi, T.V., Quensen, III J. F., Fukuda M., Tiedje J.M. (**1999**). Degradation of anaerobic reductive dechlorination products of Aroclor 1242 by four aerobic bacteria. Biodegradation, 10, 363–371.

Master, E., Lai, V., Kuipers, B., Cullen, W., Mohn, W. (**2002**). Sequential Anaerobic-Aerobic Treatment of Soil Contaminated with Weathered Aroclor 1260. Environ. Sci. Technol., 36 (1), 100-103.

Megharaj, M., Jovcic, A., Boul, H.L., Thiele, J. H. (**1997**). Recalcitrance of 1,1-Dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) to Cometabolic Degradation by Pure Cultures of Aerobic and Anaerobic Bacteria. Arch. Environ. Contam. Toxicol.,33, 141–146.

Montgomery, L., Vogel, T.M. (**1992**). Dechlorination of 2,3,5,6-tetrachlorobiphanyl by a phototrophic enrichment culture. FEMS Microbiol. Lett., 94, 247-250.

Müller, G. (**2003**). Review: Sense or no-sense of the sum parameter for water soluble ‘‘adsorbable organic halogens’’ (AOX) and ‘‘absorbed organic halogens’’ (AOX-S18) for the assessment of organohalogens in sludges and sediments. Chemosphere 52, 371-379.

Nakhla, G., Kochany, J., Lugowski, A. (**2002**). Evaluation of PCBs Biodegradaibility in Sludges by Various Microbial Cultures. Environmental Progress,21 (2), 85-93.

Natarajan, M. R., Wu, W-M., Wang, H., Bhatnagar, L., Jain, M.K. (**1996**). Dechlorination of polychlorinated biphenyl congeners by an anaerobic microbial consortium. Appl Microbiol. Biotechnol. 46, 673–677.

Natarajan, M. R., Wu, W-M., Wang, H., Bhatnagar, L., Jain, M.K. (**1998**). Dechlorination of spiked PCBs in lake sediments by anaerobic microbial granules. Wat. Res., 32 (10), 3013-3020.

Natarajan, M.R., Wu, W.M., Sanford, R., Jain, M.K. (**1999**). Degradation of biphenyl by methanogenic microbial consortium. Biotechnology Letters, 21, 741–745.

Pettigrew, C.A., Breen, A., Corcoran, C., Sayler, G.S. (**1990**). Chlorinated biphenyl mineralization by individual populations and consortia of freshwater bacteria. Appl Environ Microbial 1990; 56:2036-2045.

Rhee, G-Y., Sokol, R.C., Bethony, C.M., Bush, B. (**1993**). A long-term study of anaerobic dechlorination of PCB congeners by sediment microorganisms: Pathways and mass balance. Environ. Toxicol. Chem., 12, 1829-1834.

Rodrigues, J., Maltseva, O., Tosí, T., Helton, R., Quensen III, J., Fukuda, F., Tiedje, J. (**2001**). Development of a Rhodococcus Recombinant Strain for Degradation of Products from Anaerobic Dechlorination of PCBs. Environ. Sci. Technol., 35, 663-668.

Rysavy, J.P.; Yan, T., Novak P.J. (**2005**). Enrichment of anaerobic polychlorinated biphenyl dechlorinators from sediment with iron as a hydrogen source. Wat. Res., 39, 569-578.

Salkinoja-Salonen, M., Uotila, J., Jokela, J., Laine, M., Saski, E. (**1995**). Organic halogens in the environment: studies of environmental biodegradability and human exposure. Environmental Health Perspectives 103(5), 63-69.

Savant, D.V. ; Abdul-Rahman, R., Ranade, D.R. (**2006**). Review: Anaerobic degradation of adsorbable organic halides (AOX) from pulp and paper industry wastewater. Bioresour. Technol., 97, 1092-1104.

Schowanek, D., Racioppi, F., Matthijs, E., Boyko, R., Gabba, M., Buschini, A., Piero-Gardini, G. (**1996**). Quantitative in situ monitoring of organohalogen compounds in domestic sewage resulting from the use of hypochlorite bleach. Water Res., 30 (9), 2193-2205.

Schumacher, W., Holliger, C., Zehnder, A.J.B., Hagen, W.R. (**1997**). Redox chemistry of cobalamin and iron–sulfur cofactors in the tetrachloroethene reductase of Dehalobacter restrictus. FEBS Lett., 409, 421-425.

Shiu, W-Y., Mackay, D. (**1986**). A critical review of aqueous solubilities, vapor pressures, Henry's Law constants, and octanol-water partition coefficients of the polychlorinated biphenyls. J. Phys. Chem. Ref. Data, 15(2), 911-929.

Tripathi, C.S., Allen, D.G. (**1999**). Comparison of mesophilic and thermophilic aerobic biological treatment in sequencing batch reactors treating bleached Kraft pulp mill effluent. Wat. Res., 33(3), 836-846.

UMK-AG (**2001**) Organic contaminants in sewage sludge for agricultural use. Centre for Environmental Measurements, Environmental Inventories and Product Safety report, October 2001.

Van de Pas, B.A., Smidt, H., Hagen, W.R., van der Oost, J., Schraa, G., Stams, A.J., de Vos, W.M. (**1999**). Purification and molecular characterization of ortho-chlorophenol reductive dehalogenase, a key enzyme of halorespiration in *Desulfitobacterium dehalogenans*. J. Biol. Chem., 274, 20287–20292.

Van Lier, J.B., Hulsbeek, J., Stams, A.J., Lettinga, G. (**1993**). Temperature susceptibility of thermophilic methanogenic sludge: Implication for reactor start-up and operation. Bioresource Technology, 43, 227-235.

Van Lier, J.B., Rebac, S., Lettinga, G. (**1997**). High rate anaerobic wastewater treatment under psychrophilic and thermphilic conditions. Wat. Sci. Technol., 35, 199-206.

Van Pee, K-H., Unversucht S. (**2003**).Review: Biological dehalogenation and halogenation reactions. Chemosphere 52, 299-312.

WHO (**1992**). Polychlorynated biphenyls and terphenyls.Environmental Health Criteria 140, 1992, 2nd edition. World Health Oragnization Geneva, Switzerland.

Wohlfahrt, G., Diekert, G. (**1997**). Anaerobic dehalogenases. Curr. Opin. Biotechnol., 8, 290-295.

Ye D., Quensen III, J.F., Tiedje, J. M., Boyd, S.A. (**1992**) Anaerobic dechlorination of Polychlorinated biphenyls (Aroclor 1242) by pasteurized and ethanol-treated microorganisms from sediments. Appl. Environ. Microbiol., 58, 1110-1114.

Chapter 7

NPE and LAS removal in mesophilic and thermophilic anaerobic sewage sludge digestion

7. NPE and LAS removal in mesophilic and thermophilic anaerobic sewage sludge digestion[*]

Summary

In the last years, the anaerobic digestion role in sludge stabilization has been proved at industrial scale. However, the effect of anaerobic digestion under both mesophilic and thermophilic temperature range on organic micropollutants has been few investigated. In this study, a brief review of the nonylphenol ethoxylates and linear alkylbenzensulphonate biodegradation in anaerobic digestion is presented and it is analysed for a particular case, where it was found that the anaerobic digestion process was able to degrade nonylphenol monoethoxylate (NP1EO) and nonylphenol diethoxylates (NP2EO) at an acceptable level. Specifically, the observed efficiencies were 20-78% and 12-71% under thermophilic and mesophilic conditions, respectively. Besides, the maximum nonylphenol (NP) removal efficiency was 20% (thermophilic conditions) and 13% (mesophilic conditions). Short hydraulic retention times (HRT) led to a negative effect on the sum of NP, NP1EO and NP2EO (NPE) removal under thermophilic conditions, which decreased from 29% (HRT 26 days) to 16% (HRT 8 days). In the mesophilic digestion, HRT reduction effects were not clearly observed due to the low NPE removal efficiency. From linear alkylbenzensulphonate (LAS) sludge content monitoring, it was observed a LAS removal efficiency from 68% to 87% in the thermophilic anaerobic digestion. The LAS removal efficiency was more reduced under mesophilic conditions, namely 9.6-17.7%. Moreover, the LAS removal efficiency was influenced negatively by decreasing HRT at both temperature ranges.

---

[*] **Benabdallah el Hadj T., Dosta J., Mata-Álvarez J**. (2006). NPE and LAS removal in mesophilic and thermophilic anaerobic sewage sludge digestion. Environ. Sci. Technol., (submitted).

7.1. Introduction

Nowadays, one of the main problems of wastewater treatment plants (WWTPs) is the increasing amount of sludge produced. Consequently, their becomes, every time, more complicated. The most prioritized destination for these biosolids is their recycling in agriculture land as indicated by the European Directives (CEC, 2000). However, sludge use as amendment to soil must satisfy safety conditions. The major safety limiting factors designated in the 3rd draft of Directive presented to the European Commission (CEC, 2000) are heavy metals, pathogens load and organic micropollutants. In this last category, it can be distinguished detergent products. Anionic (as linear alkylbenzensulphonate) or nonionic (as nonylphenol ethoxylates) surfactants and their degraded products are the highest residual sewers contaminants and, therefore, sludge pollutants, due to their habitual use (see Chapter 5.1).

7.1.1. NPE

NonylPhenols (NP) are the degradation products of a class of nonionic surfactants known as NonylPhenol polyEthOxylates (NPEO) (Pryor et al., 2002). They refer to a complex mixture of isomers with C_9H_{19}-C_6H_4OH as their general chemical formula from a branched nine-carbon chain attached to a phenolic ring (see Figure 7.1).

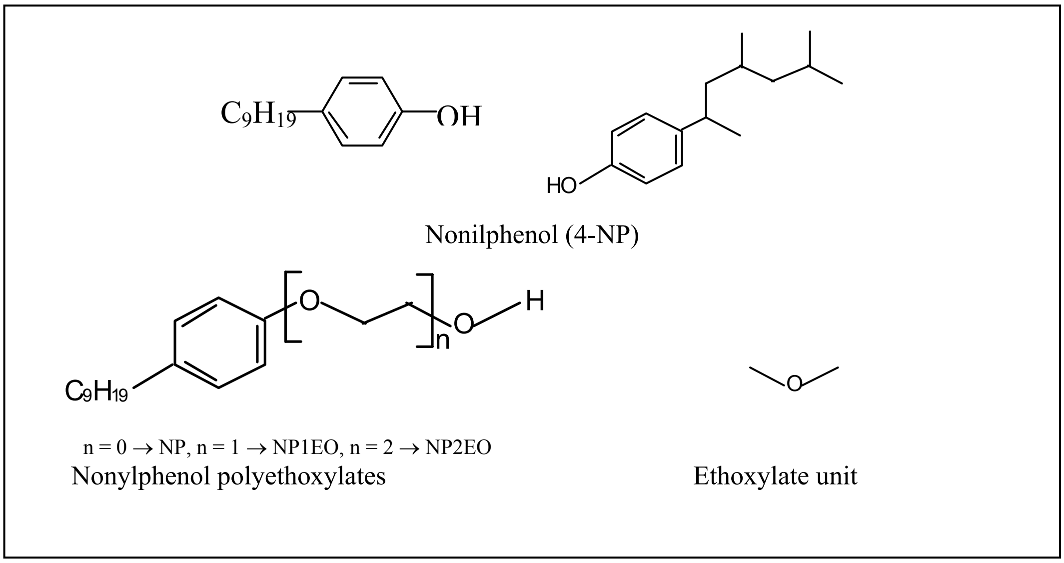


Figure 7.1. *NP and NPEO chemical structure*

Almost the total NP amount synthesized at industrial scale is destined to NPEO derivatives production with polyethoxylates chain, initially comprised of 1 to 20 ethoxy monomers (Pryor et al., 2002). NPEO amount represent 80% of the 600,000 metric tons annual worldwide production of alkylphenolethoxylates family (Tanghe et al., 1998). In Europe, NPEO is used as polymerization and emulsion agents in chemical industry, industrial and domestic detergents compounds, paper industry and paints synthesis (EU-RPA, 2000). The NP as a degraded product of NPEO was classified as corrosive and identified as estrogens and progestogens compounds, with harmful effects on the reproductive system (Bokern and Harms, 1997; Solé et al., 2000). Besides, NP was reported to be highly toxic for living organisms with inhibition effect on microbial-land activity at 50 mg NP/kg dry matter (Langenkamp et al., 2001).

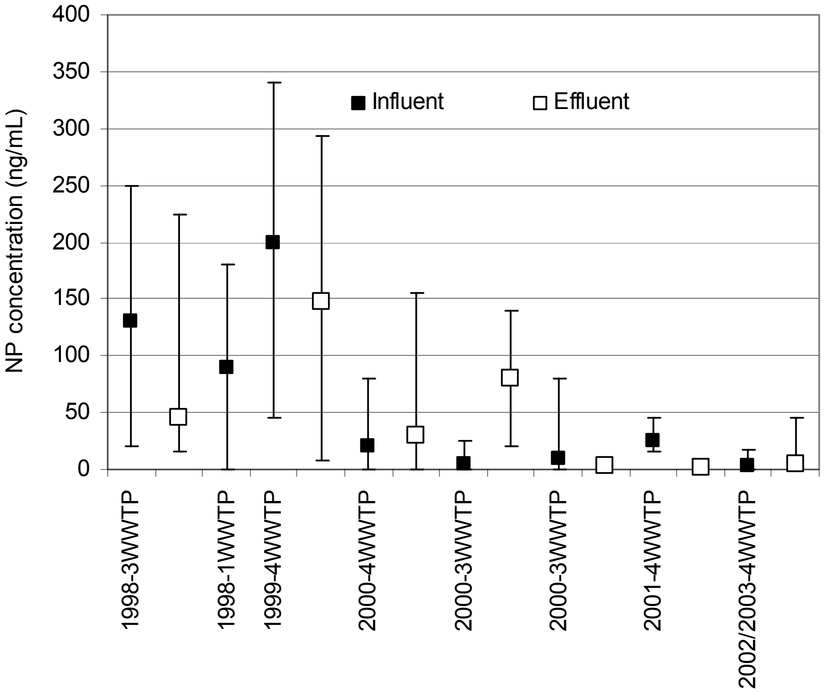


__Figure 7.2.__ NP content in influent and effluent of Catalonian WWTP during 1998-2003 period (Barceló and Petrovic, 2004).

The frequent use of NPEO in different industrial areas, make evident their detection in residual streams under various forms with shortened ethoxy chains (see Figure 7.2) as NP, nonylphenol monoethoxylate (NP1EO) and diethoxylates (NP2EO), and other hydrophobic metabolites (CEC, 2000; Ejlertsson et al., 1999; van Ginkel, 1996). Consequently, it was observed high NP, NP1EO and NP2EO (so-called NPE) concentrations at solid phase of raw sewer and sludge (Figure 7.3), up to 4000 mg/kg dry matter (dm) in Canadian's sludge (Pryor et al., 2002) and up to 8000 mg/kg dm in Germany's sludge (CEC, 2000). This enlarged range of NPE sludge content was related to the different existing sewage sludge treatment processes (Barceló and Petrovic, 2004) and the variety of NPE analysis methods (Pryor et al., 2002). In the last years, it was recorded a decreasing of the NPE sludge content which is a direct consequence of the ban of their use in detergent and paint production in some countries, like Denmark, Sweden and Switzerland (Paulsrud et al., 2000; Pryor et al., 2002). In Catalonian raw sewage sludge, the observed tendency is similar to that explained above. However, Ortiz (2004) reported that a 91.9% of the analysed sludge samples between year 2000 and 2003 not fulfilled the proposed safety conditions (50 mg NPE/kg dm) of the 3rd draft presented to the European Union Environmental Commission (CEC, 2000).

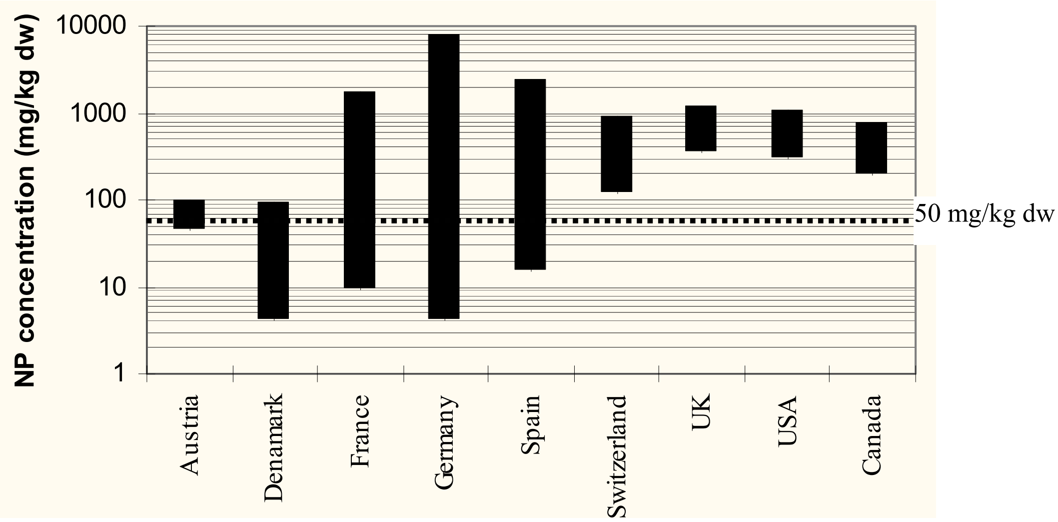


Figure 7.3. *NP content in sludge (CEC, 2000).*

The NPEO biodegradation during aerobic and anaerobic treatment can be carried out by shortening hydrophilic molecule part which leads to an increase of the NP1EO and NP2EO waste stream (Ejlertsson et al., 1999). Other molecules can be released in the medium, such as carboxylate metabolites and NP isomers (Ahel et al., 1994; Ball et al., 1989; Brunner et al., 1988; Giger et al., 1987; Jones and Westmoreland, 1998; Reinhard et al., 1982) (see Figure 7.4).

Tanghe et al. (1998) observed nearly 99% of total NP content removal in an aerobic Sequential Batch Reactor (SBR) system operated at an Hydraulic Retention Time (HRT) of 4 days and a Solid Retention Time (SRT) of 15-22 days fed with synthetic and domestic residual wastewater mixture with an organic load of 1 g COD and 2.138 mg NP/L, respectively. The NP removal rate was 0.7 mg/g VSS at 28 ºC without any solid accumulation phenomenon detected. Besides, a decrease in temperature and an increase of the organic load, to adjust the SBR conditions to real states, had little effect on the removal efficiency (86%). Hence, the positive effects of relatively high temperatures and the advantages of domestic waste microorganisms aggregation were recorded. These results demonstrate the NP biodegradation possibility in residual streams in contrast with other lab-scale studies, with a working temperature about 25 ºC (Kravetz et al., 1982a, 1982b; Rudling and Solyom, 1974; Salinitro et al., 1988) or industrial-scale experiments, with working temperatures about 15 ºC (Ahel et al., 1994; Brunner et al., 1988), where it was reported a partial degradation of NPEO to NP1EO, NP2EO and NP, and it was estimated that NP present a high stability in aerobic treatments.

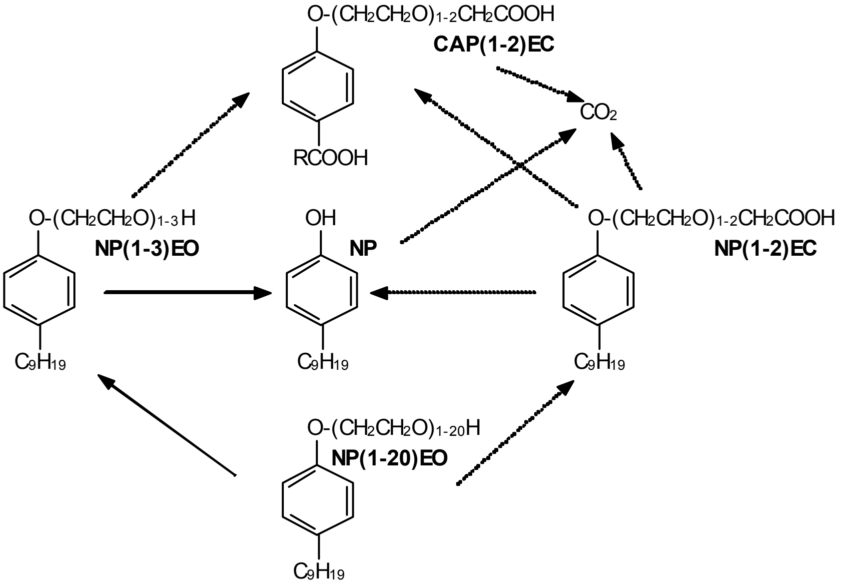


Figure 7.4. *Structures of nonylphenol ethoxylates (NPEO) and their metabolites, including nonylphenol (NP), nonylphenol carboxylates (NPEC), and alkylphenol dicarboxylates (CAPEC). Numbers in parentheses denote the range in ethoxy chain lengths. Microbial biotransformation pathways, including ethoxy chain shortening (solid lines) and oxidation (dashed lines) (Ferguson and Brownawall, 2003)*

The introduction of the anaerobic treatment as previous step in aerobic treatment processes led to removal 80% of both NP and NP2EO, and 16% and 18% accumulation in sludge from the initial amount of NP (44 g/d) and NP2EO (590 g/d), respectively (Fauser et al., 2003).

Some studies (Ahel et al., 1994; Angelidaki et al., 2000b; Ejlertsson et al., 1999; Giger et al., 1984; Marcomini et al., 1989; Ying, 2005) concluded that anaerobic digesters transform the NPEO (including NP1EO and NP2EO) to NP. This last compound tends to be adsorbed and bioaccumulated in the sludge because of its hydrophobic characteristics reflected by its high octanol-water partition index [Log $K_{ow} = 4.48$ (Ahel and Giger, 1993)]. This implied the high NP sludge contents of 1.2 g/ kg TSS, 1 g/ kg dm, 1g/ kg dm, 1.9 g / kg dm and 7.2 g NP/ kg dm recorded by Tanghe et al. (1998), Brunner et al. (1988), Giger et al. (1984), Walterson and Landner (1990) and Sundberg (1990), respectively. In fact, it is estimated that more than 50% of the total NPEO content in the residual stream can be transformed to NP adsorbed in the digested sludge (CEC, 2000) and, consequently, the NPE content can exceed the safety conditions established for their use in agricultural land (50 mg NPE/ kg dm).

Table 7.1. *Methane production from NP1-2EO (2 mg/l) incubation under anaerobic condition (Ejlertsson et al., 1999)*

	CH_4 amount (μmol)
Control bottle	190
Total conversion of ethoxylates to methane	120*
Produced methane in NP1-2EO amended bottle	630

*: Theoretical value calculated according to Reaction 7.1

$$C_9H_{19}\text{-}C_6H_4\text{-}(OCH_2)_2\text{-}OH \rightarrow C_9\text{-}H_{19}\text{-}C_6H_4\text{-}OH + CH_4 + CO_2 \qquad (7.1)$$

Ejlertsson et al. (1999) reported a biotransformation of NP1EO and NP2EO to an amount of methane that clearly exceeded the correspondent ethoxylates quantity initially added to a bottle-reactor, seeded with anaerobic sludge, which can suggest the NP mineralization (see Table 7.1). Whereas, when the bottle-reactor was inoculated with municipal solid waste landfill, the methane production was inhibited and NP accumulation in solid phase was registered, which is in accordance with the results of Battersby and Wilson (1989). Besides, Chang et al. (2005) observed a complete NP removal by anaerobic sewage sludge inoculum after 84 days of incubation period at a constant rate of 0.029 d^{-1}, which is calculated with first-order kinetics. The positive impact of temperature increase on kinetic constants has been also reported in this study (see Table 7.2).

Table 7.2. *Effects of temperature incubation factors on NP anaerobic degradation rate constants (k) and half-lives ($t_{1/2}$) in the sludge samples (Chang et al., 2005)*

Temperature (ºC)	NP initial concentration (mg/L)	k (d^{-1})	$t_{1/2}$ (d)
20	5	0.018	38.5
30	5	0.029	23.9
40	5	0.034	20.4
50	5	0.038	18.2

7.2.2. LAS

Linear AlkylbenzenSulphonates (LAS) are the anionic surfactant most widely used in laundry detergent and surface cleaners. In Europe, more than the 80% of the quantity of LAS produced (450 kt in the year 2000) is destined to detergent synthesis (Schönkaes, 1998). In Spain, the annual consumption of detergents industry reached 59.9 kt in the year 1995 (IUSE, 2003).

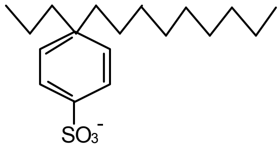


Figure 7.5. *LAS chemical structure*

The technical LAS mixture refers to various alkyl homologues and phenyl position isomers. In Europe, the mixture most frequently used in commercial products is composed by substances with alkyl chains of C10 to C13 with a proportional C10:C11:C12:C13 weight distribution of 13:30:33:24, with 11.6 as the average carbon number of linear alkyl chains and 29% of the most hydrophobic isomers (2-phenil) (Cavalli et al., 1999; Feijtel et al., 1995; Feijtel et al., 1999; Valtorta et al., 2000).

Table 7.3. *Stream waste LAS concentration in WWTP in some European countries (CEC, 2000)*

Country	WWTP	
	Influent (μg/L)	Effluent (μg/L)
Austria	400-3500	11-55
Germany	5400	67
Greece	-	129 (35-325)
Italy	4600	43
Netherlands	4000	9
Spain	**9600**	**140**
UK	15100	10

It has been reported that the LAS content in the stream waste can be influenced by different factors, such as the length of the sewer, the travel time and the degree of microbial activity present in the sewer (Matthijs et al., 1995). Consequently, an enlarged concentration range (400-15100 ng/mL) was reported for LAS content in the influent (raw sewage) of different European Sewage Treatment Plants (STPs) (see Table 7.3).

On the other hand, the LAS sewage effluent content depends on the treatment process applied. In STPs with activated sludge processes, balance matter studies stated that the 80-90% of the initial LAS content is degraded, 10-20% is adsorbed and around 1% can be released to surface water (Berna et al., 1989; Brunner et al., 1988; Cavalli et al., 1993; DiCorcia et al., 1994; Painter and Zabel, 1989;).

Table 7.4. *LAS content in sewage sludge in some European countries and USA (mg/kg dw) (CEC, 2000).*

Country	Median	Min.	Mean	Max.	Sludge Type/year
Austria	8107	2199	7579	17955	1994/1995
Germany	5000	50		16000	1985-87
Denmark	2700	11	530	16100	
Spain	**-**	**100**	**-**	**500**	**Aerobic**
	-	**12100**	**-**	**17800**	**Anaerobic**
Finland	9700	-	-	-	
Italy	-	11500	-	14000	
UK	8700	60	10400	18800	
USA	152	-	-	-	Aerobic
	4680	1680	-	7000	Anaerobic

In the solid effluent generated in WWTPs, the LAS concentration depends on the sludge treatment process (see Table 7.4). In Europe, the LAS aerobic sludge content was estimated to be below 0.5 g/kg dm. However, in the treated anaerobic sludge, the LAS content was in the range of <1 to 30 g/kg dm depending on the residual sewer content, the operational conditions of the plant and the water hardness (Berna et al., 1989; Cavalli et al., 1999; DeWolf and Feijtel, 1989; Jensen, 1999). It was reported that precipitation of LAS is correlated with water hardness, since the solubilities of the calcium and magnesium salts of LAS are very low; the solubility products ranged from 2.2×10^{-10} for C_{10} LAS to 6.2×10^{-13} for C_{13} LAS (Berna et al., 1989). The higher the water hardness, the higher LAS precipitation in the primary settler and, therefore, the lower the amount of LAS which is exposed to the biological treatment in the sewage treatment plant. High contents of LAS in the sludge (up to 30 g/kg) did not inhibit the anaerobic digestion process (Berna et al., 1989), probably because LAS were present as calcium and magnesium salts and therefore had reduced bioavailability.

Table 7.5. *Sludge LAS content (mg/kg dw) in Spain.*

References	Aerobic	Anaerobic
Prats et al., 1999	580	8270-13250
Berna et al., 1989	100-500	7000-30200
Prats et al., 1997	-	15200-16700
Waters and Feijtel, 1995	-	9400

High levels of LAS concentration (around 30 g/kg dw) were registered by Berna et al. (1989) in some Spanish regions with high water hardness (namely, more than 500 mg $CaCO_3$/ L). However, these values are considered an exceptional case (see Table 7.5), since more than the 66% of the Spanish population disposed water with hardness below 300 mg $CaCO_3$/L (CED, 1989).

The LAS homologues distribution (C10:C11:C12:C13) in the sludge was estimated to be 7:24:39:30 (Berna et al., 1989; Cavalli et al., 1993; DiCorcia et al., 1994) with an average alkyl chain length around C11.9, as a consequence of preferential adsorption of LAS homologues with a longer alkyl chain.

For LAS aerobic degradation, it was reported that SulfoPhenil Carboxylate (SPC) is the intermediate product of the primary biodegradation (Swisher, 1987). The parent molecules (LAS) can be removed with more than 99% efficiency. Whereas, the complete conversion of SPC to inorganic substances (H_2O, CO_2, Na_2SO_4), the so-called final biodegradation (Karsa et al., 1995), can be achieved with 80 to 95% in the continuous activated sludge simulation test (HERA, 2004). On the other hand, Kimerle et al. (1977) reported that SPC presence did not inhibit the biodegradation process and their toxicity is lower than parent molecules.

Due to the important LAS sorption phenomenon on the solid matter and the extended anaerobic methanogenic process for sludge stabilization, recent studies have been carried out to enhance the LAS biodegradation potential. At lab-scale, Prats et al. (2000a) reported a LAS removal efficiency from 30% to 93% in the first 250 days and 50% in the following 90 days. Using continuous stirred reactors, Angelidaki et al. (2000a) and Haggensen et al. (2002) observed an elimination level in the range of 14-25%, whereas in bed reactors a LAS degradation efficiency of 44% was reported (Sanz et al., 1999). Moreover, in a thermophilic Upflow Anaerobic Sludge Blanket (UASB) digester seeded with granulated anaerobic inoculum and fed with 6 μmol LAS/(L day) with an HRT of 12 h, a LAS removal efficiency of 40% was observed after a period of 8 days (Mogenesen and Ahring, 2002). These results confirmed that the LAS biodegradation occurs during the anaerobic digestion treatment and the operating temperature range plays an important role, since the maximum removal efficiency for mesophilic anaerobic systems was in the range 20-25% (Brunner et al., 1988; CEC, 2000). The positive temperature effect could also be deduced from composting removal efficiency of resistant LAS, which was estimated to be around 98% (Petersen, 1999; Prats et al., 2000b).

However, the biodegradation mechanism of LAS is not well defined. Balson and Felix (1995) and Sarrazin et al. (1997) suggested that LAS degradation involves three successive steps: the degradation of the straight alkyl chain, the sulphonate group and finally the benzene ring (see Figure 7.6). Other researchers proposed the anaerobic desulphonation as the first step, followed by the straight alkyl chain carboxylation and, at the end, the bacterial mineralization of aromatic ring (Campos-Garcia et al., 1999) (see Figure 7.7).

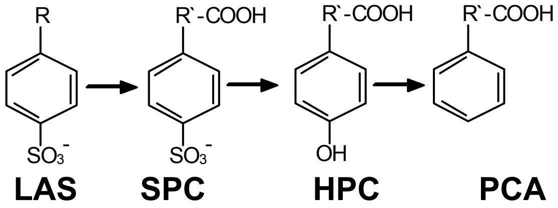


Figure 7.6. *Anaerobic LAS biodegradation pathways. LAS: linera alkybezensulfonate, SPC: Sulfophenylcarboxylic acids, HPC: Hydroxyphenylcarboxylic acids, PCA: Phenylcarboxylic acids (Sarrazin et al., 1997)*

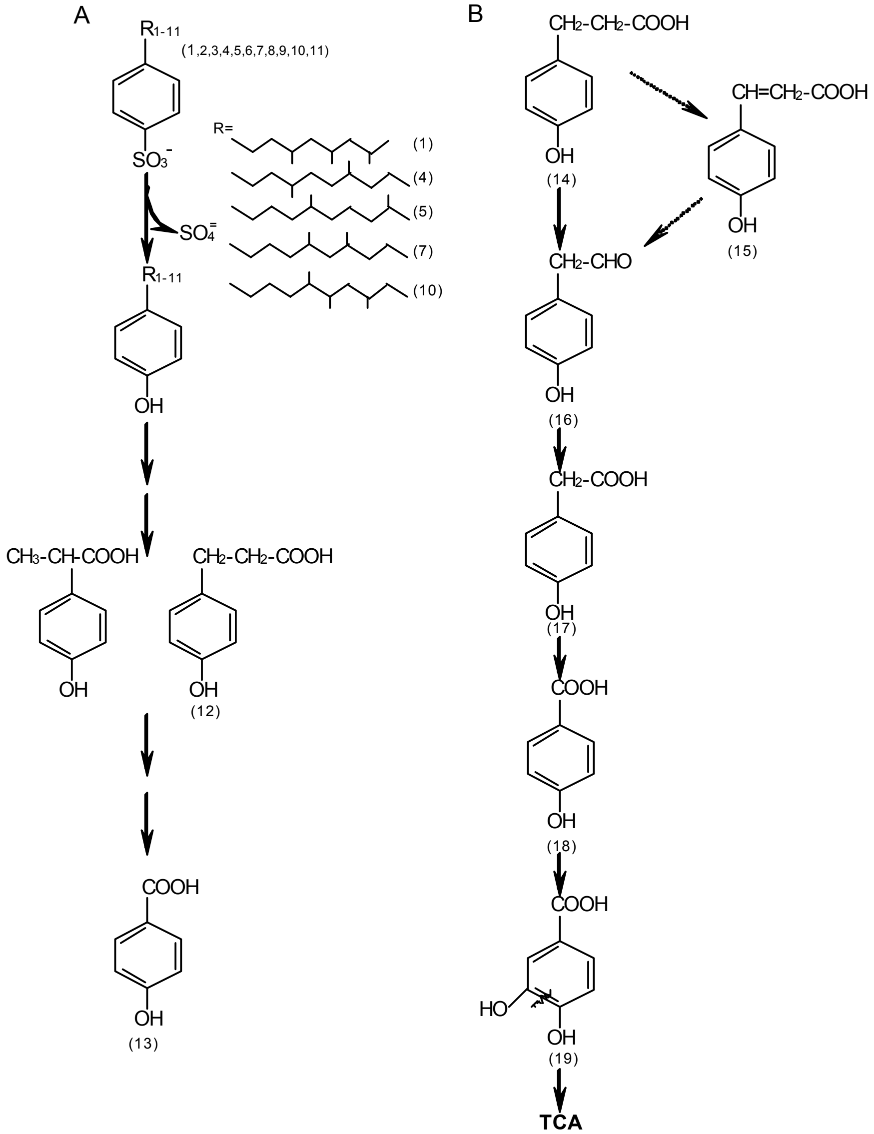


Figure 7.7. *Schematic representation of the proposed degradation route of dodecylbenzene sulfonates (A) and 4-hydroxyphenylpropionate (B) by P. aeruginosa W51D. TCA, tricarboxylic acid cycle (Campos-García et al., 1999).*

Due to the little existing information about anaerobic degradation of anionic and nonionic detergent compounds, the aims of this chapter were the estimation of the methanogenic removal of LAS and NPE under thermophilic and mesophilic conditions. Moreover, the effect of decreasing HRT at both temperature ranges was examined.

7.2. Materials and methods

In this study, Nonylphenols (NPE) were analysed only in dried sludge. However, LAS were monitored in supernatant and dry matter because it is considered the less hydrophobic compounds compared with other organic pollutants (PAH, DEHP, PCB and NPE) (see Figure 1.4 in Chapter 1).

7.2.1. Experimental set-up

Two completely mixed and jacketed anaerobic digesters were used in this study. Each one was seeded with 3.5 L of Waste Activated Sludge (WAS) from a municipal WWTP of the Barcelona Metropolitan Area (see Chapter 4).

7.2.2. Analytical methods

Analyses of total chemical oxygen demand (CODt), soluble chemical oxygen demand (CODs), total solids (TS), volatile solids (VS), pH, alkalinity and ammonium nitrogen (NH_4^+-N) Individual volatile fatty acids (VFA) and gas composition were performed as described in chapter 3.

NPE Analysis

Table 7.6. *NPE identification in GC/MS analysis.*

Compound	m/z	Typical RT (min.)
NP	220	13.3-14.01
NP1EO	264	16.3-17.2
NP2EO	308	20.7- 22.1

The SCAN mode was employed for identifying the NPE, corresponding to picks in the ion range 50-550 m/z, correspondent to retention time range from about 5 min to 57.7 min (see Table 7.6 and Annex IV, Figures IV.1 and IV.2.). NP, NP1EO and NP2EO compounds were recovered in the third and fourth fractions (iii and iv) of elution solvents used (DCM:AcEt and DCM:MeOH) as shown in Table 7.7. Calibration curves were prepared in the range 0-100 mg/L for NP and 0-100 mg NP1EO/L from NP1EO-NP2EO reagent mixture. Sub-samples were doped with a NP amount corresponding to 100 mg/kg dw to assess the analysis method recovery index (see Table 7.8).

Table 7.7. *Portion of recuperated NP/NPEOs in the third fraction relatively to the total amount recuperated*

	HRT (days)	26	22	18	12	8
NP1EO + NP2EO	Feed (%)	89.91 ± 2	85.66 ± 5	91.35 ± 3	98.57 ± 3	94.13 ± 2
	Mesophilic (%)	95.72 ± 3	93.54 ± 3	95.11 ± 7	-	-
	Thermophilic (%)	97.85 ± 2	92.01 ± 2	98.87 ± 3	98.97 ± 2	96.60 ± 4
NP	Feed (%)	72.67 ± 1	81.34 ± 6	89.56 ± 5	85.44 ± 1	84.96 ± 5
	Mesophilic (%)	88.43 ± 0	90.12 ± 4	92.31 ± 9	-	-
	Thermophilic (%)	96.05 ± 4	95.87 ± 1	92.64 ± 2	93.90 ± 0	91.55 ± 4

It is reported that NP and NPEO sludge content varied according to the treatment process followed at each WWTP, with or without activated sludge phase treatment, to site and to analysis method employed for NPE determination (Pryor et al., 2002).

Table 7.8. *Recovery grade (RI) and relative Standard deviation (RSD) from NP analysis at tested HRTs.*

HRT (days)	Feed					Thermophilic					Mesophilic		
	26	22	18	12	8	26	22	18	12	8	26	22	18
RI (%)	83.1	98.7	121.6	73.81	66.2	117.0	126.4	91.3	62.9	78.5	127.2	94.3	86.2
RSD (%)	23.3	11.9	7.0	13.5	5.4	8.3	4.6	16.2	7.3	12.9	3.0	6.2	1.4

LAS Analysis

LAS analysis in solid and liquid samples were performed as described in Chapter 3 (see Figure IV.3 in annex IV). In order to estimate the recovery level index, additional feed samples were spiked with an amount corresponding to 2 mg LAS/L in supernatant and 400 mg LAS/kg dm in lyophilized sludge. The results obtained in the analytical procedure recorded a recovery index, for sum amount of LAS homologues, between 74% and 108% (see Table IV.1 of Annex IV). These results have a relative standard deviation in the range of 3%-35% (see Table IV.1. of Annex IV).

7.3. Results and discussion

7.3.1. NP/E degradation

In Figure 7.8, the NPE, NP, NP1EO and NP2EO content in the fresh and the treated sludge and their removal efficiencies in the mesophilic and the thermophilic digesters at the different HRTs tested are presented (in Table IV.2 of annex IV, the detailed values are presented). In this Figure, it is observed that the anaerobic digestion treatment led to a decrease of these compounds. In the fresh sludge tested, the NPE content was in the range of 1097-2100 mg/kg dw and it decreased to the range of 773-1827 mg/kg dw after its treatment in the lab-scale anaerobic digesters.

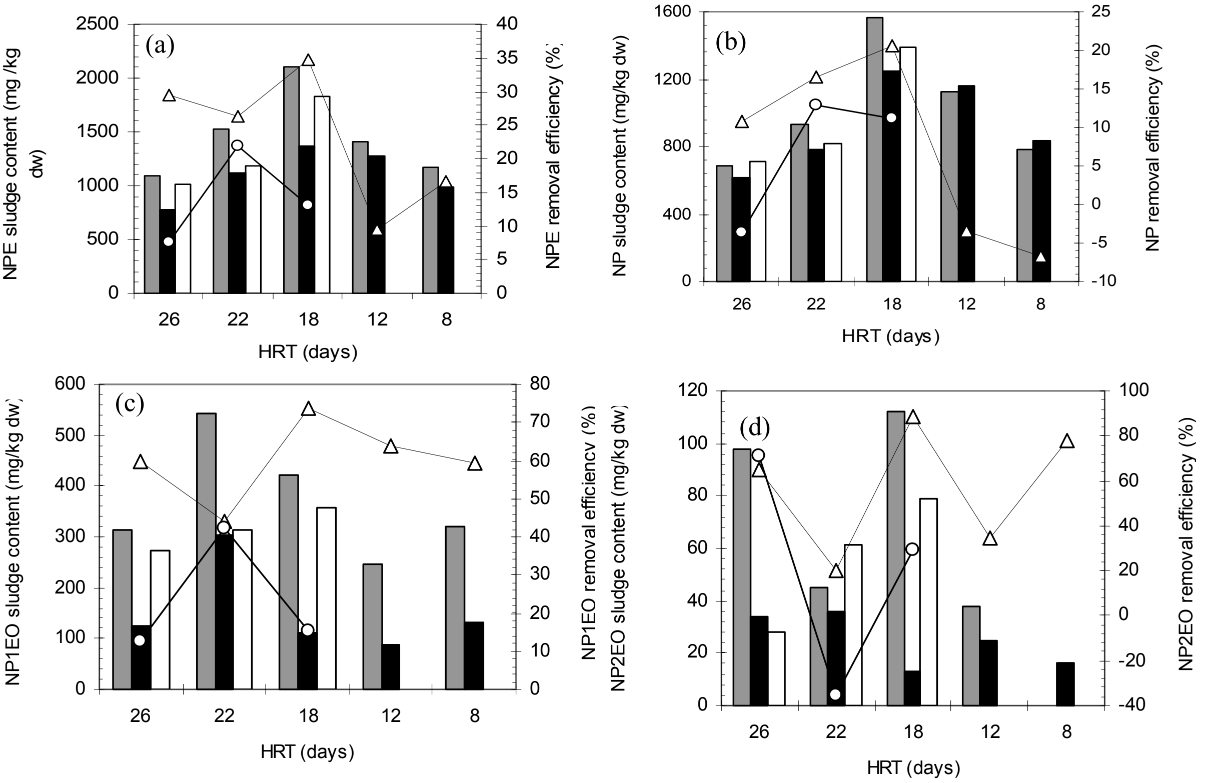


Figure 7.8. *Fresh (▨), thermophilic (■) and mesophilic (□) sludge content and the removal efficiency in the thermophilic (-Δ-) and the mesophilic (-○-) digesters of (a) total NPE, (b) NP, (c) NP1EO and (d) NP2EO at tested HRTs.*

Therefore, the maximum NPE sludge content proposed in the 3rd draft presented to the European Commission (CEC, 2000) for their use as land conditioner (50 mg/kg dw) was largely exceeded. The sludge generated under thermophilic conditions contained an average NPE content of 1104 mg NPE/kg dw, whereas in the mesophilic sludge, it was slightly higher, namely 1343 mg NPE/kg dw. These values lied in the same range reported by Pryor et al. (2000) for the sewage works of New York (1500 mg/ kg dw). However, Bennie et al. (1998) reported a larger range (22-4000 mg/kg dw) in Canadian's depurated biosolids. In Catalonia (Spain), it is frequent to detect a treated sludge with high NPE concentrations that can overcome 1000 mg/kg dw (Ortiz, 2004).

In Figure 7.8, it is also observed that the removal efficiency of NPE was more pronounced under thermophilic conditions (9.4%-34.81%) than under mesophilic conditions (7.66%-21.94%) at the tested HRTs (see Table IV.3 of annex IV). When the thermophilic digester operated at low HRTs (12 and 8 days), reduced efficiencies were registered. This can be explained by the high NPE content in the fresh sludge at 18d and their extended effects to 12d HRT period and /or by the drastic change in the kinetics process below 18 d. The recovery of the removal efficiency to 16.5% at 8d as HRT suggests that the high NPE influent content was the main cause of the NPE removal efficiency decline at 12 d HRT. As general tendency stated in the thermophilic digester, the NPE removal decreased when HRT was reduced. However, in the mesophilic digester, this tendency can not be confirmed.

From the plot of the NP content in the fresh and the treated sludge at different HRTs (Figure 7.8b), it is observed that in several cases, the NP content in the treated sludge is higher than in the fresh sludge. This observation can be explained by the fact that NPEO present in the influent sludge was degraded partially to NP as reported by Ejlertsson et al. (1999). The same observation can be appreciated for NP2EO mesophilic sludge content (see Figure 7.8d). In this case, it can be estimated that the high ethoxylated nonylphenols present were degraded to NP2EO which was not accompanied with a sufficient conversion rate to NP1EO and NP (see Figure 7.4). Due to this bioaccumulation, the percentage of removal of NP and NP2EO was sometimes negative.

On the other hand, the maximum removal efficiency values were registered for NP2EO content elimination, except for some singular points at HRTs of 12 and 22 days in the thermophilic digester and at an HRT of 22 days in the mesophilic digester. The lowest removal efficiencies were observed for NP elimination. Since EOs groups are more hydrophilic than NP, the NP are more recalcitrant because of their hydrophobic characteristics which complicates their availability to anaerobic bacterial groups and thus their bioassimilation (Ahel et al., 1994; Angelidaki et al., 2000a; Ejlertsson et al., 1999; Giger et al., 1984; Marcomini et al., 1989;).

Both thermophilic and mesophilic NPE removal efficiencies were low (35% as the maximum value). However, NPE removal efficiency was clearly enhanced under thermophilic conditions (see Figure 7.8). The change in the degrader bacterial community groups at high temperature, the change in kinetic process or the increase in

NPE availability to microorganisms, can be (separately or jointly) the reasons of this improvement.

7.3.2. LAS degradation

In Figure 7.9, the LAS content in the fresh and the treated sludge at the HRTs tested is presented. The LAS content in the fresh sludge was around 179-250 mg/L. However, the anaerobic treatment decreased this content to a range of 32-69 mg/L (thermophilic conditions) and 142-165 mg/L (mesophilic conditions). As shown in Figure 7.9, the thermophilic digestion showed much higher LAS removal efficiencies (68.1-87.2 %) than the mesophilic digestion (20.7-34.1 %) (see Table IV.1 of annex IV). These results are in contrast with the poorly primary biodegradation level observed by García et al. (2005), which could be a consequence of using external LAS reagents to spike batch anaerobic digesters. Although the aqueous phase of the anaerobic effluent had a higher LAS content than the influent, both supernatants were in the range of 5-15 mg/L (see Figure 7.10), concordantly with Feijtel et al. (1995) and Matthijs et al. (1999) results (in Table IV.4 of annex IV, the detailed results are presented). The increase of LAS content in the liquid phase after anaerobic treatment is considered a result of the transference of LAS homologues from the solid to the aqueous phase, as explained by Mogensen and Ahring (2002).

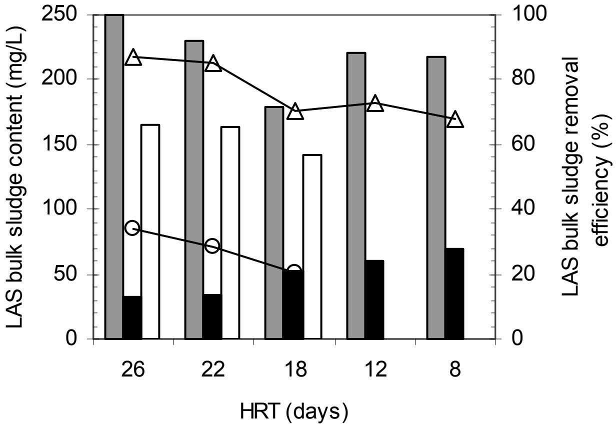


Figure 7.9. *Total LAS amount in fresh bulk feed (■), thermophilic (■) and mesophilic (□) anaerobic Bulk effluent and the removal efficiency in the thermophilic (-Δ-) and the mesophilic (-○-) digesters at the HRTs applied (for detailed results, see Table IV.1 of annex IV)*

The increase in the LAS content of the liquid phase was higher under mesophilic conditions than under thermophilic conditions and higher when decreasing the HRT. On the other hand, average carbon units in liquid phase of fresh feed were 10.56 (see Annex IV, Table IV.5), which is similar to the reported carbon number (10.8) by Cavalli et al. (1993), DiCorcia et al. (1994), Prats et al. (1993) and Tabor et al. (1996). In the thermophilic and the mesophilic liquid effluent the number was higher, namely 11.30 at 35 ºC and 11.40 at 55 ºC (see Figure 7.11). Therefore, it can be concluded that, besides the negative effect on LAS removal in liquid phase, increasing their amount, anaerobic treatment enriched their LAS contained in longer chain homologues and, consequently, increase their average carbon number. This last effect was more pronounced at high temperature (thermophilic temperature range) and low HRT.

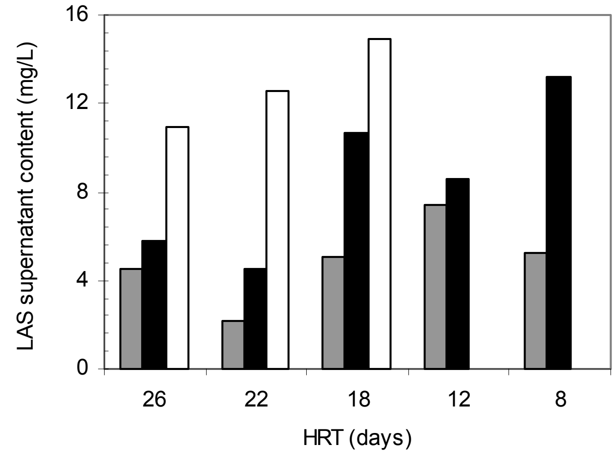


Figure 7.10. *Total LAS amount in fresh supernatant feed (▨) Thermophilic (■) and Mesophilic (□) anaerobic supernatant effluent at tested HRTs.*

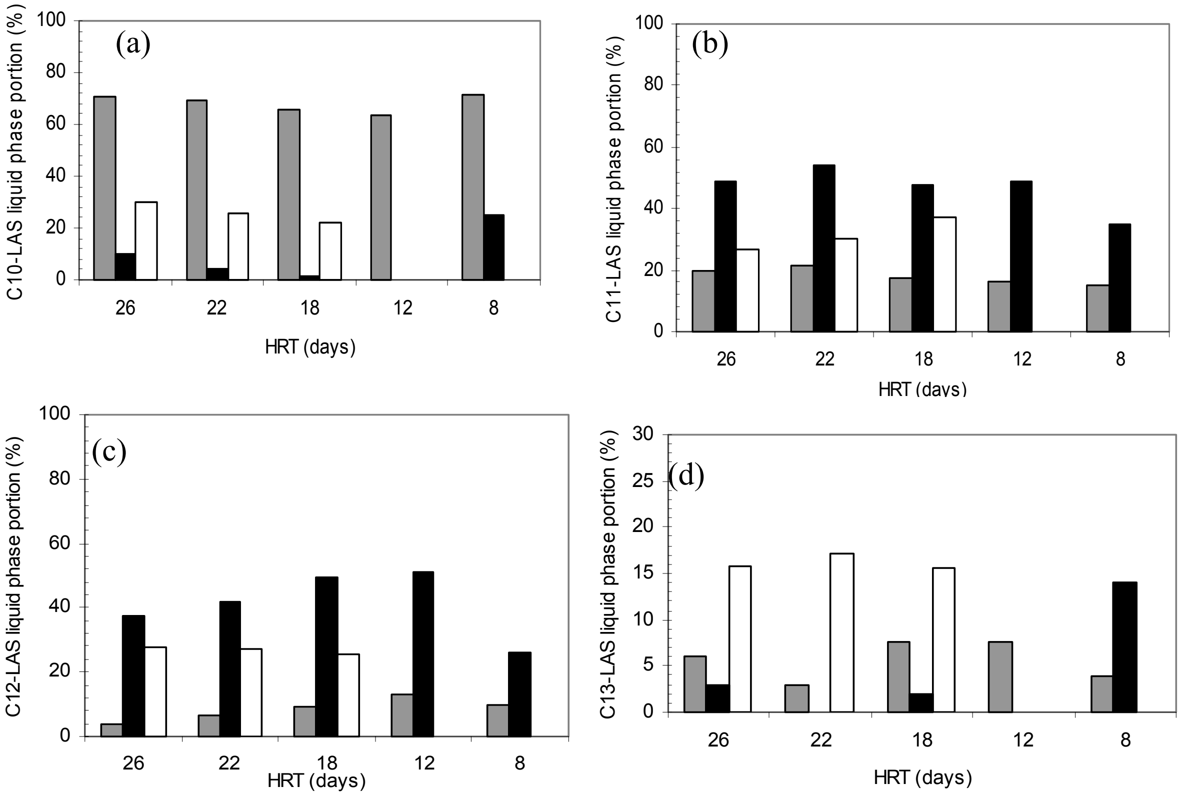


Figure 7.11. *Proportional weight distribution (%) in liquid phase of LAS homologues (a) C10, (b) C11, (c) C12 and (d) C13 in the fresh feed (▨), Thermophilic (■) and Mesophilic(□) anaerobic effluent at the tested HRTs.*

In the solid phase, where more than 90% of the total LAS amount was concentrated, an important decrease was observed. In the thermophilic sludge (Figure 7.12), at all the HRTs applied, the LAS content in the sludge was below the cut-off limit (2600 mg/kg dm) proposed in the 3rd draft presented to European Union environmental commission (CEC, 2000). However, in the mesophilic sludge all samples analyzed, correspondent at every HRT applied, had a higher LAS content (4048-4923 mg/kg dm) (in TableIV.6 of annex IV, the detailed results are presented).

On the other hand, an increase in C10_LAS concentration was observed in the thermophilic sludge from 18 days HRT after anaerobic digestion (Figure 7.13a). However, longer alkyl chain concentrations decreased at all the HRTs applied HRT in both digesters (Figure 7.13).

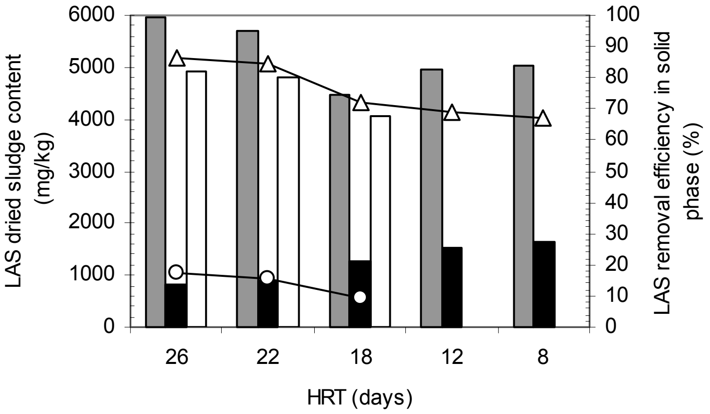


Figure 7.12. *Total LAS amount in dried matter of fresh Feed (■) Thermophilic (□) and Mesophilic (■) anaerobic effluent and the removal efficiency in the thermophilic (-Δ-) and the mesophilic (-○-) digesters at tested HRTs.*

From mesophilic sludge samples analysis, the decrease in high lineal chain LAS (C12_LAS and C13_LAS) amount was detected as shown in Figures 7.13c and 8d. Therefore, LAS composition in thermophilic sludge developed a lineal alkyl chain shorting from C11.78 in fresh feed to around C10.7. However, in the mesophilic sludge the average carbon number of lineal alkyl chain (11.56-11.80) was in the same range of solid influent (see Annex IV, Table IV.7).

Figures 7.13a and 7.13b illustrates a low removal for both C10_LAS (5.5-12.9 %) and C11_LAS ((-5.65)-10.54%) under mesophilic treatment. However, negative removal efficiencies were recorded at an HRT of 26 days for C11_LAS in the mesophilic digestion and at 18, 12 and 8 days as HRT for C10_LAS in the thermophilic digestion (see Table IV.8 of annex IV). This observation indicates an increase in the amount of the cited homologues in the treated effluent with respect to the influent.

The successive shorting of alkyl chains as the degradation process of LAS was discarded because a higher hydrophobicity due to longer alkyl chains induce to suggest that SPC and/or alkylbenzenehydroxyl (after desulphonation step) were the primary metabolites as reported by several researchers (Campos-García et al., 1999; Sarrazin et al., 1997; Swisher, 1987) (see Figures 7.6 and 7.7). The negative removal efficiency obtained can be explained by the fact that some aged LAS homologues were available to release during extraction process (after anaerobic digestion) and therefore influenced negatively the efficiency removal values as observed by Trably et al. (2003).

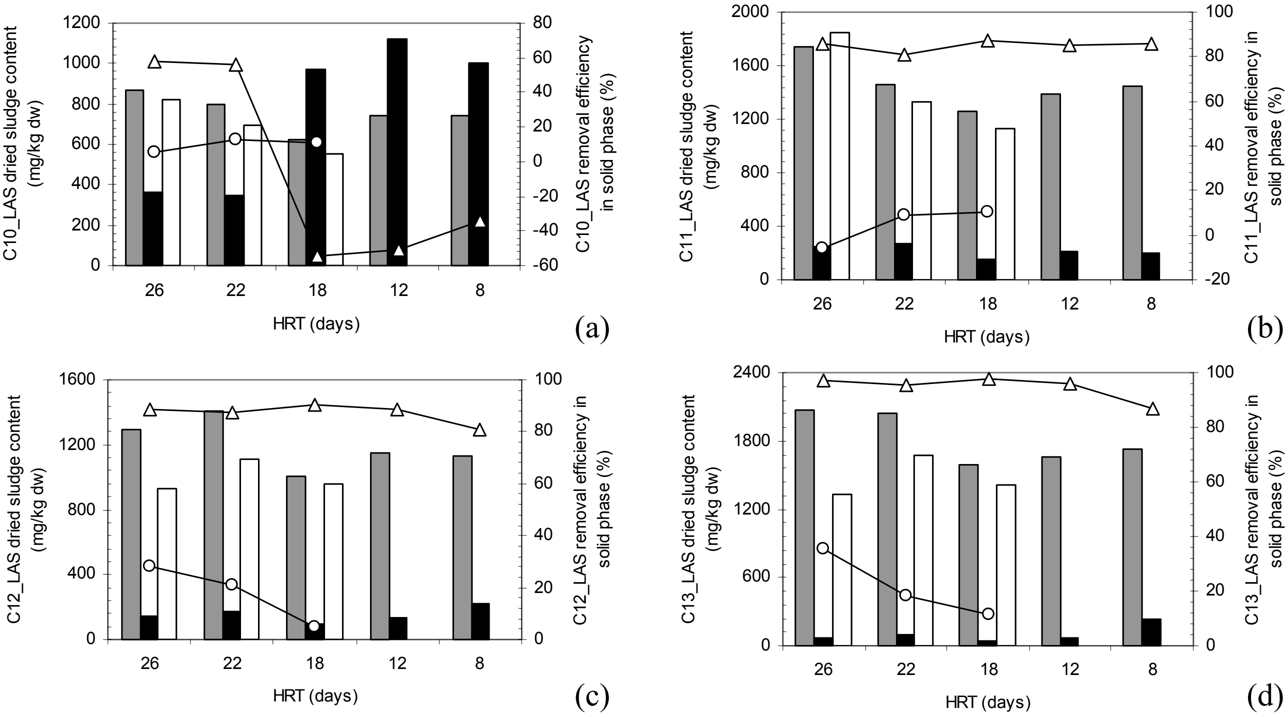


Figure 7.13. *Content of LAS homologues [C10 (a), C11 (b), C12 (c) and C13 (d)] in dried sludge of fresh Feed (■), Thermophilic (■) and Mesophilic (□) anaerobic effluent and the removal efficiency in the thermophilic (-Δ-) and the mesophilic (-○-) digesters at tested HRTs.*

Besides, the removal efficiency for the rest of LAS homologues was higher under thermophilic conditions than at mesophilic conditions, which leads to an increase in total LAS amount removal. Otherwise, the efficiency removal was affected negatively by decreasing HRT (Figure 7.13).

From the results described above, the thermophilic anaerobic digestion was able to reduce LAS sludge content. Hence, their potential could decrease slightly with HRT reduction. However, the mesophilic anaerobic digestion had a low degrader potential and did not affected LAS homologues composition. Moreover, the HRT reduction aggravated substantially the removal efficiency as shown in Figure 7.12.

7.4. Conclusions

In this study, a biological removal efficiencies of mono- and diethoxylate nonylphenol (NP1EO and NP2EO) were reached up to 78% and 71%, respectively, in both thermophilic and mesophilic anaerobic digestion. However, for nonylphenol (NP) the anaerobic treatment had removal efficiency values below 20%. Moreover, the effect of decreasing the hydraulic retention time (HRT) was clearly observed under thermophilic conditions for the sum of NP, NP1EO and NP2EO (NPE) biodegradation. Their removal efficiency was reduced by a 43% when HRT decreased from 26 to 8 days. Besides, the HRT reduction effects were not clearly stated under mesophilic conditions. The results showed here suggest that NPEO biodegradation under anaerobic conditions led to NP accumulation after EO units removal. However, it could be verified the causes of the low NP removal, determining their metabolites if it is possible and, therefore, propose their probable biodegradation pathways. In addition, tested different pretreatments, like physical, chemical, biological or combination of them, to improve NP biodegradation and similar hydrophobic xenobiotic compounds is also considered prioritized research ways.

The LAS content in the dried sludge decreased substantially under thermophilic conditions, which released a biosolids with 67-86% below the initial LAS content. In contrast, the mesophilic treatment had a LAS removal efficiency below 20% at all the HRTs applied. Hence, the HRT reduction led to an increase in the LAS content of the digested sludge.

It seems that thermophilic anaerobic digestion enhances longer straight alkyl chain LAS removal. However, the LAS biodegradation mechanism needs to be elucidated since today the proposed degradation pathways are not clear (if they can occur jointly or not and if they are the same for longer and shorter alkyl chain LAS). On the other hand, thermophilic conditions can be considered as an essential way for investigation their effects on the fate of similar organic micropollutants and hence, to generalise their use on sludge stabilization.

7.5. References

Ahel M., Giger W. (**1993**) Partitioning of alkylphenols and alkylphenol polyethoxylates between water and organic solvents. Chemosphere 26, 1471-1478.

Ahel M., Giger W., Koch M. (**1994**).Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment. I. Occurrence and transformation in sewage treatment. Wat. Res., 28, 1131-1142.

Angelidaki I., F Haagensen, BK Ahring. (**2000a**). Anaerobic transformation of LAS in continuous stirred tank reactors treating sewage sludge, 5th World Cesio congress V.2: 1551-1557, 2000, Firenze, Italy.

Angelidaki I., Mogensen A.S., Ahring B.K. (**2000b**). Degradation of organic contaminants found in organic waste. Biodegradation, 11**,** 377–383.

APHA (**1995**) Standard Methods for the Examination of Water and Wastewater. 19th edn, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.

Ball H. A., Reinhard M., McCarty P. L. (**1989**). Biotransformation of halogenated and nonhalogenated octylphenol polyethoxylate residues under aerobic andanaerobic conditions. Environ. Sci. Technol., 23, 951-961.

Balson T., Felix M.S.B. (**1995**). Biodegradability of non-ionic surfactants. In Karsa D.R., Porter M.R. (eds). Biodegradability of surfactants. Blackie Academia & Profesional, Glasgow, United Kingdom (review): 204-230

Barceló D., Petrovic M. (**2004**). Los microcontaminantes orgánicos: Problemática y situación en Cataluña bajo la perspectiva da le Directiva Europea**.** Jornada sobre gestión y tratamiento de lodos en estaciones depuradoras de aguas residuales. Barcelona, 1 Diciembre 2004.

Battersby N. S., Wilson V. (**1989**). Survey of the anaerobic biodegradation potential of organic chemicals in digesting sludge. Appl. Environ. Microbiol., 55, 433-439.

Bennie D.T., Sullivan C.A., Lee H-B., Maguire R.J. (**1998**). Alkylphenol polyethoxylatemetabolites in Canadian sewage treatment plant effluent streams. Water Qual. Res. J. Can., 33, 231-252.

Berna J.L., Ferrer J., Moreno A., Prats D., Ruiz B.F. (**1989**). The fate of LAS in the environment. Tenside Surf. Det., 26 (2), 101-107.

Bokern M., Harms H.H. (**1997**). Toxicity and Metabolism of 4-*n*-Nonylphenol in Cell Suspension Cultures of Different Plant Species. Environ.Sci. Technol., 31(7), 1849-1854.

Brunner P.H., Capri S., Marcomini A., Giger W. (**1988**) Occurrence and behaviour of linear alkylbenzenesulphonates, nonylphenol, nonylphenol mono- and nonylphenol diethoxylates in sewage and sewage sludge treatment. Wat. Res., 22, 1465-1472.

Campos-García J., Esteve A., Vázquez-Duhalt R., Ramos J-L., Soberón-Chávez G. (**1999**). The Branched-Chain Dodecylbenzene Sulfonate Degradation Pathway of *Pseudomonas aeruginosa* W51D Involves a Novel Route for Degradation of the Surfactant Lateral Alkyl Chain. Appl. Environ. Microbiol., 65, 3730-3734.

Cavalli L., Clerici R., Radici P., Valtorta L. (**1999**). Update on LAB/LAS. Tenside Surf. Det. 36: 254-258.

Cavalli L., Gellera A., Landone A. (**1993**). LAS removal and biodegradation in wastewater treatment plant. Environ. Toxic. Chem., 12, 1777-1788.

CEC (**2000**). Working Document on Sludge (3rd Draft), Commission of the European Communities Directorate-General Environment, ENV.E.3/LM, 27 April 2000, Brussels.

CED. Comité Español de detergencia (**1989**).

Chang B.V., Chiang F., Yuan S.Y. (**2005**). Anaerobic degradation of nonylphenol in sludge. *Chemosphere* 59, 1415-1420.

Comellas L., Portillo J.L., Vaquero M.T. (**1993**). Development of analytical procedure to study linear alkylbenzenesulphonate (LAS) degradation in sewage sludge-amended soil. J. Chromatogr. A. 637, 35-31.

De Wolf W., Feijtel T.C.J. (**1989**).Terrestrial risk assessment for LAS in sludge-amended soils. Chemosphere 36, 1319-1343.

Di Corcia A., Samperi R., Marcomini A. (**1994**). Monitoring aromatic surfactants and their biodegradation intermediates in raw and treated sewages by solid phase extraction and liquid chromatography. Environ. Sci. Technol., 28, 850-858.

Ejlertsson J., Nilsson M-L., Kylin H., Bergman Å., Karlson L., Öquist M., Svensson B.H. (**1999**). Anaerobic Degradation of Nonylphenol Mono- and Diethoxylates in Digestor Sludge, Landfilled Municipal Solid Waste, and Landfilled Sludge. Environ. Sci. Technol., 33,301-306.

EU-RPA. (2000). Nonylphenol risk and policy analysts. Final report for department of the environment, transport and regions. Revision september.

Fauser P., Vikelsøe J., Sørensen P.B., Carlsen L. (**2003**). Phthalates, nonylphenols and LAS in an alternately operated wastewater treatment plant—fate modelling based on measured concentrations in wastewater and sludge. Wat. Res., 37, 1288–1295.

Feijtel T.C.J., Struijs J., Matthijs E. (**1999**). Exposure modelling of detergent surfactants. Prediction of 90th percentile concentrations in the Netherlands, Environ.Toxicol. Chem., 18, 2645-2652.

Feijtel T.C.J., van de Plassche E.J. (**1995**). Environmental risk characterization of 4 major surfactants used in the Netherlands, RIVM/NVZ, Report No. 679101 025.

García M.T., Campos E., Ribosa I., Latorre A., Sánchez-Leal J. (**2005**). Anaerobic digestion of linear alkyl benzene sulfonates: Biodegradation kinetics and metabolite analysis. Chemosphere, 60, 1636-1643.

Giger W., Ahel M., Koch M., Laubscher H. U., Scha.ner C., Schneider J., (**1987**). Behaviour of alkylphenol polyethoxylate surfactants and of nitrilotriacetate in sewage treatment. Wat. Sci. Technol. 19, 449-460.

Giger W., Brunner P. H., Schaner C. (**1984**). 4-Nonylphenol in sewage sludge: accumulation of toxic metabolites from nonionic surfactants. Science 225, 623-625.

Haggensen F., Mogensen A.S., Angelidaki I. (**2002**). Anaerobic treatment of sludge: focusing on reduction of LAS concentration in sludge. Water Sci. Technol. 46: 159-165.

Harwood C.S., Gibson J. (**1997**). Shedding light on anaerobioc benzene ring degradation: a process unique to Prikaryotes? J. Bacteriol., 179, 301-309.

HERA (**2004**). Human and Environmental Risk Assessment. LAS, Linear Alkylbenzene Sulfonate (CAS Nº 68411-30-3), p 10-11. Version 2.0, May. www.heraproject.com.

IUSE. (**2003**). Final Report, Anaerobic biodegradation of detergents surfactants (Annex A). Fraunhofer-Institut für Umwelt-, Sicherheits- und Energietechnik (UMSICHT), July.

Jensen J. (**1999**). Fate and effects of LAS in the terrestrial environment. Sci. Tot. Environ., 226, 93-111.

Jones F.W., Westmoreland D.J. (**1998**). Degradation of nonylphenol ethoxylates during the composting of sludges from wool scour effluents. Environ. Sci. Technol., 32, 2623-2627.

Karsa D.R., Porter M.R. (**1995**). Biodegradability of surfactants, Chapman & Hall, 1995.

Kimerle R.A., Swisher R.D. (**1977**). Reduction of aquatic toxicity of LAS by biodegradation. *Water Res.,* 11, 31.

Kravetz L., Chung H., Guin K. F., Shebs W. T., Smith L. S., Stupel H. (**1982a**). Ultimate biodegradation of an alcohol ethoxylate and a nonylphenol ethoxylate under realistic conditions. Household and Personal Products Industry 19, 46-72.

Kravetz L., Chung H., Guin K. F., Shebs W. T., Smith L. S., Stupel H. (**1982b**) Ultimate biodegradation of an alcohol ethoxylate and a nonylphenol ethoxylate under realistic conditions. Household and Personal Products Industry 20, 62-70.

Langenkamp H., Part P., Erhardt W., Prüeß A. (**2001**). Organic contaminants in sewage sludge for agricultural use. European Commission joint research centre institute for environment and sustainability soil and waste unit.

Llabrés P., Mata-Alvarez J. (**1988**). The hydrolytic step in a dry digestion system. *Biological Wastes*, 23(1), 25-37.

Marcomini A., Cape P.D., Lichtensteiger T., Brunner P. H., Giger W. (**1989**). Behaviour of aromatic surfactants and PCBs in sludge- treated soil and landfills. J. Environ. Qual., 18, 523-528.

Marcomini A., Giger W. (**1988**). Behaviour of LAS in sewage treatment, Tenside Surf. Det., 25, 226-229.

Matthijs E., Debaere G., Itrich N., Masscheleyn P., Rottiers A., Stalmans M., Federle T.W. (**1995**). The fate of detergent surfactants in sewer systems. Wat. Sci. Tech., 31, 321-328.

Matthijs E., Holt M.S., Kiewiet A., Rijs G.B. (**1999**). Environmental Monitoring for LAS, AE, AES, AS, and soap. Environ. Toxicol. Chem., 18, 2634-2644.

Mogensen A.S., Ahring B.K. (**2002**). Formation of metabolite during biodegradation of linear alkylbenzene sulfonate in an upflow anaerobic sludge bed reactor under thermophilic conditions. Biotechnol. Bioeng., 5, 483-488.

Ortiz S. (**2004**). La gestión de biosólidos en la agència catalana de l´aigua. Jornada sobre gestión y tratamiento de lodos en estaciones depuradoras de aguas residuales. Barcelona, 1 Diciembre 2004.

Painter H.A., Zabel T. (**1989**). The behaviour of LAS in sewage treatment. Tenside Surf. Det., 26, 108-115.

Paulsrud B., Wien A., Nedland K. T. (**2000**). A survey of toxic organics in Norwegian sewage sludge, compost and manure.-Aquateam, Norwegian Water Technology Centre Asoslo.

Petersen P.H. (**1999**). Degradation of xenobiotics by composting, Ramboll, 1999, presented in a workshop(SPT/EPA,1999).

Prats D., Rodriguez M., Llamas J.M., DeLaMuela M.A., Ferrer J., Moreno A., Berna J.L. (**2000a**). The use of specific analytical methods to assess the anaerobic biodegradation of LAS. 5th World Cesio Congress **V.2**, 1655-1658, 2000, Firenze, Italy.

Prats D., Rodriguez M., Muela M.A., Llamas J.M., Ferrer J., Moreno A., Berna J.L., Nielsen A.M., Naylor C. (**2000b**). Elimination of LAS in sewage biosolids by composting. 5th World Cesio Congress **V.2**, 1475-1488, 2000, Firenze, Italy.

Prats D., Rodríguez M.A., Llamas J.M., Moreno A., Ferrer J, Berna J.L.(**1999**). Elimination of xenobiotic during composting. Tenside Surf. Deterg., 36(5), 294-298.

Prats D., Ruiz F., Vázquez B., Rodriguez-Pastor M. (**1997**). Removal of anionic and noionic surfactant in a waste water treatment plant with anaerobic digestion. Acomparative study . Wat. Res., 31(8), 1925-1930.

Prats D., Ruiz F., Vazquez B., Zarzo D., Berna J.L. (**1993**). LAS Homolog Distribution Shift during Wastewater Treatment and Composting: Ecological Implications. Environmental Toxicology and Chemistry, 12(9), 1599-1608.

Pryor S.W., Hay A.G., Walker L.P. (**2002**). Nonylphenol in anaerobically digested sewage sludge from New York State. Environ. Sci. Technol., 36, 3678-3682.

Reinhard M., Goodman N., Mortelmans K. E. (**1982**). Occurrence of brominated alkylphenol polyethoxy carboxylates in mutagenic wastewater concentrates. Environ. Sci. Technol., 16, 351-362.

Rimkus R., Rayan R., Cook E. (**1982**). Full-scale thermophilic at the west-southwest sewage treatment works, Chicago, Illinois. Journal WPCF, 54 (11), 1447-1457.

Riu J., Martínez E., Barceló D., Ginebreda A., Tirapu L.L. (**2001**). LC-MS determination of linear alkylbenzene sulfonates and their carboxylic degradation products in influent and effluent water samples and sludges from sewage-treatment plants Fresenius J. Anal. Chem., 371 (4),448-455.

Rudling L., Solyom P. (**1974**). The investigation of biodegradability of branched nonyl phenol ethoxylates. Wat. Res. 8, 115-119.

Salinitro J.P., Langston G.C., Dorn P.B., Kravetz L. (**1988**) Activated sludge treatment of ethoxylate surfactants at high industrial use concentrations. Wat. Sci. Technol. 20, 125-130.

Sanz J.L., Rodriguez M., Amils R., Berna J.L., Moreno A. (**1999**). Anaerobic biodegradation of LAS. Inhibition of the methanogenic process. Riv. It. Sostanze Grasse, **LXXVI**, 307-311.

Sarrazin L., Arnoux A., Reboillon P. (**1997**), High-performance liquid chromatographic of a linear alkylbenzenesulfonate ant its environmental biodegradation metabolites. J Chromatogr. A. 760, 285-291.

Schönkaes U. (**1998**). LAS-A modern classic surfactant. Chimica Oggi, September, 9-13.

Solé M., De Alda M-L., Castillo m., Porte C., Ladegaard-Pedersen K., Barceló D. (**2000**). Estrogenicity determination in sewage treatment plants and surface waters from the Catalonian area (NE Spain). Environ. Sci. Technol., 34, 5076-5083.

Sundberg K. (**1990**). Naturvårdsverket Rapport 3752. In Chemicals with estrogen-like effects, TemaNord 1996:580, Nordic Council of Ministers, Copenhagen, p. 278.

Swisher R.D. (**1987**). Surfactant biodegradation. 2° Edition, Marcell Dekker, New York.

Tabor C.F., Barber L.B. (**1996**). Fate of LAS in the Mississippi river. Environ. Sci. Technol., 30, 161-171.

Tanghe T., Devriese G., Verstraete W. (**1998**). Nonylphenol degradation in lab scale activated sludge units is temperature dependent. Wat. Res., 32 (10), 2889-2896.

Torres R. (**1992**). Estudi de la digestió anaeròbica en una i dues fases dels residus ramaders. Estudi en planta pilot d´un sistema en dues fases. PhD Thesis, University of Barcelona. Department of Chemical Engineering. (in Catalan).

Trably E., Patureau D., Delgenes J.P. (**2003**). Enhancement of polycyclic aromatic hydrocarbons removal during anaerobic treatment of urban sludge. Wat. Sci. Tech., 48 (4), 53–60.

Valtorta L., Radici P., Calcinai D., Cavalli L. (**2000**). Recent development of LAB/LAS. Riv. It. Sostanze Grasse **LXXVII**, 73-76.

Van Ginkel C. G., (**1996**). Complete degradation of xenobiotic surfactants by consortia of aerobic microorganisms. Biodegradation 7, 151-164.

Walterson, Landner (**1990**). Kemikalier i Dalälvens avrinninngsområde, Naturvårdsverket Rapport 3936. In Chemicals with estrogen-like Eeffects. TemaNord 1996:580, Nordic Council of Ministers, Copenhagen, p. 278.

Waters J., Feijtel T.C.J. (**1995**). AISE/CESIO environmental surfactant monitoring programme: outcome of five national pilot studies on LAS. Chemosphere, 30 (10), 1939-1956.

Ying G-G. (**2005**). Review: Fate, behavior and effects of surfactants and their degradation products in the environment. Environ. Int., in press.

Chapter 8

Effect of ultrasound pretreatment in mesophilic and thermophilic anaerobic digestion with emphasis on naphthalene and pyrene removal

Chapter 8

8. Effect of ultrasound pretreatment in mesophilic and thermophilic anaerobic digestion with emphasis on naphthalene and pyrene removal[*]

Summary

In many anaerobic digestion processes for the treatment of the sludge produced in wastewater treatment plants, the hydrolysis of the organic matter has been identified as the rate limiting step. This study is focused on the effect of ultrasonic pretreatment of raw sewage sludge before being fed to the mesophilic and the thermophilic anaerobic digestion. From particle size reduction, COD disintegration degree and biodegradability test, 11000 kJ/kg TS was estimated as the optimal specific energy in ultrasonic pretreatment. Moreover, the use of pretreated sludge improved significantly the COD removal efficiency and biogas production in lab-scale anaerobic digesters when compared with the performance without pretreatment, especially under mesophilic conditions.

During ultrasonic pretreatment, the micropollutants content reduction in the pretreated sludge was observed. Thus, the transfer of Polycyclic Aromatic Hydrocarbons (PAH) compounds to the aqueous is stated. With sonication, napthalene was better removed than without this pretreatment, particularly in the mesophilic digester. However, pyrene removal remained at same efficiency level with or without ultrasonic pretreatment.

---

[*] **Benabdallah el Hadj T., Dosta J., Mata-Álvarez J**. (2006). Effect of ultrasound pretreatment in mesophilic and thermophilic anaerobic digestion with emphasis on naphthalene and pyrene removal. Water Research, (submitted).

8.1. Introduction

Mechanical and physical pretreatment like colloid mill process (Harrison, 1991), high pressure homogenisation (Harrison, 1991; Dichtl et al., 1997; Neis et al., 1997), high thermal exposition (Li and Noike, 1992; WQI, 1996; Tanaka et al., 1997) and ultrasound cavitation (Rivard and Nagle, 1996; Dichtl et al., 1997; Neis et al., 1997) are the most solicited techniques to be incorporated in residual effluent treatment processes to enhance matter solubilization and, therefore, hydrolysis step (Weamaes and Verstraete, 1998). One of the main advantages of these techniques is that the use of external chemical agents is prevented and, therefore, the increase of the effluent volume is avoided (see Table 8.1).

Table 8.1. *Pretreatment advantages and disadvantages (Weemaes and verstraete, 1998)*

Method	*% Cell desintegration*	*Major advantage*	*Major Disadvantage*
Ultrasound	**100**	Complete desintegration	Energy intensive
Thermal	30-55	Proces flexibility	Corrosion, odour
Thermochemical	5-60	Relatively simple	Corrosion, odour, subsequent neutralisation
Biologic	5-50	Simple operation, low cost	Very low yields, odour
Oxidative	90	High desintegration efficiency	Low pH, corrosive, high cost

In recent years, some research groups have investigated the ultrasonic application on sludge before being fed to the anaerobic digestion process. The study of the effects of the sonication energy and frequency on biosolids and their impact on the anaerobic digestion process have been the main axles of their studies (Rivard and Nagle, 1996; Chiu et al., 1997; Neis et al., 1997; Mueller et al., 1998; Tiehm et al., 2001).

Ultrasonic process leads to cavitation bubble formation in the liquid phase. These bubbles grow and then violently collapse when they reach a critical size. Cavitational collapse produces intense local heating and high pressure on liquid-gas interface, turbulence and high shearing phenomena in the liquid phase. Because of the extreme local conditions, OH•, HO2•, H• radicals and hydrogen peroxide can be formed. Thus, sonication is a combination of different phenomena: chemical reactions using radicals, pyrolysis, combustion and shearing (Bougrier et al., 2005).

Pörtenlänge (1999) concluded that low frequencies (<100 kHz) promote mechanical and physical phenomena. However, high frequencies intensify the sonochemical effects (Hua and Hoffman., 1997; Petrier and Francony, 1997). Besides, Tiehm et al. (1997) and Neis and Blume (2002) reported 20-40 kHz as the optimal frequency range to achieve strong mechanical forces.

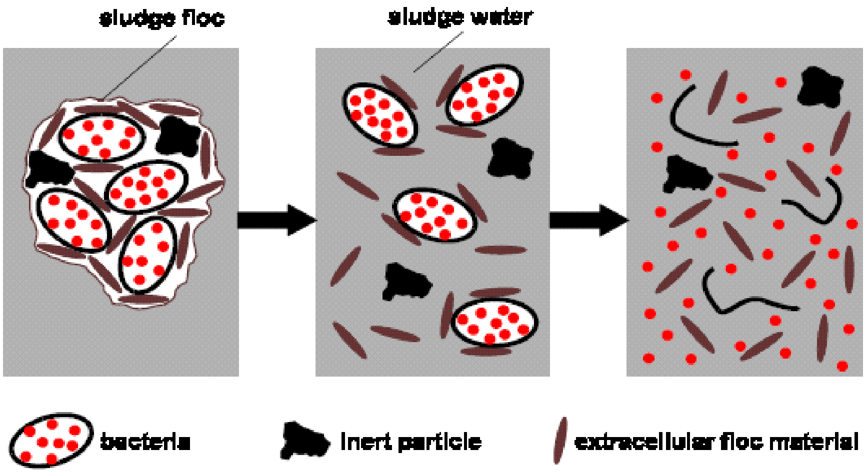


Figure 8.1. *Model of sludge desintegration with increasing ultrasonic specific energy.*

On the other hand, high energy intensity enhances the disintegration of particulated matter (Neis and Tiehm, 1997; Neis and Blume, 2002; Wang et al., 2005), which is evidenced by a reduction in particle size and an increase in the soluble matter fraction (see Figure 8.1). These characteristics lead to a positive impact on the anaerobic digestion process, specially for Waste Activated Sludge (WAS) stabilization (Lehne et al., 2001; Nickel, 2002; Bougrier et al, 2004), since the hydrolysis of WAS has been identified as the rate-limiting step (Eastman and Ferguson, 1981; Sans, 1992; Shimizu et al., 1993; Sans et al., 1995; Del Borghi et al., 1999). An enhancement of the organic matter removal and, therefore, the biogas and methane rate production as a consequence of ultrasonic pretreatment of sludge have been also reported in literature (Neis et al., 2001; Tiehm et al., 2001; Nickel, 2002). Moreover, Lafitte-Trouqué and Forster (2002) concluded that this improvement is more pronounced under thermophilic conditions than under mesophilic conditions.

Recently, some research was focused in the ultrasonic pretreatment effects on environmental micropollutants (Tiehm, 1999; Gonze et al., 1999). The intensification of the organic matter solubilization induced by the ultrasonic action, can lead to an increase of the bioavailability of some micropollutants to the degrader consortium (Neis and Tiehm, 1999; Tiehm, 1999). An important refractory family compounds are the Polycyclic Aromatic Hydrocarbons (PAH), which have a hydrophobic character and a low water solubility, that limit their anaerobic biodegradation (Pavlostathis and Jaglal, 1991; Holliger and Zehnder, 1996; Trably et al., 2003; Subramaniam et al., 2004). Tiehm (1999) reported that ultrasonic treatment enhances considerably the transference of phenanthrene and naphthalene to the aqueous phase and the sonochemical reaction products were successfully biodegraded (see Figure 8.2).

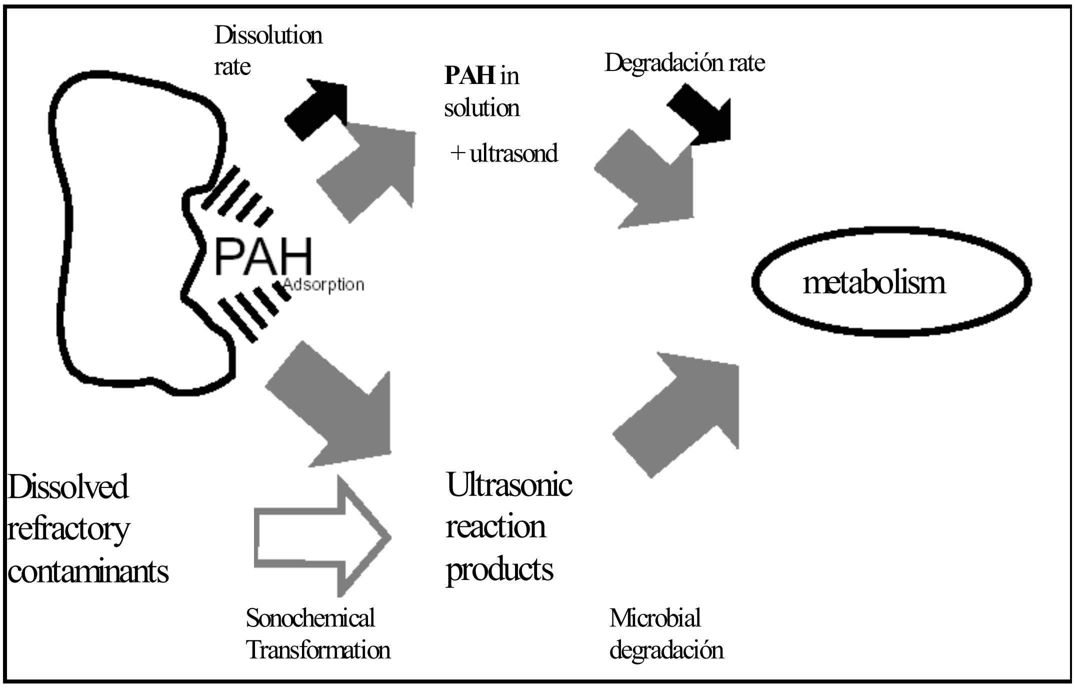


Figure 8.2. *Proposed mechanisms causing enhanced PAH bioavailability (Tiehm, 1999).*

The objective of this chapter was to detect the modification of sewage sludge characteristics due to an ultrasonic pretreatment and its effects on the anaerobic biodegradability of the sludge under both mesophilic and thermophilic conditions. After the determination of the optimal sonication conditions (specific energy), the effect of this pretreatment was evaluated at lab-scale anaerobic digesters. Besides, the impact of ultrasonic pretreatment on napthalene and pyrene removal was investigated.

8.2. Materials and methods

8.2.1. Ultrasonic treatment

The ultrasonic apparatus used in this study was an ultrasonic homogenizer HD2070

(Sonoplus Bandelin). This apparatus was equipped with a MS 73 titanium microtip probe and worked with an operating frequency of 20 kHz and supplied power of 70 W (see Figure 8.3).

Figure 8.3. *SONOPLUS BANDELIN (Ultrasound apparatus).*

Batch experiments were carried out in a cylindrical vessel with 5 cm inner diameter and without temperature regulation. The treated sample had a maximum volume of 50 mL as recommended in the apparatus manual. Specific supplied energy ranged from 0 to 15000 KJ/kg TS to determine the optimal conditions (Eder et al., 2002; Bougrier et al, 2005; Grönroos et al., 2005). The specific energy (E_S) is defined as the product of the ultrasonic power (P) and the ultrasonic time (t) divided by the sample volume (V) and the initial total solids concentration (TS_0):

$$E_S = \frac{(P\,t)}{(V\,TS_0)} \tag{8.1}$$

Particle size

Particle size analysis was conducted with a laser dispersion sensor equipped with a Universal liquid module (UML) which can detect particle size from 0.04 up to 2000 µm. Particle counts and size distribution were calculated and displayed automatically.

Turbidity

The turbidity of sludge samples (after centrifugation for 30 min at 40,000 rpm (Tiehm et al., 2001)) was estimated by measuring their light transmission with a Turbiscan apparatus (MA 2000). Deionized water was assumed to have a 100% of light transmission and was used as a patron.

Matter composition

Total and volatile solids (TS and VS) were determined in both sludge and solids of centrifugation samples according to the standard methods (APHA, 1995). These analyses allowed to obtain the total and particulated concentrations of TS and VS. The TS and VS content of the supernatant of centrifugation, considered the soluble phase, (TSs and VSs) were subsequently deduced. The VS solubility improvement (X_{VSs}) was calculated as the difference between VSs after ultrasonic treatment and the initial soluble VS (VSs_0) as shown in Equation 8.2. The same definition was used for TS, biogas production, COD removal and Specific Biogas Production (SBP).

$$X_{VS_S}(\%)=\frac{\left(VS_S-VS_{S0}\right)}{\left(VS_{S0}\right)}\cdot 100 \tag{8.2}$$

Chemical oxygen demand (COD)

COD was measured in the total sludge and in the supernatant fraction (soluble COD, CODs) according to the standard methods (APHA, 1995). By difference between COD and CODs, the particulated COD (CODp) was calculated. The COD Solubilization (S_{COD}) represents the transfer of COD from the particulated fraction of the sludge to the soluble fraction. S_{COD} was calculated by using the difference between CODs after ultrasonic treatment and the initial CODs ($CODs_0$) related to the initial particulated COD ($CODp_0$):

$$S_{COD}(\%)=\frac{\left(COD_S-COD_{S0}\right)}{\left(COD_{P0}\right)}\cdot 100 \tag{8.3}$$

The degree of disintegration (DD_{COD}) was defined by Müller and Pelletier (1998) as the comparison between CODs after ultrasonic pretreatment and the maximum CODs obtained by alkaline hydrolysis (COD_{NaOH}), as presented in Equation 8.4. For alkaline hydrolysis, sludge was mixed with NaOH (0.5 mol/L) for 24 h, at room temperature as described by Tiehm et al. (2001), Neis and Blume (2002) and Gonze et al. (2003).

$$DD_{COD}(\%)=\frac{\left(COD_S-COD_{S0}\right)}{\left(COD_{NaOH}-COD_{S0}\right)}\cdot 100 \tag{8.4}$$

8.2.2. Biodegradability test

Anaerobic biodegradability test was carried out to compare methane production for different E_s applied to the same substrate with the same inoculum under mesophilic (35 ºC) and thermophilic (55 ºC) conditions (see Figure 8.4).

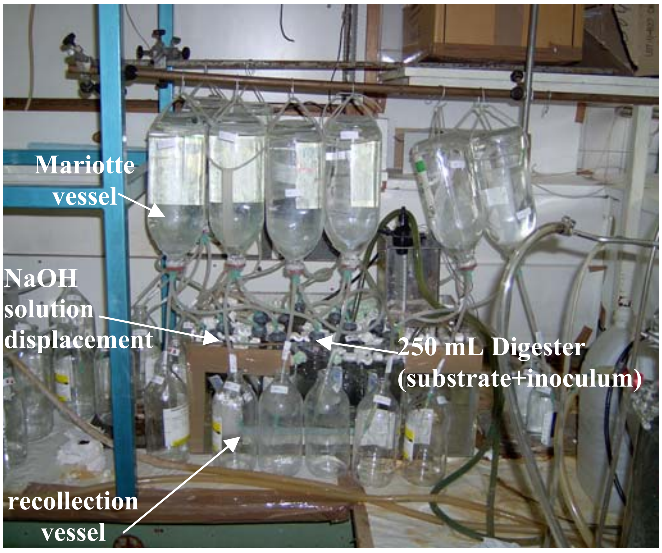


Figure 8.4. *Biodegradability test installation*

The substrate and inoculum used were collected from the feed and the outlet of the mesophilic and thermophilic lab-scale digesters, working under steady state conditions with a HRT of 20 and 15 days, respectively (see Table 8.2).

In order to determine the optimal specific energy, the substrate samples were sonicated at 0, 5000, 8000, 11000 and 15000 kJ/kg TS_0 before their addition to bottle-reactors. Each bottle-reactor (250 mL) was filled with mesophilic or thermophilic inoculum, a substrate quantity in accordance with the substrate/inoculum ratio 0.5 g DQO/g VS as reported by Bougrier et al. (2004), 1 mL of $Na_2S \cdot 9H_2O$ reductive solution (138 g/L) and 1 mL of $NaHCO_3$ alkaline solution (172.5 g/L) (Baraza, 2002; Soto et al., 1992, 1993). All bottles were diluted with deionized water to achieve a final volume of 200 mL. Blank samples were made substituting substrate by deionized water. The volume of methane produced was measured by a displacement of NaOH (25g/L) solution device (Baraza, 2002; Soto et al., 1992, 1993). For each supplied energy level, the assay was reproduced in triplicates. Mesophilic and thermophilic conditions were maintained inside the bottles by means of two heating systems (Haake DC 40).

Table 8.2. *Average feed and digested sludge characteristics and operational parameters.*

Parameter	***Mesophilic***	***Thermophilic***
Working conditions		
Temperature (ºC)	35	55
Flow rate (mL/day)	175	233
HRT (days)	20	15
Feed composition		
TS (g TS/L)	29.77 ± 0.71	
VS (g/L)	22.79 ± 0.47	
CODt (g COD/L)	45.44 ± 1.06	
$N\text{-}NH_4^+$ (mg/L)	628 ± 41	
pH	6.30 – 6.50	
Treated Effluent Characteristics		
TS (g/L)	23.04 ± 0.48	22.69 ± 0.52
VS (g/L)	13.31 ± 0.32	12.23 ± 0.31
CODt (g COD/L)	25.50 ± 0.71	23.08 ± 0.43
$N\text{-}NH_4^+$ (mg/L)	878 ± 198	1195.28 ± 63.30
pH	6.90 – 6.96	6.95 – 7.32
Removal Efficiency		
TS degradation (%)	22.60	23.76
VS degradation (%)	41.60 ± 0.5	46.34 ± 0.7
CODt degradation (%)	43.89 ± 1.7	49.21 ± 1.6
Biogas characteristics		
Daily biogas production L/(L_r day)	0.360 ± 0.021	0.578 ± 0.034
Methane content (% CH_4)	63.6 ± 1.9	67.6 ± 2.6
SBP (L_{BIOGAS}/g SVr)	0.76 ± 0.035	0.821 ± 0.026
SBP (L_{BIOGAS}/g CODr)	0.36 ± 0.008	0.388 ± 0.011

8.2.2. Lab-scale reactors

Two completely mixed and jacketed anaerobic digesters (5L) were used in this study. Mesophilic and thermophilic conditions were maintained by means of two heating systems (Haake DC 40). Both digesters were stabilized at HRTs of 20 days (mesophilic) and 15 days (thermophilic) as shown in Table 8.2. It can be observed that some operational parameters were changed from initial conditions described in Chapter 4. Thus, the total solids content of the inlet and outlet were reduced to avoid some technique problems due to pipes obstruction. However, the same efficiency was registered in both digesters (specific biogas production and volatile solids removal). After the determination of the optimum specific energy of the sonication pretreatment of sludge, the feed was pretreated at this energy value.

8.2.4. Naphthalene and Pyrene analysis

PAHs were analysed using solvents and chemicals of analytical grade (Merck, Barcelona). Naphthalene, deutered naphthalene (d_8), pyrene and deutered pyrene (d_{10}) were supplied by Sigma-Aldrich Química, S.A (Barcelona). Sample analysis were carried out as follows: 10 g of lyophilized samples, initially spiked with naphthalene d_8 and pyrene d_{10} at a final concentration of 0.125 mg/ kg ST for each surrogate, were reflushed in a Soxhlet apparatus filled with a mixture of Dichloromethane:n-Hexane (DCM:Hex) in a ratio 1:1 during 24 hours. The extract was purified and dried on an alumina-sodium sulphate column by means of two elution solvents with different polarities, namely, (*i*) Hexane and (*ii*) Hex:DCM 1:1. Then, eluateds were concentrated under a gentle N_2 stream and were reconstitued in 1 mL Hex, before their injection into GC/MS system. The GC was equipped with an HP-5MS column. PAH compounds were recovered in the second fraction of the elution solvent used (DCM: Hex).

Table 8.3. *PAH compounds identification at GC/MS analysis.*

Compound	M/Z	TR (min)
Naphthalene	128	8.61
Naphthalene d_8	136	8.55
Pyrene	202	19.76
Pyrene d_{10}	212	19.71

For PAHs analysis, the samples were injected split-less into a gas-chromatograph (Shimadzu QP2010) by an automatic sampler (Shimadzu AOC-20i+S). The injector temperature was 280ºC and the flow 1 mL/min. The oven was set to an initial temperature of 60 ºC for 1 min followed by an increasing temperature (10 ºC/min) up to 320 ºC which was kept for 5 min. Helium was used as gas carrier. The MS was run in SIM mode and PAHs compounds were identified as shown in Table 8.3.

8.3. Results and discussion

8.3.1. Ultrasonic pretreatment

Particle size and turbidity. Figure 8.5 shows the particle size distribution of the untreated samples, that ranged from 0.4 to 150 µm. Volume distribution was centred in the 30-50 µm size interval, with a diameter mean value of 33.8µm. In the treated samples, The volume occupied by small particles was incremented with the increasing

E_S dose. Hence, the 90% of the volume was occupied by particle with diameter size below or equal to 28 µm. Furthermore, the average size particle decreased to 10.12-13.26µm.

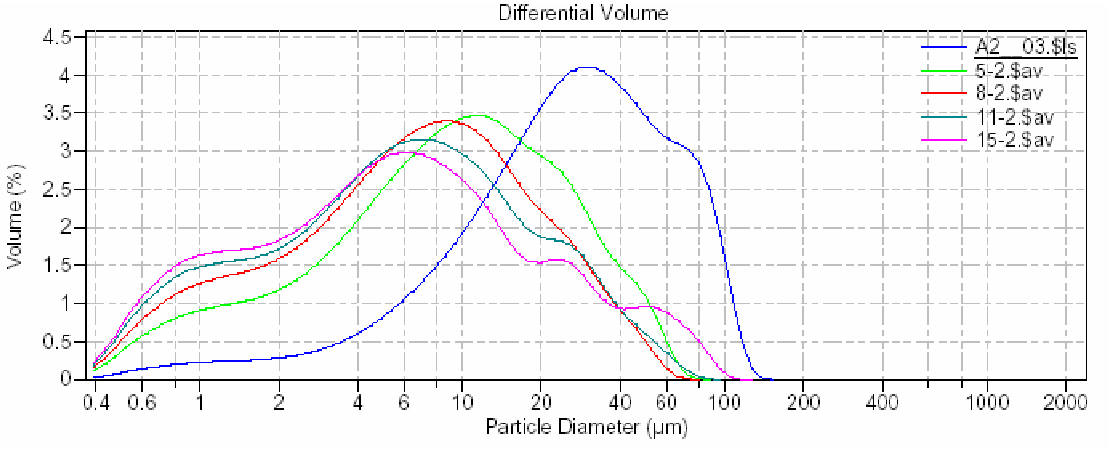


Figure 8.5. Particles size distribution for the selected specific energies applied.

Besides, particles with more than 50 µm were reduced to below quarter part after sonication. This can be explained by the fact that the substrate mixture contained a high primary sludge amount (more than 75%) as elucidated by Blume and Neis (2001). As stated in Figure 8.5, the application of high E_s doses in ultrasonic pretreatment of sludge led to an increase in the released matter to the aqueous medium. Figure 8.6 shows the effect of the tested ultrasonic E_S on the aqueous phase turbidity. In this plot it is clearly observed a reduction of the light transmission when E_S increased, since more particulated organic matter is released to the aqueous media.

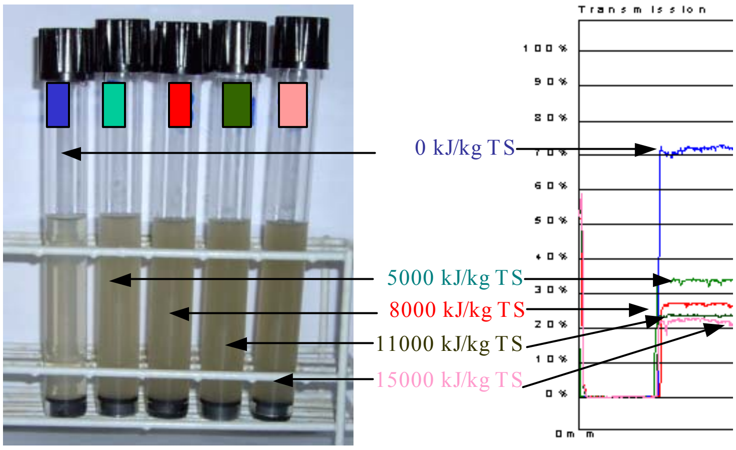


Figure 8.6. Effect of ultrasound E_S on aqueous phase turbidity.

On the other hand, the volume of particles with size 40 µm or more was higher for samples treated with 15000 kJ/kg TS than other samples treated with a lower E_S dose.

This phenomenon, known as re-flocculation, was caused by a regrouping of intra- and extra-cellular material after the ultrasonic treatment (Gonze et al., 2003).

TS, VS and COD disintegration and solubilization. During the ultrasound pretreatment of sludge, TS and VS were maintained almost constant around 32.90 g/L and 23.49 g/L, respectively (see Figure 8.7). Hence, the evaporation and mineralization phenomenon was not induced by the sonication treatment. However, the particulated and soluble matter were affected by the E_S applied. The increase of Es until 11000 kJ/kg TS induced to a sustainable improvement of organic matter solubilization (expressed as VS) by 40 % respect to non-sonicated sample, as stated in Figure 8.7. An optimum E_S value of 11000 kJ/kg TS was observed, since little difference in VS solubilization improvement was detected between 11000 (42.2 %) and 15000 kJ/kg TS (43.4 %).

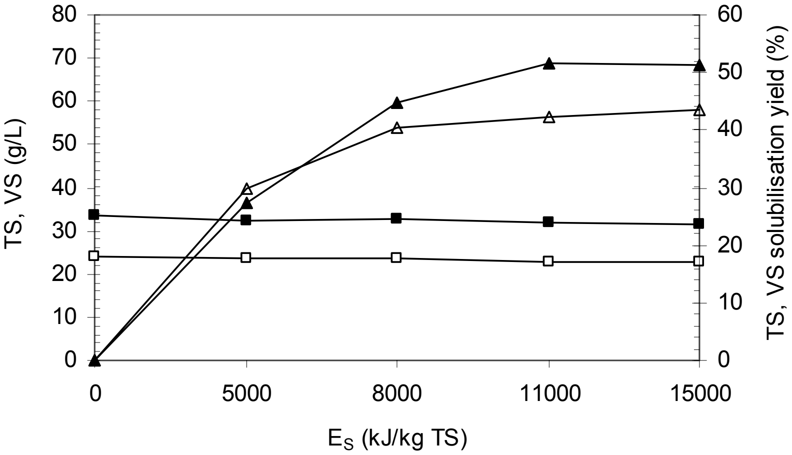


Figure 8.7. *Effect of ultrasonic pretreatment on TS (■) and VS (□) concentration and TS (▲) and VS (Δ) solubilization yield at E_s doses tested.*

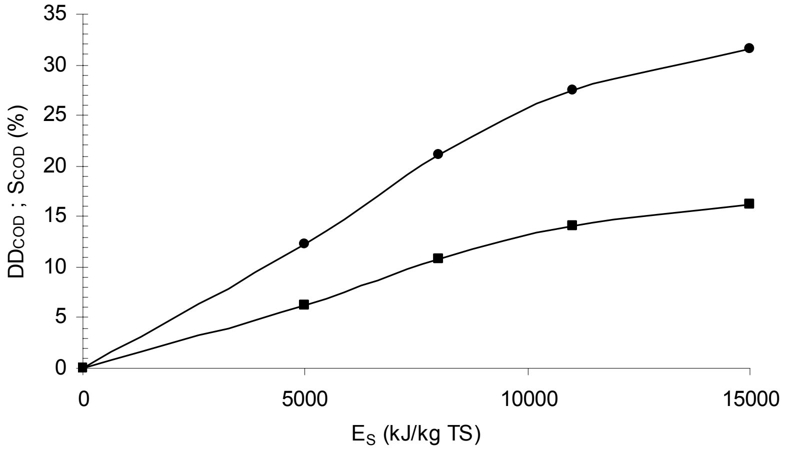


Figure 8.8. *Solubilization (■) and degree of disintegration (●) of COD at the tested specific energies.*

Similarly to TS and VS removal results, the total COD was maintained constant during the ultrasonic pretreatment. The transfer of COD from the particulated to the soluble fraction was clearly improved by increasing the ultrasonic E_S (see Figure 8.8). It was observed an intense increase of the disintegration degree (DD_{COD}) until 11000 kJ/kg TS was reached, but it was maintained in the range 27.5-31.5% at higher E_S tested.

From solid distribution and organic matter solubilization, optimum results were obtained at an E_S of 11000 kJ/kg TS. However, the disintegration values observed in this study are low, when compared with other studies (75% at 20000 kJ/kg TS (Müller and Pelletier, 1998), 60% at 40000 kJ/kg TS (Lehne et al., 2001) and 60% at 15000 kJ/kg TS (Bougrier et al., 2005)). This fact is probably related to the high percentage of primary sludge in the substrate mixture tested (Neis et al., 1997; Nickel, 2002; Grönroos et al., 2005) and to the high VS/TS ratio (Bougrier et al., 2004), since it has been reported that the ultrasonic effects are stronger in WAS treatment process and/or with a VS/TS ratio equal or below 75%.

8.3.2. Biodegradability test

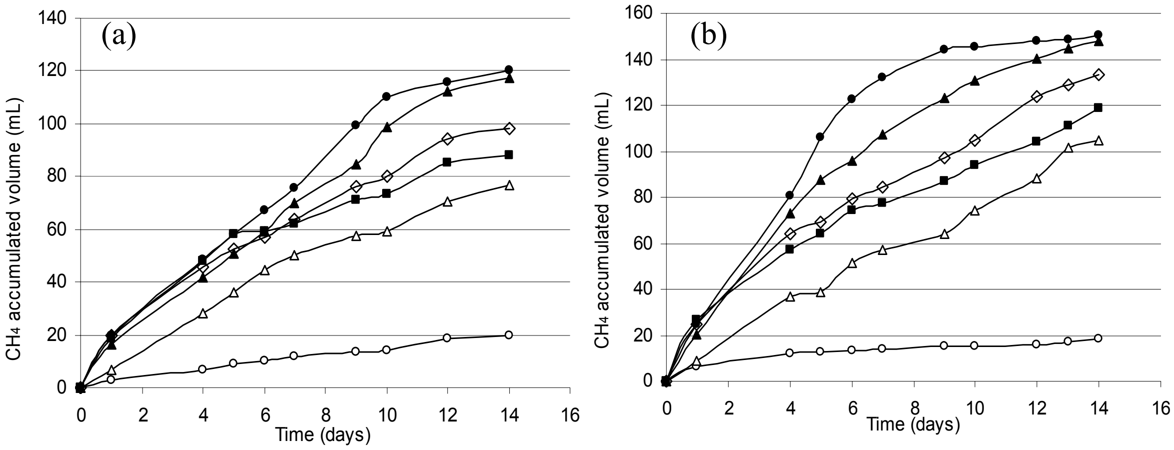


Figure 8.9. *Methane accumulated volume in the biodegradability test under (a) mesophilic and (b) thermophilic conditions. ((○) Blank; (Δ) E_s = 0 kJ/kg TS; (■) E_s = 5000 kJ/kg TS; (◊) E_s = 8000 kJ/kg TS; (▲) E_s = 11000 kJ/kg TS; (●) E_s = 15000 kJ/kg TS).*

The impact of the different E_S applied to the raw substrate on anaerobic digestion efficiency was evaluated by the methane accumulated amount in the biodegradability test. Figure 8.9 shows the average accumulated methane production in the bottle-reactors fed with the same amount of substrate previously treated at the selected E_S. Under both mesophilic and thermophilic conditions, the biogas production was improved with increasing E_S inputs. At low E_S, the total methane produced increased significantly between 12.5-17.5 % and 11.0-19.7% under thermophilic and mesophilic conditions, respectively. However, the enhancement in the biogas yield was only 1.6% (thermophilic) and 2.5 % (mesophilic) when the applied E_S was changed from 11000 to 15000 kJ/kg ST.

Recorded solubilization matter results and methane improvement at the tested E_S, show that 11000 kJ/kg TS was the optimum E_S for the actual substrate. Thus, this E_S dose was applied on the mixed sludge before its insertion to the lab-scale digesters.

8.3.3. Anaerobic Digestion performance

Table 8.4. *Average treated sludge characteristics and operational parameters with combined ultrasonic and anaerobic digestion treatment.*

Parameter	***Mesophilic***	***Thermophilic***
Treated effluent characteristics		
VS (g/L)	12.14 ± 0.07	11.55 ± 0.068
COD (g COD/L)	21.84 ± 0.26	21.88 ± 0.44
pH	6.90 – 6.96	7.18
Organic matter removal efficiency		
VS removal (%)	46.76 ± 1.2	49.30 ± 0.8
COD removal (%)	51.93 ± 2.1	52.53 ± 1.9
Biogas production and methane content		
Daily biogas production ($L_{BIOGAS}/(L_r$ day))	0.47 ± 0.020	0.673 ± 0.012
Methane content (% CH_4)	63.9 ± 2.1	67.8 ± 3.5
SBP ($L_{BIOGAS}/g\ VS_r$)	0.883 ± 0.032	0.898 ± 0.28
SBP ($L_{BIOGAS}/g\ COD_r$)	0.399 ± 0.014	0.423 ± 0.016

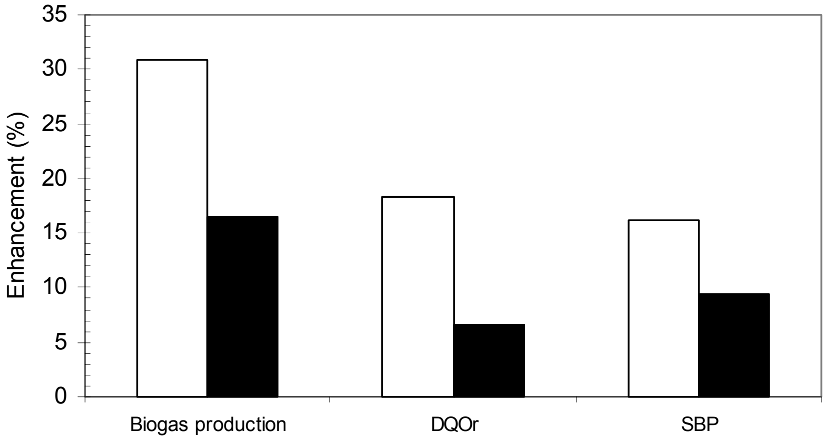


Figure 8.10. *Improvement in biogas production, COD removal and specific biogas production when using pretreated sludge in the mesophilic (□) and the thermophilic (■) digestion.*

After a lag time equivalent to three times HRT from the starting sonicated substrate feeding, the steady-state operational parameters were recorded during a period of two months (see Table 8.4). Comparing these results, with those of Table 8.2 some conclusions about the effect of ultrasonic pre-treatment on anaerobic sludge digestion can be obtained. Thus, Figure 8.10 shows the improvement of biogas production, COD removal and specific biogas production obtained. In the mesophilic digester, the COD removal and the biogas production were enhanced more than in the thermophilic digester, when pretreated mixture sludge was supplied as the influent. However, the methane biogas content was maintained at same level in both reactors as observed by Kim et al. (2003). Consequently, it seemed that the mesophilic anaerobic fermentation was better influenced than the thermophilic anaerobic fermentation. Since the biological hydrolysis of organic matter is enhanced under thermophilic conditions (de la Rubia et al., 2002), the solubilisation by ultrasound pretreatment would affect more the mesophilic digester performance because the hydrolysis step under mesophilic conditions is more limited when compared with the thermophilic conditions. However, no information or data were available from other research to confirm this hypothesis.

8.3.4. PAH removal in ultrasonic pretreatment and anaerobic digestion

Table 8.5 shows the average naphthalene and pyrene content in the feed and treated sludge samples during the operational periods with and without using the ultrasound pretreatment. Figure 8.11 presents the naphthalene and pyrene removal efficiencies in both operational periods.

Table 8.5. *Average naphthalene and pyrene content in sludge samples within the non-sonicated substrate feeding period and within the pretreated substrate feeding period.*

	Samples	***Naphthalene*** (µg/kg dw)	***Naphthalene recovery index*** (%)	***Pyrene*** (µg/kg dw)	***Pyrene recovery Index*** (%)
Non-sonication substrate period	Substrate	55.87 ± 9.23	80.3 ± 7.4	451.58 ± 13.82	75.85 ± 7.6
	Mesophilic Sludge	27.81± 11.65	67.8 ± 14.5	198.72 ± 18.93	69.30 ± 7.2
	Thermophilic Sludge	37.09 ± 3.38	49.25 ± 9.3	310.39 ± 29.67	76.05 ± 3.0
Sonication period	Substrate	48.58 ± 13.54	64 ± 10.3	586.47 ± 25.64	89.55 ± 1.8
	Sonicated substrate	36.83 ± 7.34	72.9 ± 16.6	521.20 ± 17.08	62.45 ± 24.5
	Sonicated mesophilic sludge	22.39 ± 6.85	96 ± 16.0	395.41 ± 13.87	73.60 ± 22.8
	Sonicated thermophilic sludge	17.71 ± 4.03	78.5 ± 14.7	274.96 ± 17.31	67.70 ± 14.4

When using a non-pretreated influent, the naphthalene and pyrene contents decreased by 34% and 31%, respectively, in the mesophilic digester. The thermophilic digester showed a high removal efficiency, releasing an effluent with a 50% of the naphthalene and only a 44% of the pyrene inlet amount. The ultrasonic substrate pretreatment reduced both micropollutants content in the sludge by 11% (pyrene) and 24% (naphthalene). Since the organic matter removal by sonication was discarded (Bougrier et al., 2005), the low micropollutants content in the treated sludge was due to a transference from the solid to the aqueous phase (Thomas et al., 1986; Tiehm, 1999). Angelidaki et al. (2000) and Christensen et al. (2004) reported that the high level of naphthalene transference is probably related to its low molecular weight and its low octanol partition index (namely, K_{ow} = 2300 and $2·10^5$ for naphthalene and pyrene, respectively (IARC, 1983)).

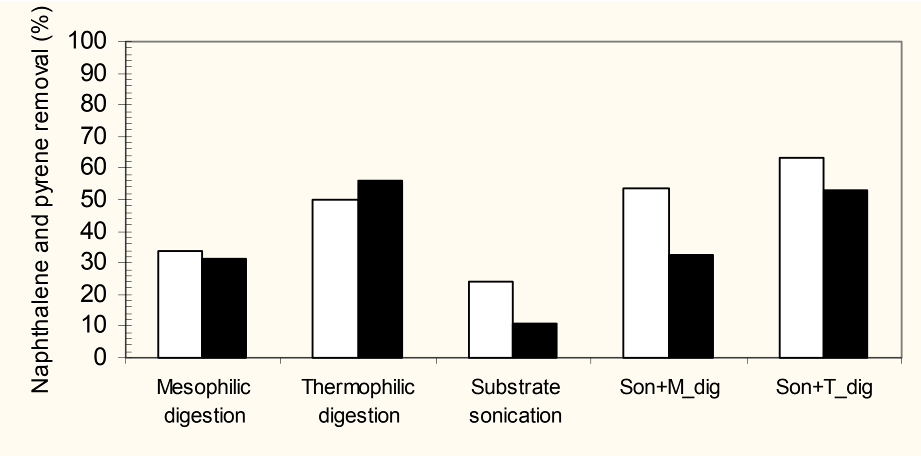


Figure 8.11. *Naphthalene (□) and pyrene (■) removal in dry matter sludge after the studied treatments. (Son+M_dig: combined sonication and mesophilic anaerobic digestion treatment; Son+T_dig: combined sonication and thermophilic anaerobic digestion treatment).*

Digestion of sonicated substrate led to an enhancement of the naphthalene removal at both temperature ranges. However, this efficiency improvement was higher in the mesophilic digestion than in the thermophilic digestion. Moreover, the anaerobic removal of pyrene from sonicated substrate remained at the same efficiency registered for non pretreated substrate. Thus, the combined ultrasonic-anaerobic digestion treatment provided the same pyrene biodegradation efficiency obtained in the anaerobic digestion without pretreatment, at both temperature ranges.

It can be concluded that ultrasonic pretreatment under the studied conditions of this work was not able to improve the pyrene bioavailability to the anaerobic consortium. This fact was probably due to that pyrene, as a high molecular weighted PAH, has a high sorption coefficient (K_{ow}) which is inversely proportional to the diffusion and bioavailability phenomenon (Coates et al., 1996; Rockne and Strand, 1998).

8.4. Conclusions

In this work, the ultrasound pretreatment has proved to be an effective technique to disintegrate the particulated matter. An optimal specific energy of 11000 kJ/kg TS promoted the best solubilization yield and enhanced biogas production in the subsequent anaerobic stabilization under both mesophilic and thermophilic conditions.

The high efficiency of thermophilic anaerobic digestion was slightly improved when it is combined with ultrasonic pretreatment. However, the feeding of a pretreated sludge highly favoured the biogas production in mesophilic anaerobic digestion when compared with the performance without pretreatment.

The diffusion of Polycyclic Aromatic Hydrocarbons (PAH) compounds to the aqueous phase was stated by a reduction in the sonicated sludge pollutants content. High naphthalene removal efficiencies were recorded by the combined ultrasonic-thermophilic anaerobic digestion treatment. However, the naphthalene removal enhancement by combined ultrasonic and anaerobic digestion treatment was more pronounced under mesophilic conditions. On the other hand, the obtained pyrene removal was nearly the same with and without ultrasonic pre-treatment, which was probably due to the high sorption coefficient of pyrene.

8.5. References

Angelidaki I., Mogensen A.S., Ahring B.K. (**2000**). Degradation of organic contaminants found in organic waste. Biodegradation, 11, 377–383.

APHA (**1995**) Standard Methods for the Examination of Water and Wastewater. American Public Health Association. 19th ed., Washingthon.

Baraza J. (**2002**). Contribución al estudio del tratamiento integrado de residuos municipales en un vertedero. PhD Thesis, University of Barcelona. Department of Chemical Engineering. (in Spanish).

Blume T., Neis U. (**2001**). “Improved wastewater disinfection by ultrasonic pre-treatment”. Conference Applications of Power Ultrasound in Physical and Chemical Processing, (3rd, Paris, 13-14 December). <http://ultrawaves.de/pub/Ultrawaves_Improved_Wastewater_Disinfection.pdf> [Consulted on 2/3/2005].

Bougrier C., Carrère H., Battimelli A., Delgenès J.P. (**2004**). Effects of various pre-treatments on waste activated sludge in order to improve matter solubilisation and anaerobic digestion. In: Proceedings of the 10th World congress. Montreal, Canada. p. 998-1003.

Bougrier C., Carrère H., Delgenès J.P. (**2005**). Solubilisation of waste-activated sludge by ultrasonic treatment-169.

Chiu Y.C., Chang C.N., Lin J. Chem. Eng. J., 106, 163.G., Huang S.J. (**1997**). Alkaline and ultrasonic pretreatment of sludge before anaerobic digestion. Water Sci. Technol., 36(11), 155-162.

Christensen N., Batstone D.J., He Z., Angelidaki I. y Shmidt J.E. (**2004**). Removal of polycyclic aromatic hydrocarbons (PAHs) from sewage sludge by anaerobic degradation. Wat. Sci. Technol., 50 (9), 237-244.

Coates J. D., Anderson R. T.and Lovley D. L. (**1996**). Oxidation of polycyclic aromatic hydrocarbons under sulphate-reducing conditions. Appl. Environ. Microbiol., 62, 1099-1101.

De la Rubia M.A., Pérez M., Romero L.I., Sales D. (**2002**). Anaerobic Mesophilic and thermophilic municipal sludge digestion. Chem. Biochem. Eng. Q., 16 (3), 119-124.

Del Borghi A., Converti A., Palazzi E., Del Borghi M. (**1999**). Hydrolysis and thermophilic anaerobic digestion of sewage sludge and organic fraction of municipal solid waste. Bioprocess Engineering, 20, 553-560.

Dichtl N., Müller J., Braunschweig E. E., Günthert F. W., Osswald M. O. (**1997**). Disintegration von Klärschlammein aktueller úberblick. Korrespondez Abwasser, 44, 1726-1739.

Eastman J.A., Ferguson J.F. (**1981**). Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. J. WPCF 53 (3) 352–366.

Eder, B.; F.W. Günthert (**2002**). Practical experience of sewage sludge disintegration by ultrasound. TU Hamburg-Harburg Reports on Sanitary Engineering, 35, 173-187.

Gonze E., Fourel L., Gonthier Y., Boldo P., Bernis A. (**1999**). Wastewater pretreatment with ultrasonic irradiation to reduce toxicity Chem. Eng. J.,73,93-100.

Gonze E., Pillot S., Valette E., Gonthier Y., Bernis A. (**2003**). Ultrasonic treatment of an aerobic activated sludge in a batch reactor. Chem. Eng. and Process., 42, 965-975.

Grönroos A., Kyllönen H., Korpijärvi K. (**2005**). Ultrasound assited method to increase soluble chemical oxygen demand (SCOD) of sewage sludge for digestion. Ultrasonics Sonochemistry, 12, 115-120.

Harrison S. T. L. (**1991**) Bacterial cell disruption : a key unit operation in the recovery of intracellular products. Biotechnol. Adv., 9, 217-240.

Holliger C., Zehnder A. JB. (**1996**). Anaerobic biodegradation of hydrocarbons. Current Opinion in Biotech., 7, 326-330.

Hua I., Hoffmann M. R. (**1997**). Optimization of ultrasonic irradiation as an advanced oxidation technology. Environ. Sci. Technol., 31, 2237-2243.

IARC (Internal Agency for Research on Cancer). (**1983**). Polynuclear Aromatic Compounds, Part 1, Chemical, Environmental and Experimental Data', Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 32, International Agency for Research on Cancer, Lyon, pp. 33–91.

Kim J., Park C., Kim T-H. (**2003**). Effects of various pretreatments for enhanced anaerobic digestion with waste activated sludge. J. Biosci. and Bioeng., 95(3), 271-275.

Lafitte-Trouqué S., Forster C.F. (**2002**). The use of ultrasound and g-irradiation as pre-treatments for the anaerobic digestion of waste activated sludge at mesophilic and thermophilic temperatures. Bioresource Technol., 84, 113-118.

Lehne G., Muller A., Schwedes J. (**2001**). Mechanical disintegration of sewage sludge. Wat. Sci. Technol., 43 (1), 19-26.

Li Y. Y., Noike T. (**1992**). Upgrading of anaerobic digestion of waste activated sludge by thermal pretreatment". Wat. Sci. Technol., 26, 857-866.

Müller J., Lehne G., Schwedes J., Battenberg S., Näveke R., Kopp J., Dichtl N., Scheminski A., Krull R., Hempel D. C. (**1998**). Disintegration of sewage sludges and influence on anaerobic digestion. Wat. Sci. Technol., 38(8-9), 425-433.

Müller J., Pelletier L. (**1998**). L'eau, l'industrie, les nuisances 217. p. 19-26.

Neis U., Blume T. (**2002**). The effect of ultrasound on particulate matter, especially micro-organisms in complex water and waste water media. <http://ultrawaves.de/pub/Ultrawaves_Effect_of_Ultrasound_on_Particulate_Matter.pdf> [Consulted on 4/3/2005].

Neis U., Nickel K., Tiehm A. (**1997**). Intensievierung der schlammfaulung durch Klärschlammaufschluβ mit ultraschall. Korrespondez Abwasser, 44, 1850-1855.

Neis U., Nickel K., Tiehm A. (**2001**) in: Mason T.J., Tiehm A. (Eds.), Ultrasound in Environmental Protection, vol. 6, Jai Press Inc., 2001, pp. 59–90.

Neis U., Tiehm A. (**1997**). Particle size analysis in primary and secondary waste water effluents. Wat. Sci. Technol., 36(4), 151-158.

Neis U., Tiehm A. (**1999**) Ultrasound in waste water and sludge treatment. In: Tiehm A. and Neis U. (eds.) Technical University of Hamburg-Harburg Reports on Sanitary Engineering vol. 25: Ultrasound in Environmental Engineering, pp 39-61.(ISSN 0724- 0783; ISBN 3-930400-23-5).

Nickel K. (**2002**). Ultrasonic disintegration of biosolids- benefits, consequences and new strategies. TU Hamburg Reports on Sanitary Engineering, 35, 189-200. <http://ultrawaves.de/pub/Ultrawaves_Disintegration_Benefits.pdf> [Consulted on 4/3/2005].

Pavlostathis S.G., Jagalal K. (**1991**). Desorption behavior of trichloroethylene in contaminated soil. Environ. Sci. Technol., 25,274.

Petrier C., Francony A. (**1997**). Incidence of wave frequency on the reaction rates during ultrasonic waste water treatment. Wat. Sci. Technol., 35(4), 175-180.

Portenlänger G. (**1999**). "Mechanical and radical effects of ultrasound. En Ultrasound in Enviromental Engineering, TU Hamburg-Harburg Reports on Sanitary Engineering, eds Tiehm A. and Neis U., vol. 25, pp.139-151.

Rivard C. J., Nagle N. J. (**1996**). Pre-treatment technology for the beneficial reuse of municipal sewage sludges. Appl. Bioch. Biotechnol., 57-58, 983-991.

Rockne K. J., Strand S. E. (**1998**). Biodegradation of bicyclic and polycyclic aromatic hydrocarbons in anaerobic enrichments. Environ. Sci. Technol., 32, 2962-2967.

Sans C. (**1992**). Estudi de la fermentació acidogènica de la fracció orgànica dels residus sòlids urbans a nivell de planta pilot. PhD Thesis, University of Barcelona. Department of Chemical Engineering. (in Catalan).

Sans C., Mata-Álvarez J., Cecchi F., Pavan P. (**1995**). Acidogenic fermentation of organic urban wastes in plug-flow reactor in themophilic conditions. Bioresource Technol., 51, 89-96.

Shimizu T.. Kudo K.. Nasu Y.. Anaerobic waste activated sludge digestion—a bioconversion and kinetic model. Biotechnol. Bioeng., 41 (**1993**) 1082–1091.

Soto J.M., Méndez R., Lema J.M. (**1992**). Determinación de la toxicidad y biodegradabilidad anaerobia de aguas residuales. Tecnología del Agua, Febrero, 70-81. (in spanish).

Soto J.M., Méndez R., Lema J.M. (**1993**). Methanogenic and non-methanogenic activity test. Theorical basis and experimental set-up. Wat. Res., 27(8), 1361-1376.

Subramaniam K., Stepp C., Pignatello J.J., Smets B., Grasso1D. (**2004**). Enhancement of polynuclear aromatic hydrocarbon. Desorption by complexing agents in Weathered Soil. Environ. Eng. Sci., 21(4), 515-523.

Tanaka S., Kobayashi T., Kamiyama K.I., Bildan L.N.S. (**1997**). Effects of thermochemical pretreatment on the anaerobic digestion of waste activated sludge. Wat. Sci. Technol., 35(8), 209-215.

Thomas J.M., Yordy J.R., Amador J.A., Alexander M. (**1986**). Rates of dissolution and biodegradation of water-insoluble organic compounds. Appl. Environ. Microbiol., 52, 290-296.

Tiehm A. (**1999**). “Combination of ultrasound and biodegradation: enhanced bioavailability of polycyclic aromatic hydrocarbons”. En: Tiehm A. and Neis U. (eds). Technical University of Hamburg-Harburg Reports on Sanitary Engineering, vol. 25: Ultrasound in Environmental Engineering, p. 167-180. ISSN 0724-0783. <http://www.tu-harburg.de/aww/publications/tiehm3.pdf> [Consulted on 10/4/2005].

Tiehm A., Nickel K., Neis U. (**1997**). The use of ultrasound to accelerate the anaerobic digestion of sewage sludge. Wat. Sci. Technol., 36(11), 121-128.

Tiehm, A.; K. Nickel; M. Zellhorn; U. Neis (**2001**). “Ultrasonic waste activated sludge disintegration for improving anaerobic stabilization”. Wat. Res., 35(8), 2003-2009.

Trably E., Patureau D., Delgenes J.P. (**2003**). Enhancement of polycyclic aromatic hydrocarbons removal during anaerobic treatment of urban sludge. Wat. Sci. Technol., 48(4), 53-60.

Wang F., Wang Y., Ji M. (**2005**). Mechanisms and kinetics models for ultrasonic waste activated sludge disintegration. J. Hazar. Mat., B123, 145-150.

Weemaes M.P.J., Verstraete W.H. (**1998**). Review: Evaluation of current wet sludge disintegration techniques. J. Chem. Technol. Biotechnol., 73, 83-92.

WQI (**1996**). Thermal test. Water Quality International, July/August, pp. 24-25

Conclusions and recommendations

Chapter 9

9. Conclusions and recommendations

9.1. Conclusions and recommendations

The anaerobic digestion role in sludge stabilization has been proved at industrial scale during the last years. However, the increasing amount of sludge from wastewater treatment plants (WWTP), the general concern about the presence and the behaviour of organic micropollutants and the restrictive legislations on their management and final destination invite to enhance the actual treatment processes and/or to find a reliable alternative. The anaerobic thermophilic digestion could be a feasible alternative to anaerobic mesophilic digestion in order to improve the sludge quality, to reduce vector attraction and to minimize the pathogenic load. However, the effect of anaerobic digestion under both temperature conditions on micropollutants has been few investigated.

In the present study, the fate of organic micropollutants in sludge during anaerobic digestion has been reported, paying a special attention to temperature conditions (mesophilic and thermophilic) and hydraulic retention time (HRT) reduction effects. The main conclusions of every set of experimental results are presented:

Start-up and HRT reduction

- The use of Waste Activated Sludge (WAS) as the inoculum to anaerobic digesters start-up represents a good option, especially, under thermophilic conditions.

- The gradual substitution of synthetic substrate by real substrate (mixture of primary and secondary sewage sludge) led to a steady state with 35 and 30 days as HRT after 60 and 85 days in the mesophilic and the thermophilic digester, respectively. Since the non-availability of thermophilic inoculum and the long lag time reported in the literature for anaerobic digesters start-up, specially for thermophilic one, the use of WAS as inoculum and the gradual substitution as start-up procedure represent a good alternative to conventional thermophilic anaerobic digesters start-up based on mesophilic anaerobic inoclum acclimatation.

- The HRT in the thermophilic digester was reduced to 8 days without major alterations, with, approximately, the similar values of operational parameters registered in the mesophilic digester at 18 days as HRT. These HRTs are in the same HRTs range reported in the literature for both thermophilic (6-15 days) and mesophilic digesters (15-30 days). Also, the efficiency digesters values are at same order of large bibliographic values.

- Application of an HRT below 8 days resulted inadequate for thermophilic digester, since the VFAs accumulation was observed.

PAH and DEHP removal

- Both PAHs and DEHP were able to biodegrade up to certain degree under anaerobic conditions.

- An enhancement in Polycyclic Aromatic Hydrocarbons (PAH) removal at the thermophilic temperature range (50-65%) was observed with respect to the mesophilic temperature range (38-44%).

- The negative effect of HRT reduction was observed only for Low Molecular Weighted PAH (LMWPAH). It was recorded the decrease in the removal values from 82% at 26 d to 60% at 8d as HRT in the thermophilic digester and from 57% (26 d) to 53% (18 d) in the mesophilic digester.

- Excepted the HRT of 26 d in the mesophilic digester, the PAH content in dry matter of both mesophilic and thermophilic digester effluent are reduced to below the maximum concentration proposed in the 3rd Draft of European Directive (6 mg/kg dm) for sludge land use.

- The ability of anaerobic digestion for reduction PAH sludge content is variable depending on some operational conditions (temperature and HRT) and differs depending on the PAH nature HMW (high hydrophobic character) and LMW (more soluble). Hence, the composition of PAH in the sewage sludge can determine, at a high degree, the efficiency of anaerobic digestion and, then, the release of healthiness sludge able to be recycled in the agriculture land.

- Under thermophilic conditions, the Di(2-Ethyl-Hexyl)-Phthalate (DEHP) removal (45-47%) was increased by 25% to 50% with respect to mesophilic conditions (22-38%) at same applied HRTs (18, 22 and 26 days).

- It can differentiated two removal efficiency levels under thermophilic temperature conditions, high efficiency (45-47%) for high HRTs (18, 22 and 26 days) and low efficiency (32 %) for low HRTs (8and 12 days). However, under mesophilic conditions the removal efficiency was decreased sustainably with HRT reduction, due to the low biodegradation rate and the high long period that mesophilic anaerobic digestion needs to achieve the organic matter mineralization and reduction.

- The application of thermophilic conditions and relatively high HRT, 26-18 days, has decreased the DEHP sludge content to around the limit proposed for sludge use as conditioner (100 mg/kg dm, CEC, 2000). However, the mesophlic conditions showed their deficiency in DEHP reduction content under anaerobic digestion to satisfy the safety requirements.

- Taking in to account biogas production and composition results, it can be concluded that HRTs between 12 d and 18 days offer the optimum operational conditions for PAH and DEHP removal from sewage sludge under thermophilic conditions.

- Although some studies show the ability of some PAHs compounds mineralization, especially LMWPAH, but there are no evidences of them for the rest of PAH. Besides, DEHP metabolites are also not investigated and little information is available on their anaerobic removal mechanism. Hence, it is necessary to verify the fate and toxicity of the PAH and DEHP anaerobic biodegradation metabolites.

PCB and AOX removal

- The biodegradation or biotransformation of the compounds referred as AOX and PCB was enhanced under thermophilic conditions. The total PCB removal efficiency was in the range of 59.4-83.5 % and 33.0-58.0 % under thermophilic and mesophilic conditions, respectively.

- The HRT reduction impacted negatively on the total PCB amount removal efficiency. Additionally, a bioaccumulation of Lightly Chlorinated PCBs was detected in the mesophilic digester, due to reduction of Highly Chlorinated PCBs and no accompaniment of the Lightly Chlorinated PCBs biodegradation this reductive dechlorination mechanism.

- The PCB dry matter content of thermophilic digester effluent was decreased below the cut-off limit proposed in the 3rd Draft (6 mg/kg dm) at all applied HRTs. However, in the mesophilic digester, only 26 d as HRT allowed to release a safety sludge for agricultural use.

- The AOX removal efficiency was in the range of 40.4-50.3 % for thermophilic conditions and 30.2-43.2% for mesophilic conditions.

- High HRTs promoted an improvement of the AOX removal capacity of the anaerobic digestion. This can be explained by the fact that dehalogenation pathways, proposed as the main biodegradation mechanism for halogenated compounds under anaerobic conditions, needs a long time contact to be carried out with acceptable efficiency, which corresponds to high HRTs.

NPE and LAS removal

- NPE (sum of Nonylphenol, mono- and diethoxylate nonylphenol (NP, NP1EO and NP2EO)) removal efficiency oscillated between 9.4-34.8% and 7.7-21.9% under mesophilic and thermophilic conditions, respectively, which dependent basically NP removal efficiency. This last decreased noticeably (NP accumulation was observed, -3 and -7%) at low HRT (8 and 12 days) under thermophilic conditions.

- Under thermophilic condition, the maximum NP removal was around 20% at HRT of 20 days, however the NP1EO and NP2EO removal efficiency achieved was up to 75% at some applied HRTs (18 days).

- The effect of HRT decrease was clearly observed under thermophilic conditions for the NPE biodegradation. Their removal efficiency was reduced by a 43% when HRT decreased from 26 to 8 days.

- The HRT reduction effects on NPE removal efficiency were not clearly stated under mesophilic conditions. This is probably because mesophilic conditions do not enhance sufficiently the NPEO reduction/transformation, and, in a lesser extent to, the decrease of the more recalcitrant compound, such as NP. Hence, the temperature effect has more impact than the treatment time.

- At all applied HRTs, it was observed that the NP content of dried matter of both thermophilc and mesophilic digestion effluents do not fulfill the safety conditions proposed in the 3rd Darft (50 mg/kg dm), recording values in the range 1097-2100 mg/kg dm, 1013-1827 mg/kg dm and 773-1369 mg/kg dm in the fresh sludge and mesophilic and thermophilic anaerobic digester sludge.

- Due to NP amount accumulation (negative NP removal values) at some applied HRTs (at 26d in the mesophlic digester and at 12 and 8d in the thermophilic digester), it can be deduced that the nonylphenol ethoxylates were converted to NP during anaerobic digestion.

- In this study, low NP removal values were registered (-3.6-12.8% in the mesophlic digester and -6.8-20.6% in the thermophilic digester). However, it would be necessary to identify the NP intermediates in order to know if a real biodegradation is produced and not just a biotransformation.

- LAS removal was in the range 9-18% and 67-87% under mesophlic (HRT between 18 and 26 days) and thermophilic (HRT between 8 and 26 days) conditions, respectively.

- HRT reduction had a negative impact on LAS anaerobic biodegradation under both temperature conditions. Besides, C10_LAS and C11_LAS accumulation was recorded.

- High LAS content was observed in dry matter of fresh sludge (4477-5977 mg/kg dm) and digested sludge (4048-4922 mg/kg dm in the mesophilic) and (821-1650 mg/kg dm). Hence, the fresh and mesophilic sludge are not fulfilled the maximum limit proposed in the 3rd Draft (2600 mg/kg dm).

- Two LAS anaerobic biodegradation mechanisms are proposed, the first consists in the degradation of the straight alkyl chain, the sulphonate group and the benzene ring, and the second corresponds to desulphonation, straight alkyl chain carboxylation and, at the end, the bacterial mineralization of aromatic ring. However, the LAS biodegradation mechanism needs to be elucidated since today the proposed degradation pathways are not clear (if they can occur jointly or not and if they are the same for longer and shorter alkyl chain LAS).

Ultrasonic effects

- An optimal specific energy of 11000 kJ/kg TS (at 20 kHz as ultrasound frequency) promoted the best solubilization yield (30% and 42% for soluble COD and soluble VS) and enhanced biogas production in the subsequent anaerobic stabilization under both mesophilic (31and 16% for biogas production and specific biogas production, respectively) and thermophilic (16 and 10% for biogas production and specific biogas production, respectively) conditions.

- The high efficiency of thermophilic anaerobic digestion was slightly improved when it was combined with ultrasonic pre-treatment at 15 days as HRT. For volatile solid removal and specific biogas production, it passes from 46% and 0.82 L/g VS_r with 67% of methane content to 49% and 0.90 L/g VS_r with 69% as methane content, respectively.

- The feeding of a pretreated sludge highly favoured the biogas production in mesophilic anaerobic digestion when compared with the performance without pretreatment at 20 days as HRT . For volatile solid removal and specific biogas production, it pass from 42% and 0.76 L/g VS_r with 64% of methane content to 48% and 0.88 L/g VS_r with 64% as methane content, respectively.

- High naphthalene removal efficiencies were recorded by the combined ultrasonic-thermophilic anaerobic digestion treatment (with an average mean of 63.5%). However, the naphthalene removal enhancement by combined ultrasonic and anaerobic digestion treatment was more pronounced under mesophlic conditions. It pass from 33.6% in mesophlic anaerobic digestion without pretreatment to 53.9% in combined treatment.

- The obtained pyrene removal was nearly the same with and without ultrasonic pre-treatment for both thermophilic (56 and 53%) and mesophlic (31 and 33%) anaerobic digestion.

- The solubilization of both PAH compounds was stated with ultrasonic treatment by the reduction their content in the sonicated fresh sludge (24% for naphthalene and 11% for pyrene). Hence the high hydrophobic character of pyrene resisted to the ultrasound effect. The naphthalene solubilization are enhanced the subsequent biodegradation/transdormation under anaerobic digestion and probably it is mineralized partially as reported by several researchers.

To summarise, anaerobic treatment plays an important role in sludge stabilization. the comparative study presented in this work shows a significant enhancement of anaerobic digestion under thermophilic conditions. Moreover, the thermophilic working temperature improve some problematic organic micropollutants removal such as PAHs, DEHP, PCBs, NPEOs and AOX. However, the removal enhancement was variable and did not always satisfy the health and safety conditions proposed in EU for sludge destined to be reused in the agricultural soil. It can be deduced that, generally, the HRT between 12 and 18 days at thermophilic conditions and equal or large than 20 days at mesophlic temperature represents the optimum operational conditions for anaerobic digestion.

From this study, the main recommendations can be summarized as follows:

In the light of the obtained results (thermophilic degradation/transformation), determination of the intermediate compounds formed during DEHP anaerobic biodegradation can be considered a very interesting way to clarify biodegradation metabolites to better understand anaerobic DEHP reduction pathways. Besides, clarification of the anaerobic biodegradation PAH metabolites, including HMWPAH, can elucidate the removal PAH pathways and, hence prevent any undesirable effects.

The reductive dehalogenation is reported as the main organo halogen compound degradation mechanism. Some anaerobic degradation metabolites generated during dehalogenation pathways are mineralized easily under aerobic exposition conditions. However, other large halogenated compounds, their metabolites recalcitrance and final biodegradation are not enough investigated and need to be elucidated. Hence, to ensure the correct estimation of anaerobic sewage sludge digestion efficiency, it is very important to understand the anaerobic degradation mechanism of the main compounds (within 500 compounds) included under the AOX parameter.

For PCB, the reductive dechlorination was also reported as the dominant biodegradation mechanism, allowing to high rate dechlorination of High Chlorinated PCB respect to Low Chlorinated PCB under anaerobic conditions. Their complete removal is estimated possible, since their metabolites are suspected to be mineralized. However, today, not any study has confirmed this hypothesis.

High LAS content was observed in the sewage sludge during this study. The thermophilic anaerobic digestion seemed to be able to reduce their content below the cut-off limit, however, LAS concentration in sludge after anaerobic digestion is still higher and overcame the expected data. For this, to find their causes (massive use in consumption products, water alkalinity, aerobic process effects, etc.) is considered as primary step to investigate their fate during any treatment. Two main LAS pathways degradation are proposed, however both mechanisms do not explain the different biodegradation rates registered for LAS with long alkyl chain and LAS with short alkyl chain. Additional research should be carry out to clarify LAS anaerobic biodegradation mechanism.

The NP shows high recalcitrance character and resistance to anaerobic biodegradation/transformation. The low removal efficiency registered in this study (if it is confirmed) can promote an advanced research to identify their metabolites to understand their biodegradation mechanism and, therefore, to endorse a new strategy to enhance their removal from sludge and fulfil the safety and healthiness requirements for sludge use on land.

Anaerobic digestion is considered the main treatment technology reserved for sewage sludge stabilization. Different pretreatments mechanical (ultrasonic, etc), thermal, chemical, biological or combination of them are proposed to enhance their efficiency. The use of these pretreatments is at investigation level for major techniques. Positive results were reported on the efficiency enhancement regarding conventional yield parameters. However, little studies were developed on the effect of pretreatment combined with anaerobic digestion on organic micropollutants.

LAS high content in sludge and the recalcitrant nature of NP and DEHP are significant problems in the removal of micropollutants during anaerobic digestion of sewage sludge. Additional studies specially on the behaviour of these chemicals and the effects of other treatments (like the anaerobic digestion combined with pretreatments and/or post-treatments) must be carried out to achieve sludge-use on land. The ultrasonic pretreatment as a previous step of anaerobic digestion leads to a positive effect on biogas production and naphthalene degradation. The results obtained in this combined treatment (ultrasound + anaerobic digestion) are promising and suggest the convenience of other investigations of other combined treatments to enhance the stabilized sludge quality.

9.2. Summary Tables

The main results obtained at different conditions are presented in following tables:

Table 9.1. *Yield parameters values in the mesophilic digester*

Parameters/HRT (d)		26	22	18	12	8
VS_r	-	51.7 ± 0.2	50.7 ± 0.8	49.9 ± 1.2	-	-
COD_r	-	47.4 ± 0.9	46.9 ± 0.3	46.8 ± 0.4	-	-
SBP (mL/ g VS_f)	-	295 ± 14	328 ± 2	308 ± 10	-	-
Biogas CH_4 content (%)	-	72.6 ± 0.9	74.9 ± 1.4	73.2 ± 0.9	-	-
EU-PAH removal (%)	LMWPAH	57.07 ± 1.2	56.71 ± 2.3	52.60 ± 1.5	-	-
	HMWPAH	41.17 ± 0.8	35.25 ± 1.1	39.18 ± 0.6	-	-
	ABPAH	56.13 ± 0.9	53.96 ± 1.5	49.97 ± 0.5	-	-
	EU-PAH	43.20 ± 3.6*	36.56 ± 2.3	41.67 ± 0.7	-	-
DEHP removal (%)	-	37.8 ± 2.1*	27.7 ± 2.5*	21.7 ± 3.1*	-	-
PCB removal (%)	LCB	-4.67 ± 1.5	-16.14 ± 2.1	-39.11 ± 3.1	-	-
	HCB	69.30 ± 2.3	67.03 ± 3.5	43.27 ± 2.3	-	-
	Total PCB	58.40 ± 4.1	47.02 ± 4.6*	33.02 ± 4.1*	-	-
AOX removal (%)	-	38.2 ± 3.5	43.2 ± 7.1	30.2 ± 1.4	-	-
NPE removal (%)	NP	-3.6 ± 1.0	12.8 ± 1.6	11.2 ± 0.9	-	-
	NP1EO	12.5 ± 3.6	42.4 ± 4.3	15.2 ± 0.3	-	-
	NP2EO	71.4 ± 9.4	-35.6 ± 4.5	29.5 ± 1.5	-	-
	NPE	7.7 ± 1.3*	21.9 ± 1.9*	13.0 ± 1.8*	-	-
LAS removal (%)	C10-LAS	5.5 ± 0.3	12.9 ± 0.6	11.2 ± 0.6	-	-
	C11-LAS	-5.7 ± 0.6	9.0 ± 0.0	10.5 ± 0.0	-	-
	C12-LAS	28.4 ± 3.2	21.1 ± 2.1	4.6 ± 0.0	-	-
	C13-LAS	35.7 ± 2.1	18.1 ± 1.6	11.4 ± 0.2	-	-
	Total LAS	17.7 ± 2.2*	15.8 ± 1.2*	9.6 ± 0.5*	-	-

*: non-fulfilment the safety conditions for agriculture use (CEC, 2000).

Table 9.2. *Yield parameters values in the thermophilic digester*

Parameters/HRT (d)		26	22	18	12	8
VS_r	-	50.9 ± 0.6	51.7 ± 0.4	52.3 ± 1.2	50.9 ± 0.6	50.3 ± 1.0
COD_r	-	58.6 ± 0.5	57.0 ± 3.9	59.6 ± 0.4	56.9 ± 1.1	55.1 ± 1.0
SBP (mL/ g VS_f)	-	461 ± 15	412 ± 16	420 ± 31	337 ± 6.0	290 ± 18
Biogas CH_4 content (%)	-	71.5 ± 3.5	67.7 ± 3.7	60.7 ± 2.1	54.1 ± 1.6	54.0 ± 1.6
EU-PAH removal (%)	LMWPAH	81.72 ± 2.1	75.65 ± 3.5	72.03 ± 3.6	60.95 ± 2.9	59.82 ± 3.1
	HMWPAH	63.08 ± 1.9	53.72 ± 2.4	62.44 ± 1.3	54.02 ± 5.6	48.52 ± 2.1
	ABPAH	83.50 ± 1.8	84.69 ± 1.3	74.53 ± 3.4	71.60 ± 6.3	62.17 ± 2.8
	EU-PAH	65.46 ± 1.7	55.06 ± 2.5	64.22 ± 2.6	59.07 ± 4.6	51.01 ± 3.3
DEHP removal (%)	-	45.4 ± 3.1	46.2 ± 2.5*	46.7 ± 3.5	31.7 ± 5.1*	32.3 ± 2.3*
PCB removal (%)	LCB	18.42 ± 1.2	11.55 ± 0.5	14.11 ± 2.5	7.82 ±1.4	7.26 ± 2.4
	HCB	94.80 ± 2.1	92.52 ± 2.3	88.39 ± 3.4	82.16 ± 4.1	79.20 ± 2.3
	Total PCB	83.54 ± 1.8	73.04 ± 2.2	79.14 ± 2.9	69.67 ± 3.9	59.41 ± 2.4
AOX removal (%)	-	50.2 ± 4.1	44.0 ± 3.2	45.7 ± 2.2	49.6 ± 3.5	40.4 ± 5.2
NPE removal (%)	NP	10.8 ± 3.2	16.5 ± 3.5	20.6 ± 3.3	-3.4 ± 1.1	-6.8 ± 1.4
	NP1EO	59.6 ± 5.2	43.9 ± 4.5	73.6 ± 6.5	63.8 ± 5.1	59.2 ± 4.2
	NP2EO	65.3 ± 6.3	20.0 ± 2.1	88.4 ± 7.2	34.2 ± 3.0	78.3 ± 9.2
	NPE	29.5 ± 3.3*	26.4 ± 2.8*	34.8 ± 5.2*	9.4 ± 1.3*	16.5 ± 2.8*
LAS removal (%)	C10-LAS	57.8 ± 3.5	56.4 ± 4.7	-54.6 ± 6.2	-50.3 ± 6.2	-34.6 ± 4.1
	C11-LAS	86.1 ± 6.3	81.2 ± 5.6	87.5 ± 4.6	85.0 ± 5.4	86.1 ± 9.2
	C12-LAS	88.6 ± 4.5	87.7 ± 6.1	90.7 ± 8.1	88.6 ± 3.8	80.8 ± 5.5
	C13-LAS	96.9 ± 6.3	95.5 ± 5.5	97.8 ± 7.7	96.2 ± 4.6	86.7 ± 6.1
	Total LAS	86.3 ± 9.6	84.5 ± 7.2	72.0 ± 8.3	69.2 ± 5.7	67.3 ± 9.5

*: non-fulfilment the safety conditions for agriculture use (CEC, 2000).

Ultrasonic pretreatment experiment

Table 9.3. *Operational parameters of anaerobic digestion without ultrasonic pretreatment.*

Parameter	***Mesophilic***	***Thermophilic***
HRT (d)	20	15
VS_r (%)	41.60 ± 0.5	46.34 ± 0.7
COD_r (%)	43.89 ± 1.7	49.21 ± 1.6
Daily biogas production ($L_{BIOGAS}/(L_r$ day))	0.360 ± 0.021	0.578 ± 0.034
Methane content (% CH_4)	63.6 ± 1.9	67.6 ± 2.6
SBP (L_{BIOGAS}/g VS_r)	0.76 ± 0.035	0.821 ± 0.026
SBP (L_{BIOGAS}/g COD_r)	0.36 ± 0.008	0.388 ± 0.011

Table 9.4. *Operational parameters with combined ultrasonic and anaerobic digestion treatment.*

Parameter	***Mesophilic***	***Thermophilic***
HRT (d)	20	15
VS_r (%)	46.76 ± 1.2	49.30 ± 0.8
COD_r (%)	51.93 ± 2.1	52.53 ± 1.9
Daily biogas production ($L_{BIOGAS}/(L_r$ day))	0.470 ± 0.020	0.673 ± 0.012
Methane content (% CH_4)	63.9 ± 2.1	67.8 ± 3.5
SBP (L_{BIOGAS}/g VS_r)	0.883 ± 0.032	0.898 ± 0.28
SBP (L_{BIOGAS}/g COD_r)	0.399 ± 0.014	0.423 ± 0.016

Table 9.5. *Naphthalene and pyrene removal (%) under the different applied treatments*

	Mesophilic	***Thermophilic***	***Sonication***	***Son+Meso***	***Son+Thermo***
Naphthalene	33.61 ± 0.6	50.23 ± 0.4	24.19 ± 0.2	53.91 ± 0.3	63.54 ± 0.7
Pyrene	31.27 ± 0.9	55.99 ± 1.2	11.13 ± 01	32.58 ± 0.5	53.12 ± 0.9

Son+Meso: a combined ultrasonic and mesophilic anaerobic digestion treatment.
Son+Thermo: a combined ultrasonic and thermophilic anaerobic digestion treatment.

Table 9.6. *Improvement (%) in operational yield and naphthalene and pyrene removal when using sonicated substrate in the mesophilic and thermophilic digester*

Parameter	***Mesophilic***	***Thermophilic***
VS_r	12.40 ± 0.5	6.39 ± 0.4
COD_r	18.32 ± 0.8	6.69 ± 0.9
Biogas production volume	30.88 ± 1.1	16.44 ± 0.7
Naphthalaene removal	60.38 ± 2.3	26.52 ± 1.5
Pyrene removal	4.20 ± 0.2	-5.14 ± 0.6

Annexes

List of Tables

Annex IV for Chapter 4

Table I.1. *Physico-chemical characteristics of concentrate waste activated sludge used as seed.*
Table I.2. *Influent characteristics*
Table I.3. *Data during Synthetic (acetate and glucose) feeding in the mesophilic digester*
Table I.4. *Data during Synthetic (acetate and glucose) feeding in the thermophilic digester*
Table I.5. *Inlet and outlet sludge characteristics in the mesophilic digester*
Table I.6. *Yield parameters values in the mesophilic digester*
Table I.7. *Inlet and outlet sludge characteristics in the thermophilic digester*
Table I.8. *Yield parameters values in the thermophilic digester*
Table I.9a. *VFAs evolution in the mesophilic digester (mg/L).*
Table I.9b. *VFAs evolution in the mesophilic digester (mg HAc/L)*
Table I.9c. *VFAs evolution in the thermophilic digester (mg/L).*
Table I.9d. *VFAs evolution in the thermophilic digester (mg HAc/L)*

Annex II for Chapter 5

Table II.1. *PAHs content in the inlet and outlet sludge (mg/kg dm) at selected HRTs*
Table II.2. *PAHs characterisitics [USEPA, IARC]*
Table II.3. *Individual and total PAHs removal (%) at the tested HRTs*
Table II.4. *PAH categories removal at different conditions*
Table II.6. *DEHP content in the inlet and outlet sludge (mg/kg dm) and removal efficiencies (%) at selected HRTs*

Annex III for Chapter 6

Table III.1. *AOX content in the fresh and digested sludge and AOX removal efficiency under different*

Annex IV for Chapter 7

Table IV.1. *LAS content in Bulk sludge (mg/L) under different conditions and Recovery index (RI) in supernatant and dry matter LAS analysis.*
Table IV.2. *NP, NP1EO, NP2EO and NPE content in dried sludge (mg/kg) under different anaerobic conditions*
Table IV.3. *NP, NP1EO, NP2EO removal efficiency (%) under different anaerobic conditions*
Table IV.4. *LAS amount in liquid phase (mg/L) at different conditions*
Table IV.5. *LAS homologues distribution (%) and average carbon number of linear alkyl chains in liquid phase.*
Table IV.6. *LAS content in the dried matter of sludge at different conditions (mg/kg dm)*
Table IV.7. *LAS homologues distribution (%) and average carbon number of linear alkyl chains in solid phase.*
Table IV.8. *LAS removal from the dried matter of sludge at different conditions (%)*

List of Figures

Annex IV for Chapter 4

Figure 1. *Biogas production per organic matter unit added (COD_f) and methane content in both digesters at HRT reduction period. (□ mesophilic specific biogas production; ■ thermophilic specific biogas production; Δ Methane biogas content in the mesophilic digester; ○ methane biogas content in the thermophilic digester).*
Figure 2. *Evolution of COD removal (%) at each HRT. (□ Mesophilic digester; ■ thermophilic digester)*

Annex II for Chapter 5

Figure II.1. *PAH chromatogram at 20 mg/L each*
Figure II.2. *DEHP chromatogram at 50 mg/L*
Figure II.3. *Example of calibration curves for Acenaphthene (I and II) and Benzo (ghi) perylene (III)*

Annex III for Chapter 6

Figure III.1. *Chromatogram of the PCBs at 100 ppbs each and PCB180 spectrum*

Annex IV for Chapter 7

Figure IV.1. *GC/MS NP chromatogram at 100 mg/L*
Figure IV.2. *GC/MS NP1EO-NP2EO mixture (75:25) chromatogram at 400 mg/L*
Figure IV.3. *LAS HPLC/UV-Fluorescence chromatogram of commercial LAS (Petresul® 550) at 100 mg/L. PIC 1, 2, 3 and 4 correspond to C10, C11, C12 and C13 LAS homologues*

Annex I for Chapter 4

Annex I for Chapter 4

Table I.1. *Physico-chemical characteristics of concentrate waste activated sludge used as seed.*

Parameter	Assays number	Minimum	Maximum	Average
TS (g/L)	6	29.12	30.09	29.75 ± 0.370
VS (g/L)	6	13.8	15.2	14.6 ± 0.597
VSS (%)	6	62	69	66 ± 2.75
COD_t (mg/L)	8	15713	19235	17132 ±117
COD_{dis} (mg/L)	8	3894	5134	4900 ± 43
NH_4^+_N (mg/L)	8	185	462	227 ± 52
pH	4	6.1	6.6	6.4 ± 0.1

Table I.2. *Influent characteristics.*

Parameter		Assays number	Minimum	Maximum	Average
TS (g/L)		3	38.11	44.05	41.07± 0.73
VS (g/L)		3	27.6	34.6	30.95± 0.42
VSS (%)		3	58	81	73.00± 3.23
COD_t (g/L)		5	45.39	54.60	51.17±1.20
COD_s (g/L)		3	13.67	17.24	15.30± 0.167
NH_4^+_N (g/L)		3	0.19	0.37	0.31±0.05
pH		5	5.86	7.62	6.64 ± 0.53
Metals (µg/g)	Cr	1	------	------	125
	Pb	1	------	------	78
	Ni	1	------	------	89
	Cu	1	------	------	651
	Hg	1	------	------	1.97
	Cd	1	------	------	1.54
	Zn	1	------	------	1.99

Table I.3. *Data during Synthetic (acetate and glucose) feeding in the mesophilic digester*

Observations	Time (d)	Biogas (mL)	Accumulated biogas (mL / Lr)	CH_4 content (%)	SBP (mL biogas /g COD)
1st fed 2.025gr Ac/Lr (2.187 g COD/Lr)	→0	0	0.00	N.D.	**480.02 (13)**
	1	0	0.00	N.D.	
	2	546 (4)	156.00 (4)	6 (2)	
	3	1155 (3)	330.00 (7)	N.D.	
	4	2625 (2)	**750.00** (3)	36.3 (4)	
	5	3370.5 (3)	963.00 (6)	N.D.	
	6	3674.3 (8)	1049.80 (9)	43.5 (3)	
2nd fed 2.025gr Ac/Lr (2.187g COD/Lr)	→7	0	0.00	N.D.	**520.35 (6)**
	8	1011.5 (4)	289.00 (5)	47 (5)	
	9	2632 (3)	752.00 (4)	N.D.	
	10	3409 (4)	**974.00** (4)	82 (4)	
	11	3809.75 (5)	1088.50 (7)	N.D.	
	12	3983 (6)	1138.00 (8)	72.8 (7)	
3rd fed with glucose 2.05g /Lr (2.187 g COD/Lr)	→13	0	0.00	N.D.	**540.47 (13)**
	14	591.5 (8)	169.00 (9)	56 (2)	
	15	1295 (8)	370.00 (11)	N.D.	
	16	2170 (6)	620.00 (9)	68.9 (5)	
	17	3594.5 (6)	**1027.00** (7)	N.D	
	18	3986.5 (7)	1139.00 (8)	N.D.	
	19	4137 (13)	1182.00 (14)	57 (6)	
4th fed with glucose 2.05g /Lr (2.187 g DQO/Lr)	→20	0	0.00	N.D	**550.98 (13)**
	21	735 (11)	210.00 (12)	62 (3)	
	22	2345 (10)	670.00 (11)	N.D	
	23	3801 (11)	**1086.00** (13)	69.6 (2)	
	24	4098.5 (15)	1171.00 (17)	N.D.	
	25	4217.5 (12)	1205.00 (13)	61.2 (3)	

→ : Feeding; N.D.: not determinate. RSD values are indicated in parenthesis.

Table I.4. *Data during Synthetic (acetate and glucose) feeding in the thermophilic digester*

Observations	Time (d)	Biogas (mL)	Accumulated biogas (mL / Lr)	CH_4 content (%)	SBP (mL biogas /g COD)
1st fed with 5 g Ac/Lr (5.425 g COD/Lr)	→ 0	0	0.00	0	**537.20 (14)**
	1	38 (8)	10.86 (8)	N.D	
	2	89 (6)	25.43 (6)	0	
	3	2100 (5)	600.00 (6)	N.D	
	4	4000 (11)	1142.86 (11)	5 (2)	
	5	5250 (9)	1500.00 (9)	N.D	
	6	7500 (6)	2142.86 (6)	28 (4)	
	7	8750 (3)	**2500.00** (4)	N.D	
	8	9750 (2)	2785.71 (3)	47 (3)	
	9	10100 (5)	2885.71 (6)	N.D	
	10	10200 (7)	2914.29 (8)	58 (5)	
2nd fed 5g Ac/Lr (5.425g COD/Lr)	→ 11	0	0.00	N.D	**592.49 (11)**
	12	1125 (3)	321.43 (4)	N.D	
	13	2450 (4)	700.00 (4)	48 (6)	
	14	4050 (8)	1157.14 (8)	N.D	
	15	5255 (6)	1501.43 (7)	62 (2)	
	16	7955 (5)	2272.86 (6)	N.D	
	17	9500 (8)	2714.29 (6)	75 (1)	
	18	11000 (6)	**3142.86** (7)	N.D	
	19	11250 (9)	3214.29 (11)	79 (4)	
3rd fed glucose 4.5 g/Lr (4.8 g COD/Lr)	→ 20	0	0.00	N.D	**582.14 (19)**
	21	694 (8)	198.41 (8)	N.D	
	22	1806 (8)	515.87 (8)	21 (3)	
	23	2500 (6)	714.29 (7)	N.D	
	24	3333 (1)	952.38 (3)	55 (3)	
	25	4444 (2)	1269.84 (3)	N.D	
	26	7222 (4)	2063.49 (5)	65 (5)	
	27	8889 (8)	**2539.68** (9)	N.D	
	28	9306 (6)	2658.73 (7)	71 (7)	
	29	9444 (5)	2698.41 (6)	N.D	
	30	9500 (3)	2714.29 (6)	63 (2)	
	31	9611 (5)	2746.03 (6)	N.D	
	32	9750 (11)	2785.71 (11)	N.D	
	33	9780 (13)	2794.29 (14)	62 (5)	
4th fed glucose 4.5gr/Lr (4.8 g de COD/Lr)	→ 34	0	0.00	N.D	**594.64 (16)**
	35	890 (11)	254.29 (11)	N.D	
	36	2170 (9)	620.00 (9)	32 (8)	
	37	3420 (8)	977.14 (9)	N.D	
	38	5445 (12)	1555.71 (12)	61 (3)	
	39	8000 (13)	2285.71 (13)	N.D	
	40	9170 (14)	**2620.00** (15)	79 (5)	
	41	9720 (11)	2777.14 (14)	N.D	
	42	9805 (14)	2801.43 (15)	N.D	
	43	9990 (16)	2854.29 (16)	64 (4)	

→ : Feeding; N.D.: not determinate. RSD values are indicated in parenthesis.

Table I.5. *Inlet and outlet sludge characteristics in the mesophilic digester*

Inlet sludge characteristics				Mesophilic outlet sludge characteristics		
HRT (d)	TS (g/L)	VS (g/L)	COD (g/L)	TS (g/L)	VS (g/L)	COD (g/L)
35	40.9 ± 0.9	30.3 ± 13.9	13.9 ± 0.2	30.9 ± 0.6	13.9 ± 0.1	20.9 ± 0.8
30	39.7 ± 1.2	31.2 ± 14.3	14.3 ± 0.8	31.2 ± 0.5	14.3 ± 0.2	24.4 ± 0.7
26	42.2 ± 0.8	30.5 ± 14.7	14.7 ± 0.8	31.4 ± 0.7	14.7 ± 0.1	26.2 ± 0.0
22	39.8 ± 1.1	30.3 ± 14.9	14.9 ± 2.7	31.6 ± 0.4	14.9 ± 0.1	26.5 ± 1.6
20	40.5 ± 1.0	29.7 ± 14.9	14.9 ± 1.4	31.4 ± 0.5	14.9 ± 0.1	25.6 ± 1.1
18	38.9 ± 0.9	31.5 ± 15.8	15.8 ± 0.8	31.5 ± 0.8	15.8 ± 0.2	27.7 ± 0.5

Table I.6. *Yield parameters values in the mesophilic digester*

HRT (d)	SV_r (%)	COD_r (%)	SBP (mL/ g SV_f)	CH_4 content (%)
35	54.0 ± 1.3	59.2 ± 1.5	447 ± 31	68.4 ± 0.9
30	54.0 ± 0.9	53.1 ± 0.6	363 ± 16	71.7 ± 2.3
26	51.7 ± 0.2	47.4 ± 0.9	295 ± 14	72.6 ± 0.9
22	50.7 ± 0.8	46.9 ± 0.3	328 ± 2	74.9 ± 1.4
20	49.7 ± 0.8	46.3 ± 0.8	299 ± 7	72.5 ± 1.1
18	49.9 ± 1.2	46.8 ± 0.4	308 ± 10	73.2 ± 0.9

Table I.7. *Inlet and outlet sludge characteristics in the thermophilic digester*

Inlet sludge characteristics				Thermophilic outlet sludge characteristics		
HRT (d)	TS (g/L)	VS (g/L)	COD (g/L)	TS (g/L)	VS (g/L)	COD (g/L)
30	39.7 ± 0.9	31.2 ± 0.3	52.0 ± 0.8	31.9 ± 0.2	13.8 ± 0.1	20.7 ± 0.6
26	42.2 ± 1.2	30.5 ± 0.2	49.8 ± 0.8	32.3 ± 0.3	15.0 ± 0.3	20.6 ± 0.1
22	39.8 ± 0.8	30.3 ± 0.5	49.8 ± 2.7	33.4 ± 0.5	14.6 ± 0.2	21.3 ± 0.9
20	40.5 ± 1.1	29.7 ± 0.5	47.6 ± 1.4	33.0 ± 0.9	15.0 ± 0.4	20.5 ± 0.5
18	38.9 ± 1.0	31.5 ± 0.4	52.1 ± 0.8	34.1 ± 0.8	15.0 ± 0.2	21.1 ± 0.3
16	39.4 ± 0.9	31.6 ± 0.4	52.0 ± 0.7	34.3 ± 0.4	15.2 ± 0.1	22.1 ± 0.1
15	39.7 ± 0.7	30.5 ± 1.5	53.7 ± 0.4	33.9 ± 0.5	15.3 ± 0.1	20.5 ± 1.0
14	40.4 ± 1.3	32.0 ± 1.0	51.4 ± 0.8	34.2 ± 0.4	15.2 ± 0.1	22.8 ± 0.2
13	41.8 ± 1.0	30.6 ± 0.4	47.4 ± 1.4	34.0 ± 0.3	15.3 ± 0.1	23.2 ± 2.2
12	43.2 ± 0.7	31.5 ± 0.4	51.1 ± 1.1	34.4 ± 0.3	15.5 ± 0.1	22.0 ± 0.4
11	39.7 ± 0.5	31.8 ± 0.3	47.2 ± 1.8	34.0 ± 0.2	15.5 ± 0.1	21.8 ± 0.1
10	39.0 ± 1.8	29.5 ± 1.5	52.5 ± 1.5	34.0 ± 0.7	15.4 ± 0.1	21.9 ± 1.1
9	40.2 ± 1.4	32.5 ± 0.7	53.8 ± 0.8	33.9 ± 0.6	15.5 ± 0.1	23.3 ± 0.5
8	42.2 ± 0.8	31.3 ± 0.7	51.2 ± 1.2	33.7 ± 0.8	15.6 ± 0.1	23.0 ± 0.4
7	41.1 ± 3.1	31.8 ± 2.8	51.8 ± 2.9	32.8 ± 0.9	15.4 ± 0.2	22.9 ± 1.3
8	40.1 ± 2.1	29.8 ± 1.7	53.2 ± 0.9	32.6 ± 0.4	16.1 ± 0.3	22.6 ± 0.8

Table I.8. *Yield parameters values in the thermophilic digester*

HRT (d)	SV$_r$ (%)	COD$_r$ (%)	SBP (mL/ g SV$_f$)	CH$_4$ content (%)
30	55.8 ± 0.6	60.3 ± 1.2	502 ± 1	72.3 ± 0.6
26	50.9 ± 0.6	58.6 ± 0.5	461 ± 15	71.5 ± 3.5
22	51.7 ± 0.4	57.0 ± 3.9	412 ± 16	67.7 ± 3.7
20	49.6 ± 2.0	56.9 ± 2.3	417 ± 3	63.5 ± 2.5
18	52.3 ± 1.2	59.6 ± 0.4	420 ± 31	60.7 ± 2.1
16	51.8 ± 0.4	57.5 ± 0.4	393 ± 9	59.7 ± 0.7
15	49.9 ± 2.6	61.8 ± 1.6	389 ± 15	53.5 ± 1.5
14	52.5 ± 1.2	55.6 ± 1.1	364 ± 14	54.3 ± 3.3
13	49.9 ± 0.2	51.3 ± 3.1	368 ± 9	52.3 ± 1.8
12	50.9 ± 0.6	56.9 ± 1.1	337 ± 6	54.1 ± 1.6
11	51.2 ± 0.1	53.7 ± 1.6	304 ± 4	50.3 ± 2.3
10	47.8 ± 3.2	58.3 ± 3.4	306 ± 17	50.5 ± 6.5
9	52.3 ± 0.8	56.7 ± 0.3	300 ± 15	52.0 ± 1.0
8	50.3 ± 1.0	55.1 ± 0.8	290 ± 18	54.0 ± 1.6
7	51.1 ± 3.8	55.5 ± 5.0	153 ± 37	27.1 ± 3.9
8	45.6 ± 4.0	57.5 ± 2.0	265 ± 43	44.3 ± 7.2

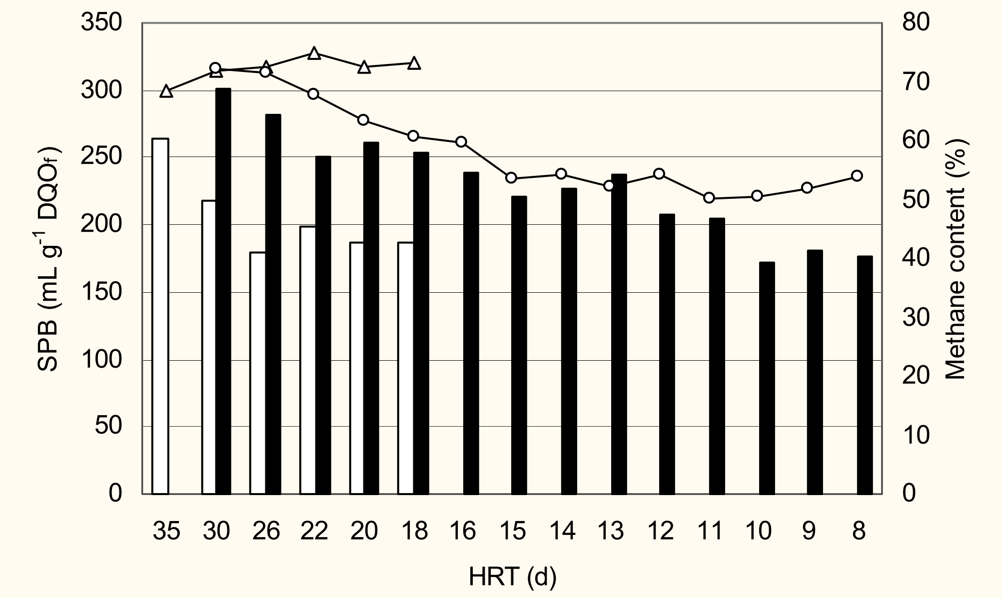


Figure I.1. *Biogas production per organic matter unit added (COD$_f$) and methane content in both digesters at HRT reduction period. (□ mesophilic specific biogas production; ■ thermophilic specific biogas production; Δ Methane biogas content in the mesophilic digester; ○ methane biogas content in the thermophilic digester).*

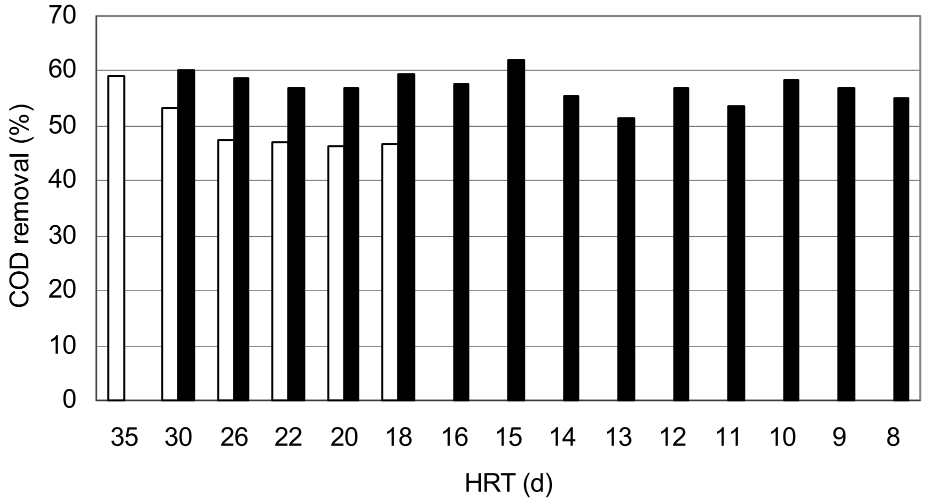


Figure I.2. *Evolution of COD removal (%) at each HRT.*
(□ mesophilic digester; ■ thermophilic digester).

Table I.9a. *VFAs evolution in the mesophilic digester (mg/L).*

HRT (d)	Ac	Pr	IBu	Bu	IVa	Va	ICa	Ca	Hep	TVFA	TVFA RI (%)
35	65.22	35.8	42.26	8.84	33.98	7.49	12.6	N.D	N.D	206.19	-
30	68.81	39.31	46.42	11.6	35.51	7.13	9.04	N.D	N.D	217.82	5.64
26	78.07	41.22	53.61	7.55	35.91	14.51	8.19	5.63	N.D	244.69	12.34
22	85.88	45.66	49.92	12.07	51.39	19.46	10.74	9.72	N.D	284.83	16.4
20	93.32	60.05	59.74	18.53	55.18	18.69	16.56	10.11	N.D	332.17	16.62
18	116.64	72.95	71.59	22.63	60.08	17.8	34.18	10.31	7.53	413.69	24.54

TVFA RI (%): relative increase in total VFAs amount respect to previous HRT VFAs amount; N.D.: not detect

Table I.9b. *VFAs evolution in the mesophilic digester (mg HAc/L).*

HRT (d)	Ac	Pr	IBu	Bu	IVa	Va	ICa	Ca	Hep	TVFA	TVFA RI (%)
35	65.22	29.03	28.82	6.03	19.99	4.41	6.52	N.D	N.D	160	
30	68.81	31.87	31.65	7.91	20.89	4.19	4.68	N.D	N.D	170	6.25
26	78.07	33.42	36.55	5.15	21.12	8.54	4.24	2.91	N.D	190	11.76
22	85.88	37.02	34.04	8.23	30.23	11.45	5.56	5.03	N.D	217.42	14.43
20	93.32	48.69	40.73	12.64	32.46	10.99	8.56	5.23	N.D	252.62	16.19
18	116.64	59.14	48.81	15.43	35.34	10.47	17.68	5.33	3.47	312.32	23.63

TVFA RI (%): relative increase in total VFAs amount respect to previous HRT VFAs amount; N.D.: not detect

***Table I.9c**. VFAs evolution in the thermophilic digester (mg/L).*

HRT (d)	Ac	Pr	IBu	Bu	IVa	Va	ICa	Ca	Hep	TVFA	TVFA RI (%)
30	144.77	96.23	37.26	27.94	33.4	7.34	10.55	3.42	3.38	364.3	
26	189.89	128.63	54.23	42.03	50.25	11.99	16.72	5.23	4.8	503.77	38.29
22	235.97	158.75	71.26	56.02	66.7	13.96	24.31	9.34	7.99	644.29	27.89
20	249.3	170.61	82.69	64.5	65.48	18.02	30	14.26	N.D	694.86	7.85
18	266.54	186.36	86.65	68.18	75.7	21.41	24.47	12.69	11.79	753.8	8.48
16	287.59	185.33	93.49	73.56	81.68	23.11	26.4	13.69	12.72	797.57	5.81
15	296.75	201.35	97.12	76.41	84.85	24	27.42	14.22	13.21	835.35	4.74
14	314.63	205.87	102.42	80.58	89.48	25.31	28.92	15	13.93	876.15	4.88
13	332.45	229.13	110.43	86.88	96.48	27.29	31.18	16.17	15.02	945.05	7.86
12	357.34	230.73	124.74	94.72	94.59	33.7	44.29	29.68	15.66	1025.45	8.51
11	364.56	237.23	135.02	102.78	97.73	34.81	45.75	30.66	16.18	1064.73	3.83
10	394.47	247.58	145.34	108.87	115.2	36.08	56.35	33.28	17.56	1154.74	8.45
9	415.68	258.71	170.15	125.47	131.31	46.57	59.66	38.05	14.79	1260.38	9.15
8	494.17	335.52	163.63	128.74	142.96	40.44	46.2	23.97	22.26	1397.88	10.91
7	707.76	457.44	175.56	46.79	21.78	7.05	12.14	4.86	N.D	1433.36	2.54
8(2)	450.76	302.4	218.54	159.27	151.13	53.6	68.66	43.79	17.02	1465.18	-2.48

TVFA RI (%): relative increase in total VFAs amount respect to previous HRT VFAs amount; N.D.: not detect

***Table I.9d.** VFAs evolution in the thermophilic digester (mg HAc/L).*

HRT (d)	Ac	Pr	IBu	Bu	IVa	Va	ICa	Ca	Hep	TVFA	TVFA RI (%)
30	144.77	78.02	25.41	19.05	19.65	4.32	5.46	1.77	1.56	300.00	
26	189.89	104.29	36.98	28.66	29.56	7.05	8.65	2.71	2.21	410.00	36.67
22	235.97	128.71	48.59	38.19	39.23	8.21	12.58	4.83	3.69	520.00	26.83
20	249.30	138.33	56.38	43.98	38.52	10.60	15.52	7.38	N.D	560.00	7.69
18	266.54	151.10	59.08	46.48	44.53	12.60	12.66	6.56	5.44	605.00	8.04
16	287.59	150.27	63.74	50.15	48.05	13.59	13.66	7.08	5.87	640.00	5.79
15	296.75	163.26	66.22	52.10	49.91	14.12	14.18	7.36	6.10	670.00	4.69
14	314.63	166.92	69.83	54.94	52.64	14.89	14.96	7.76	6.43	703.00	4.93
13	332.45	185.78	75.29	59.24	56.75	16.05	16.13	8.37	6.93	757.00	7.68
12	357.34	187.08	85.05	64.58	55.64	19.82	22.91	15.35	7.23	815.00	7.66
11	364.56	192.35	92.06	70.07	57.49	20.48	23.67	15.86	7.47	844.00	3.56
10	394.47	200.74	99.10	74.23	67.76	21.22	29.15	17.21	8.11	912.00	8.06
9	415.68	209.77	116.01	85.55	77.24	27.40	30.86	19.68	6.82	989.00	8.44
8	494.17	272.04	111.56	87.78	84.09	23.79	23.90	12.40	10.27	1120.00	13.25
7	707.76	370.90	119.70	31.90	12.81	4.14	6.28	2.51	N.D	1256.00	12.14
8(2)	450.76	245.19	149.00	108.59	88.90	31.53	35.51	22.65	7.85	1140.00	-9.24

TVFA RI (%): relative increase in total VFAs amount respect to previous HRT VFAs amount; N.D.: not detect

Annex II for Chapter 5

Annex II for Chapter 5

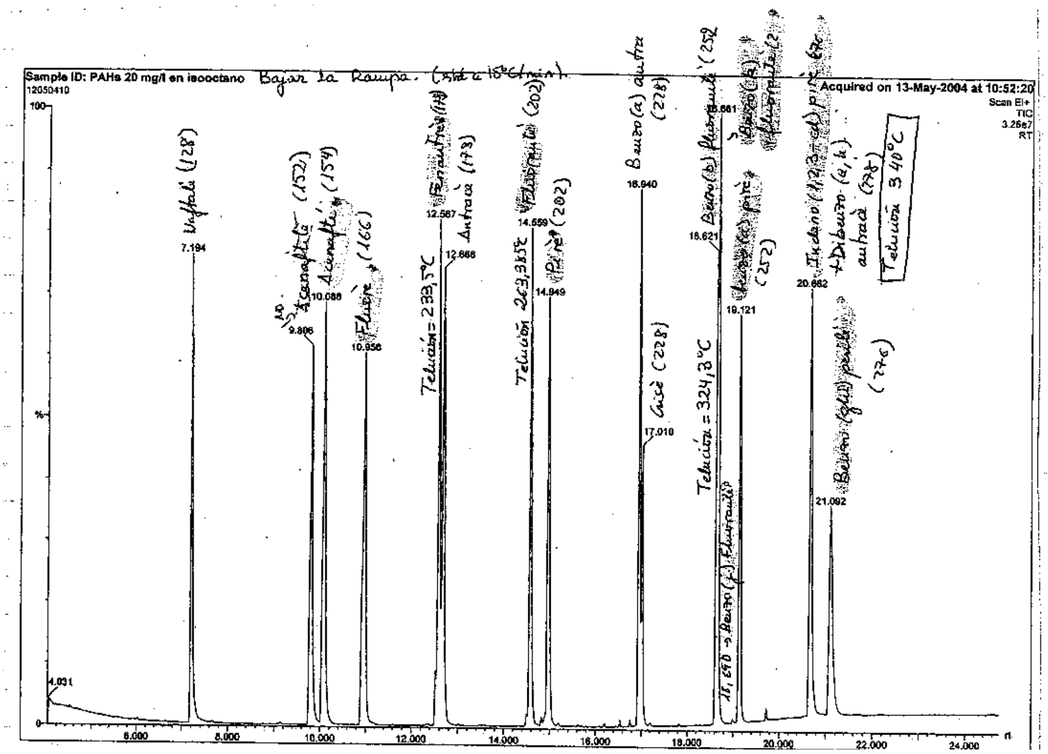


Figure II.1. PAH chromatogram at 20 mg/L each

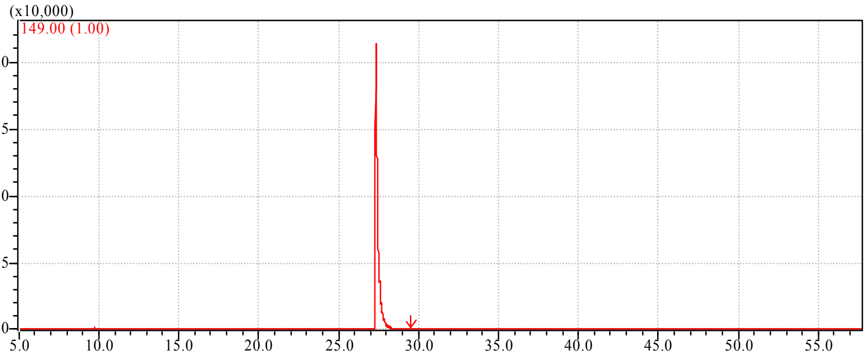


Figure II.2. DEHP chromatogram at 50 mg/L

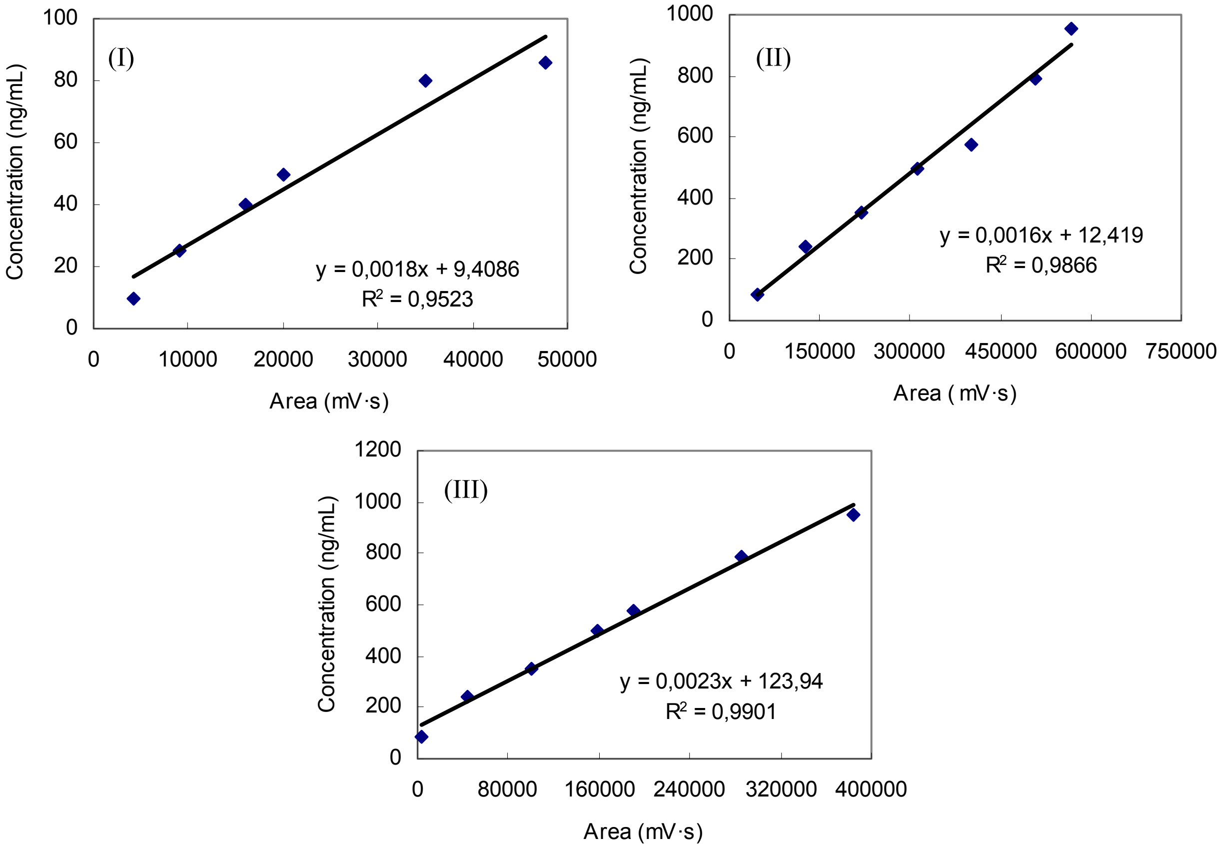


Figure II.3. *Example of calibration curves for Acenaphthene (I and II) and Benzo (ghi) perylene III)*

Table II.1. *PAHs content in the inlet and outlet sludge (mg/kg dm) at selected HRTs*

	Compound//HRT (d)	26	22	18	12	8
Feed	Naphthalene	0.37 ± 0.01	0.05 ± 0.01	0.14 ± 0.06	0.18 ± 0.05	0.03 ± 0.00
	Acenaphthylene	0.27 ± 0.0	0.06 ± 0.02	0.16 ± 0.03	0.59 ± 0.03	0.24 ± 0.01
	Acenaphthene *	0.40 ± 0.02	0.37 ± 0.02	0.49 ± 0.03	**7.78** ± 1.30	0.21 ± 0.01
	Fluorene*	0.32 ± 0.01	0.04 ± 0.02	0.19 ± 0.04	0.28 ± 0.20	0.34 ± 0.03
	Phenanthrene*	0.75 ± 0.02	0.10 ± 0.03	0.30 ± 0.06	0.37 ± 0.01	0.70 ± 0.05
	Anthracene	1.25 ± 0.08	0.17 ± 0.02	0.44 ± 0.06	0.49 ± 0.01	0.39 ± 0.04
	Fluoranthene*	1.06 ± 0.05	0.13 ± 0.01	0.54 ± 0.05	0.36 ± 0.00	0.57 ± 0.03
	Pyrene*	1.10 ± 0.06	0.09 ± 0.05	0.35 ± 0.02	0.28 ± 0.01	0.49 ± 0.09
	Benzo (a) anthracene	1.64 ± 0.07	0.17 ± 0.04	1.01 ± 0.02	0.42 ± 0.01	0.61 ± 0.05
	Crysene	1.63 ± 0.06	0.09 ± 0.04	2.31 ± 0.03	0.28 ± 0.04	0.98 ± 0.06
	Benzo (b+j+k) fluoranthene*	1.62 ± 0.02	0.30 ± 0.05	0.96 ± 0.01	0.44 ± 0.05	0.80 ± 0.03
	Benzo (a) pyrene*	1.82 ± 0.02	**6.97** ± 0.36	0.97 ± 0.05	0.64 ± 0.06	0.86 ± 0.00
	Indeno (1,2,3,cd) pyrene*	1.84 ± 0.10	0.14 ± 0.8	0.57 ± 0.05	0.46 ± 0.03	0.86 ± 0.04
	Benzo (ghi) perylene*	2.54 ± 0.03	0.34 ± 0.09	0.88 ± 0.07	0.95 ± 0.09	0.81 ± 0.06
	Dibenzo (ah) anthracene	0.00	0.42 ± 0.05	2.00 ± 0.23	0.00	0.74 ± 0.09
	EU-PAHs total	11.44 ± 0.03	8.50 ± 0.52	5.26 ± 0.41	11.57 ± 0.91	5.64 ± 0.25
	PAHs total	**16.60 ± 0.91**	**9.46 ± 0.60**	**11.32 ± 0.86**	**13.53 ± 1.21**	**8.63 ± 0.50**
Thermophilic	Naphthalene	0.12 ± 0.03	0.02 ± 0.02	0.05 ± 0.00	0.08 ± 0.01	0.01 ± 0.00
	Acenaphthylene	0.08 ± 0.02	0.02 ± 0.01	0.06 ± 0.00	0.22 ± 0.02	0.11 ± 0.00
	Acenaphthene *	0.09 ± 0.01	0.10 ± 0.00	0.15 ± 0.04	**3.11** ± 0.09	0.11 ± 0.00
	Fluorene*	0.04 ± 0.00	0.01 ± 0.00	0.05 ± 0.00	0.08 ± 0.01	0.12 ± 0.00
	Phenanthrene*	0.13 ± 0.01	0.01 ± 0.00	0.08 ± 0.00	0.11 ± 0.01	0.28 ± 0.01
	Anthracene	0.21 ± 0.05	0.03 ± 0.00	0.12 ± 0.01	0.11 ± 0.00	0.14 ± 0.01
	Fluoranthene*	0.31 ± 0.02	0.03 ± 0.00	0.17 ± 0.00	0.13 ± 0.01	0.26 ± 0.00
	Pyrene*	0.33 ± 0.03	0.03 ± 0.00	0.09 ± 0.01	0.12 ± 0.01	0.22 ± 0.01
	Benzo (a) anthracene	0.57 ± 0.07	0.07 ± 0.01	0.34 ± 0.00	0.16 ± 0.02	0.30 ± 0.01
	Crysene	0.62 ± 0.05	0.03 ± 0.00	0.95 ± 0.02	0.11 ± 0.02	0.45 ± 0.03
	Benzo (b+j+k) fluoranthene*	0.53 ± 0.04	0.14 ± 0.00	0.35 ± 0.02	0.20 ± 0.03	0.38 ± 0.02
	Benzo (a) pyrene*	0.63 ± 0.03	**3.26** ± 0.54	0.32 ± 0.02	0.24 ± 0.04	0.46 ± 0.01
	Indeno (1,2,3,cd) pyrene*	0.72 ± 0.04	0.08 ± 0.01	0.25 ± 0.05	0.21 ± 0.01	0.48 ± 0.01
	Benzo (ghi) perylene*	1.16 ± 0.00	0.15 ± 0.00	0.42 ± 0.03	0.54 ± 0.05	0.47 ± 0.00
	Dibenzo (ah) anthracene	**0.19** ± 0.05	0.22 ± 0.02	1.16 ± 0.06	**0.07** ± 0.00	0.47 ± 0.00
	EU-PAHs total	3.95 ± 0.45	3.82 ± 0.42	1.88 ± 0.09	4.73 ± 0.11	2.76 ± 0.03
	PAHs total	**5.74 ± 0.21**	**4.21 ± 0.54**	**4.55 ± 0.14**	**5.50 ± 0.20**	**4.25 ± 0.12**
Mesophilic	Naphthalene	0.17 ± 0.00	0.02 ± 0.00	0.07 ± 0.00	-	-
	Acenaphthylene	0.12 ± 0.00	0.03 ± 0.00	0.08 ± 0.00	-	-
	Acenaphthene *	0.16 ± 0.01	0.16 ± 0.01	0.22 ± 0.01	-	-
	Fluorene*	0.14 ± 0.03	0.02 ± 0.00	0.09 ± 0.01	-	-
	Phenanthrene*	0.33 ± 0.01	0.05 ± 0.00	0.16 ± 0.01	-	-
	Anthracene	0.56 ± 0.01	0.06 ± 0.00	0.19 ± 0.01	-	-
	Fluoranthene*	0.58 ± 0.01	0.07 ± 0.00	0.27 ± 0.02	-	-
	Pyrene*	0.63 ± 0.01	0.05 ± 0.00	0.20 ± 0.01	-	-
	Benzo (a) anthracene	0.93 ± 0.03	0.10 ± 0.00	0.54 ± 0.03	-	-
	Crysene	0.91 ± 0.03	0.05 ± 0.00	1.34 ± 0.11	-	-
	Benzo (b+j+k) fluoranthene*	0.98 ± 0.06	0.20 ± 0.01	0.54 ± 0.04	-	-
	Benzo (a) pyrene*	1.07 ± 0.10	**4.59** ± 0.09	0.61 ± 0.05	-	-
	Indeno (1,2,3,cd) pyrene*	1.05 ± 0.00	0.08 ± 0.00	0.35 ± 0.01	-	-
	Benzo (ghi) perylene*	1.55 ± 0.10	0.18 ± 0.00	0.63 ± 0.05	-	-
	Dibenzo (ah) anthracene	**0.12** ± 0.00	0.23 ± 0.00	1.69 ± 0.20	-	-
	EU-PAHs total	6.50 ± 0.20	5.39 ± 0.11	3.07 ± 0.41	-	-
	PAHs total	**9.30 ± 0.58**	**5.89 ± 0.23**	**6.99 ± 1.03**	-	-

*: EU-PAHs list

Table II.2. *PAHs characterisitics [USEPA, IARC]*

PAHs	Vapor Pressure (Torr at 20 ºC)	Water Solubility (mg/L)	K_{ow}
Acenaphthene	10^{-3}-10^{-2}	3.4 (a 25ºC)	21000
Acenaphthylene	10^{-3}-10^{-2}	3.93	12000
Fluorene	10^{-3}-10^{-2}	1.9	15000
Naphthalene	0,049	32	2300
Antraceno	$2\ 10^{-4}$	0.05-0.07 (a 25ºC)	2800
Fluoranthene	10^{-6}-10^{-4}	0.26 (a 25ºC)	34000
Phenanthrene	$6{,}8\ 10^{-4}$	1.0-1.3 (a 25ºC)	29000
Benzo[α] anthracene	$5\ 10^{-9}$	0.01 (a 25ºC)	$4\ 10^{5}$
Benzo[β]fluoranthene	10^{-11}-10^{-6}	-	$4\ 10^{6}$
Benzo[k]fluoranthene	$9{,}6\ 10^{-7}$	-	$7\ 10^{6}$
Crysene	10^{-11}-10^{-6}	0.002 (a 25ºC)	$4\ 10^{5}$
Pyrene	$6{,}9\ 10^{-9}$	0.14 (a 25ºC)	$2\ 10^{5}$
Benzo[ghi]perylene	$\sim 10^{-10}$	0.00026 (a 25ºC)	10^{7}
Benzo[α]pyrene	5 10	0.0038 (a 25ºC)	10^{6}
Dibenzo[α,h]anthracene	$\sim 10^{-10}$	0.005	10^{6}
Indeno[1,2,3-cd]pireno	$\sim 10^{-10}$	-	$5\ 10^{7}$

Table II.3. *Individual and total PAHs removal (%) at the tested HRTs*

	Compound//HRT (d)	26	22	18	12	8
Thermophilic	Naphthalene	66.39 ± 1.5	62.57 ± 3.2	68.39 ± 6.5	53.54 ± 4.4	57.23 ± 2.3
	Acenaphthylene	72.14 ± 2.3	69.03 ± 5.6	64.44 ± 8.2	62.95 ± 3.6	54.90 ± 4.5
	Acenaphthene *	76.93 ± 3.2	72.16 ± 4.3	69.53 ± 3.2	60.05 ± 2.5	48.23 ± 3.2
	Fluorene*	86.74 ± 2.1	79.83 ± 6.5	76.09 ± 5.3	72.32 ± 6.3	65.71 ± 2.2
	Phenanthrene*	82.13 ± 5.6	86.86 ± 7.1	73.53 ± 6.2	71.05 ± 8.1	60.45 ± 1.6
	Anthracene	83.41 ± 3.9	84.34 ± 3.2	73.41 ± 8.1	76.66 ± 3.2	63.98 ± 5.6
	Fluoranthene*	71.03 ± 2.6	73.66 ± 4.2	69.32 ± 1.2	63.23 ± 4.5	54.34 ± 3.4
	Pyrene*	69.54 ± 1.9	64.81 ± 1.0	74.56 ± 2.0	57.57 ± 6.2	55.32 ± 6.5
	Benzo (a) anthracene	65.13 ± 5.2	59.34 ± 1.1	66.23 ± 3.1	61.12 ± 4.1	50.03 ± 1.7
	Crysene	62.12 ± 4.8	63.23 ± 3.3	59.13 ± 4.1	59.85 ± 3.1	54.13 ± 3.5
	Benzo (b+j+k) fluoranthene*	67.18 ± 6.7	51.30 ± 2.5	63.22 ± 2.2	55.12 ± 2.1	52.66 ± 4.6
	Benzo (a) pyrene*	65.14 ± 5.4	53.28 ± 4.2	67.02 ± 3.3	62.23 ± 1.0	46.36 ± 6.7
	Indeno (1,2,3,cd) pyrene*	60.92 ± 7.2	48.12 ± 3.6	55.34 ± 4.5	54.33 ± 5.6	44.87 ± 5.6
	Benzo (ghi) perylene*	54.46 ± 3.7	56.77 ± 6.4	52.11 ± 6.1	43.27 ± 3.5	42.31 ± 2.3
	Dibenzo (ah) anthracene	-	46.67 ± 1.3	41.90 ± 3.5	-	36.05 ± 4.0
	PAHs total	**65.44 ± 6.2**	**55.50 ± 4.6**	**59.83 ± 6.7**	**59.37 ± 4.6**	**50.73 ± 4.2**
Mesophilic	Naphthalene	53.43 ± 3.2	58.12 ± 6.3	51.66 ± 2.1		
	Acenaphthylene	57.60 ± 2.5	51.31 ± 3.5	49.33 ± 1.3		
	Acenaphthene *	59.61 ± 6.2	57.77 ± 2.6	55.23 ± 2.1		
	Fluorene*	55.22± 3.4	62.83 ± 4.3	54.12 ± 2.0		
	Phenanthrene*	56.51 ± 2.3	49.98 ± 2.6	47.33 ± 3.5		
	Anthracene	55.01 ± 5.4	61.92 ± 1.3	56.13 ± 2.0		
	Fluoranthene*	45.32 ± 6.1	48.38 ± 4.6	49.84 ± 5.0		
	Pyrene*	42.33 ± 6.6	44.77 ± 3.2	43.23 ± 1.9		
	Benzo (a) anthracene	43.72 ± 1.4	39.32 ± 2.0	45.84 ± 4.3		
	Crysene	44.43 ± 4.2	49.02 ± 1.5	42.22 ± 6.5		
	Benzo (b+j+k) fluoranthene*	39.39 ± 3.5	32.11 ± 1.6	43.51 ± 4.3		
	Benzo (a) pyrene*	41.12 ± 6.5	34.12 ± 2.1	37.23 ± 3.3		
	Indeno (1,2,3,cd) pyrene*	42.83 ± 1.3	46.77 ± 1.3	38.12 ± 1.0		
	Benzo (ghi) perylene*	38.91 ± 3.5	48.65 ± 4.6	29.17 ± 1.0		
	Dibenzo (ah) anthracene	-	45.15 ± 5.2	15.23 ± 3.0		
	PAHs	**44.00 ± 5.6**	**37.78 ± 4.3**	**38.29 ± 3.5**		

*: EU-PAHs list; -: negative removal (accumulation).

Table II.4. *PAH categories removal at different conditions*

	Thermophilic					Mesophilic		
HRT (d)	26	22	18	12	8	26	22	18
PAHs removal (%)	65.46	55.06	64.22	59.07	51.01	43.20	36.56	41.67
LMWPAHs removal (%)	81.72	75.65	72.03	60.95	59.82	57.07	56.71	52.60
HMWPAHs removal (%)	63.08	53.72	62.44	54.02	48.52	41.17	35.25	39.18
Abiotic PAHs removal (%)	83.50	84.69	74.53	71.60	62.17	56.13	53.96	49.97

Table II.5. *PAH removal mean values (%) of a different PAH categories at the tested HRTs*

	Thermophilic					Mesophilic		
HRT (d)	26	22	18	12	8	26	22	18
PAHs removal mean	70.23	64.80	64.95	60.95	52.44	48.24	48.68	43.88
LMWPAHs removal mean	77.96	75.80	70.90	66.10	58.42	56.23	56.99	52.30
HMWPAHs removal mean	64.44	57.46	60.98	57.09	48.45	42.26	43.14	38.27
Abiotic PAHs removal mean	84.09	83.68	74.34	73.34	63.38	55.58	58.24	52.53

Table II.6. *DEHP content in the inlet and outlet sludge (mg/kg dm) and removal efficiencies (%) at selected HRTs*

	HRT (d)	26	22	18	12	8
Feed	Content	169.3 ± 3.9	195.5 ± 9.8	172.2 ± 14.1	162.7 ± 21.8	158.9 ± 18.3
	Removal efficiency	-	-	-	-	-
Thermophilic	Content	92.4 ± 6.4	105.2 ± 3.7	91.7 ± 14.8	111.1 ± 8.9	107.6 ± 3.9
	Removal efficiency	45.4 ± 3.1	46.2 ± 2.5	46.7 ± 3.5	31.7 ± 5.1	32.3 ± 2.3
Mesophilic	Content	105.3 ± 12.1	141.4 ± 19.5	134.9 ± 8.1	-	-
	Removal efficiency	37.8 ± 2.1	27.7 ± 2.5	21.7 ± 3.1	-	-

Annex III for Chapter 6

Annex III for Chapter 6

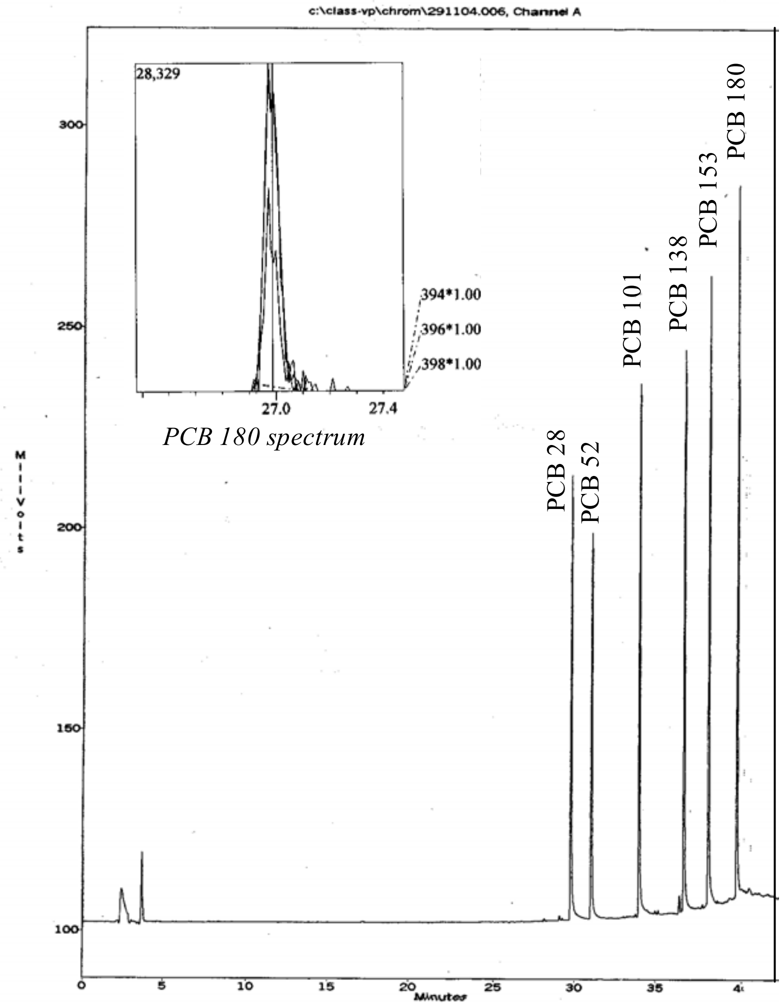


Figure III.1. *Chromatogram of the PCBs at 100 ppbs each and PCB180 spectrum*

Table III.1. *AOX content in the fresh and digested sludge and AOX removal efficiency under different conditions*

HRT (d)	26	22	18	12	8
Feed (mg/kg dw)	633.7 ± 81.2	678.8 ± 74.6	580.5 ± 95.8	721.2 ± 81.2	615.1 ± 91.6
Thermophlic outlet (mg/kg dw)	315.1 ± 51.1	380.0 ± 41.7	315.2 ± 45.2	363.7 ± 43.6	366.5 ± 52.3
Mesophilic outlet (mg/kg dw)	391.5 ± 45.6	385.4 ± 88.6	405.1 ± 45.0	-	-
Thermophilic removal (%)	50.2 ± 4.1	44.0 ± 3.2	45.7 ± 2.2	49.6 ± 3.5	40.4 ± 5.2
Mesophlic removal (%)	38.2 ± 3.5	43.2 ± 7.1	30.2 ± 1.4	-	-

Annex IV for Chapter 7

Annex IV for Chapter 7

Figure IV.1. *GC/MS NP chromatogram at 100 mg/L*

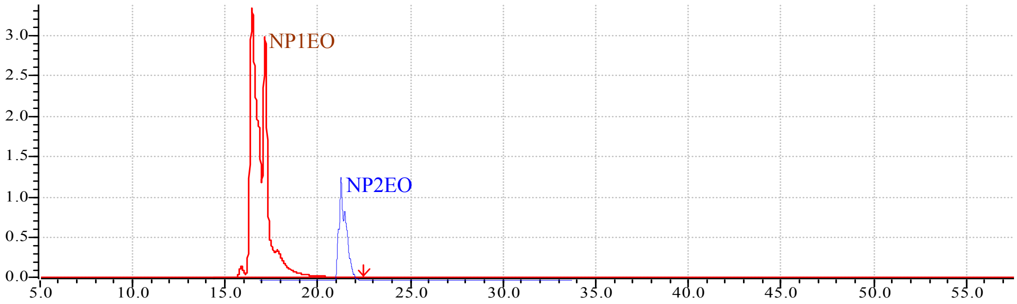


Figure IV.2. *GC/MS NP1EO-NP2EO mixture (75:25) chromatogram at 400 mg/L*

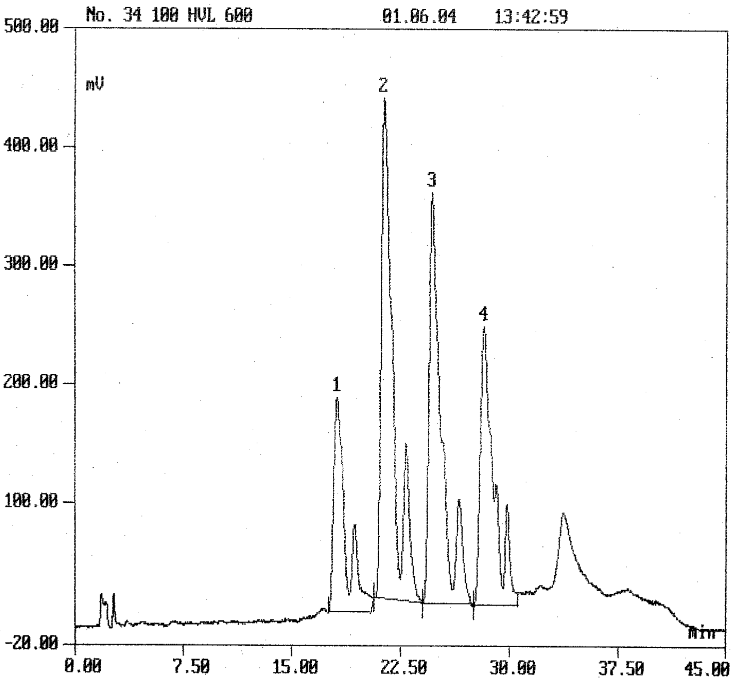


Figure IV.3. *LAS HPLC/UV-Fluorescence chromatogram of commercial LAS (Petresul® 550) at 100 mg/L. PIC 1, 2, 3 and 4 correspond to C10, C11, C12 and C13 LAS homologues*

Table IV.1. *LAS content in Bulk sludge (mg/L) under different conditions and Recovery index (RI) in supernatant and dry matter LAS analysis.*

HRT (d)	26	22	18	12	8
LAS content in fresh Bulk sludge (mg/L)	250.7 ± 87.7	229.3 ± 40.6	179.1 ± 5.4	220.8 ± 29.3	218.2 ± 35.9
LAS content in thermophilic bulk sludge (mg/L)	32.1 ± 13.6	34.0 ± 3.6	53.1 ± 16.2	60.6 ± 17.9	69.6 ± 11.3
LAS content in mesophilic bulk sludge (mg/L)	165.3 ± 8.6	163.8 ± 18.9	142.1 ± 17.9	-	-
Removal in thermophilic digestion (%)	87.2 ± 4.5	85.2 ± 3.9	70.4 ± 6.6	72.6 ± 5.3	68.1 ± 3.6
Removal in mesophilic digestion (%)	34.1 ± 2.5	28.6 ± 5.9	20.7 ± 8.3	-	-
RI in dry matter (%)	75.0 ± 4.6	73.7 ± 6.8	107.6 ± 17.8	98 6 ± 11.3	87.3 ± 14.6
RI in Supernatant (%)	80.3 ± 3.5	95.3 ± 4.3	108.6 ± 12.3	105.6 ± 5.6	95.6 ± 13.5
RI total (%)	76.9 ± 5.1	74.3 ± 7.1	108.5 ± 18.3	99.9 ± 13.1	88.1 ± 14.3

Table IV.2. *NP, NP1EO, NP2EO and NPE content in dried sludge (mg/kg) under different anaerobic conditions*

	//HRT (d)	26	22	18	12	8
Feed	NP	687 (26.5)	935 (13.5)	1567 (7.4)	1121 (15.2)	782 (5.1)
	NP1EO	312 (20.5)	542 (9.7)	421 (5.8)	246 (6.3)	321 (7.2)
	NP2EO	98 (9.7)	45 (5.2)	112 (6.1)	38 (9.8)	74 (1.3)
	NPE	1097 (23.3)	1522 (11.9)	2100 (7.0)	1405 (13.5)	1176 (5.4)
Thermophilic	NP	613 (8.7)	781 (4.6)	1245 (17.1)	1159 (7.2)	835 (13.5)
	NP1EO	126 (6.19	304 (4.4)	111 (6.1)	89 (7.6)	131 (9.5)
	NP2EO	34 (7.3)	36 (8.9)	13 (8.2)	25 (9.4)	16 (9.6)
	NPE	773 (8.3)	1121 (4.6)	1369 (16.2)	1273 (7.3)	982 (12.9)
Mesophilic	NP	712 (1.9)	815 (6.2)	1391 (1.2)	-	-
	NP1EO	273 (5.6)	312 (5.1)	357 (1.7)	-	-
	NP2EO	28 (9.5)	61 (11.2)	79 (3.2)	-	-
	NPE	1013 (3.0)	1188 (6.2)	1827 (1.4)	-	-

RSD values are indicated in parenthesis.

Table IV.3. *NP, NP1EO, NP2EO removal efficiency (%) under different anaerobic conditions*

	// HRT (d)	26	22	18	12	8
Thermophilic	NP	10.77 ± 3.2	16.47 ± 3.5	20.55 ± 3.3	**-3.39** ± 1.1	**-6.78** ± 1.4
	NP1EO	59.62 ± 5.2	43.91 ± 4.5	73.63 ± 6.5	63.82 ± 5.1	59.19 ± 4.2
	NP2EO	65.31 ± 6.3	20.00 ± 2.1	88.39 ± 7.2	34.21 ± 3.0	78.26 ± 9.2
	NPE	29.54 ± 3.3	26.35 ± 2.8	34.81 ± 5.2	9.40 ± 1.3	16.54 ± 2.8
Mesophilic	NP	**-3.64** ± 1.0	12.83 ± 1.6	11.23 ± 0.9	-	-
	NP1EO	12.50 ± 3.6	42.44 ± 4.3	15.20 ± 0.3	-	-
	NP2EO	71.43 ± 9.4	**-35.56** ± 4.5	29.46 ± 1.5	-	-
	NPE	7.66 ± 1.3	21.94 ± 1.9	13.00 ± 1.8	-	-

Table IV.4. *LAS amount in liquid phase (mg/L) at different conditions*

//HRT (d)	26	22	18	12	8
Feed	4.56 ± 0.4	2.21 ± 0.9	5.09 ± 0.3	7.42 ± 0.3	5.28 ± 0.2
Thermophilic	5.79 ± 0.8	4.53 ± 0.6	10.66 ± 0.7	8.55 ± 0.4	13.24 ± 0.6
Mesophilic	10.95 ± 0.7	12.58 ± 0.5	14.91 ± 0.5	-	-

Table IV.5. *LAS homologues distribution (%) and average carbon number of linear alkyl chains in liquid phase.*

	//HRT (d)	26	22	18	12	8
Feed	C10-LAS	70.5 ± 3.5	69.4 ± 3.1	65.6 ± 5.1	63.2 ± 2.1	71.1 ± 3.2
	C11-LAS	19.5 ± 1.2	21.3 ± 3.2	17.5 ± 3.2	16.1 ± 0.2	15.1 ± 1.1
	C12-LAS	4 ± 0.2	6.4 ± 0.2	9.2 ± 1.1	13.1 ± 0.0	9.9 ± 0.0
	C13-LAS	6 ± 0.2	2.9 ± 0.0	7.6 ± 0.2	7.5 ± 0.0	3.9 ± 0.0
	Ci-LAS	**10.46 ± 0.2**	**10.4 ± 0.3**	**10.5 ± 0.3**	**10.6 ± 0.1**	**10.5 ± 0.2**
Thermophilic	C10-LAS	10.3 ± 1.2	4.3 ± 0.4	1.2 ± 0.0	0	24.8 ± 3.2
	C11-LAS	49.0 ± 3.2	54.1 ± 5.2	47.6 ± 6.8	48.8 ± 4.1	34.7 ± 1.2
	C12-LAS	37.7 ± 2.1	41.6 ± 3.9	49.3 ± 7.2	51.2 ± 5.1	26.3 ± 1.2
	C13-LAS	2.9 ± 0.0	0	1.8 ± 0.0	0	14.1 ± 0.0
	Ci-LAS	**11.3 ± 0.1**	**11.4 ± 0.1**	**11.5 ± 0.2**	**11.5 ± 0.1**	**11.3 ± 0.3**
Mesophilic	C10-LAS	29.7 ± 2.1	25.7 ± 3.2	21.9 ± 2.1	**-**	**-**
	C11-LAS	26.7 ± 2.0	30.3 ± 2.5	36.9 ± 2.7	**-**	**-**
	C12-LAS	27.9 ± 3.0	26.9 ± 3.1	25.6 ± 3.2	**-**	**-**
	C13-LAS	15.7 ± 1.2	17.1 ± 0.5	15.5 ± 1.3	**-**	**-**
	Ci-LAS	**11.3 ± 0.1**	**11.3 ± 0.1**	**11.3 ± 0.1**	**-**	**-**

Ci-LAS: carbon number of linear alkyl

Table IV.6. *LAS content in the dried matter of sludge at different conditions (mg/kg dm)*

	//HRT (d)	26	22	18	12	8
Feed	C10-LAS	869 ± 90	799 ± 25	626 ± 10	746 ± 36	744 ± 45
	C11-LAS	1744 ± 205	1455 ± 47	1256 ± 30	1394 ± 70	1442 ± 813
	C12-LAS	1297 ± 230	1407 ± 80	1005 ± 40	1150 ± 98	1135 ± 118
	C13-LAS	2066 ± 420	2041 ± 149	1590 ± 45	1655 ± 115	1721 ± 131
	LAS	5977 ± 945	5703 ± 300	4477 ± 133	4944 ± 319	5043 ± 414
Thermophilic	C10-LAS	367 ± 31	348 ± 45	968 ± 113	1121 ± 149	1002 ± 115
	C11-LAS	242 ± 28	273 ± 19	157 ± 18	209 ± 49	201 ± 29
	C12-LAS	148 ± 35	174 ± 57	936 ± 87	131 ± 21	218 ± 15
	C13-LAS	63.± 34	91 ± 18	34 ± 9	62.3 ± 8	230 ± 34
	LAS	821 ± 128	886 ± 139	1253 ± 227	1524 ± 228	1650 ± 197
Mesophilic	C10-LAS	822 ± 56	696 ± 26	556 ± 74	-	-
	C11-LAS	1843 ± 66	1324 ± 149	1123 ± 121	-	-
	C12-LAS	929 ± 78	1110 ± 111	959 ± 96	-	-
	C13-LAS	1329 ± 124	1672 ± 135	1409 ± 115	-	-
	LAS	4922 ± 324	4803 ± 421	4048 ± 406	-	-

Ci-LAS: carbon number of linear alkyl

Table IV.7. *LAS homologues distribution (%) and average carbon number of linear alkyl chains in solid phase.*

	//HRT (d)	26	22	18	12	8
Feed	C10-LAS	14.55 ± 1.5	14.02 ± 1.0	13.99 ± 1.1	15.08 ± 1.3	14.76 ± 1.2
	C11-LAS	29.18 ± 2.1	25.52 ± 2.3	28.05 ± 3.1	28.19 ± 3.0	28.60 ± 2.3
	C12-LAS	21.71 ± 3.2	24.68 ± 3.0	22.45 ± 2.1	23.26 ± 2.1	22.51 ± 1.9
	C13-LAS	34.57 ± 3.1	35.78 ± 3.1	35.51 ± 3.2	33.47 ± 3.5	34.13 ± 2.9
	Ci-LAS	**11.76 ± 0.1**	**11.82 ± 0.1**	**11.79 ± 0.1**	**11.75 ± 0.1**	**11.76 ± 0.1**
Thermophilic	C10-LAS	44.70 ± 4.1	39.29 ± 6.3	77.27 ± 8.1	73.56 ± 7.1	60.69 ± 7.0
	C11-LAS	29.52 ± 3.5	30.84 ± 5.0	12.52 ± 1.0	13.72 ± 1.2	12.18 ± 0.6
	C12-LAS	18.03 ± 1.7	19.60 ± 2.9	7.47 ± 0.5	8.63 ± 0.8	13.21 ± 0.0
	C13-LAS	7.75 ± 0.7	10.27 ± 1.0	2.74 ± 0.1	4.09 ± 0.5	13.91 ± 1.2
	Ci-LAS	**10.89 ± 0.0**	**11.01 ± 0.2**	**10.36 ± 0.1**	**10.43 ± 0.1**	**10.80 ± 0.1**
Mesophilic	C10-LAS	16.69 ± 1.3	14.50 ± 0.1	13.73 ± 0.3	-	-
	C11-LAS	37.44 ± 0.3	27.57 ± 0.5	27.76 ± 0.1	-	-
	C12-LAS	18.88 ± 1.8	23.12 ± 0.2	23.70 ± 0.6	-	-
	C13-LAS	26.99 ± 0.6	34.81 ± 0.1	34.81 ± 0.8	-	-
	Ci-LAS	**11.56 ± 0.0**	**11.78 ± 0.1**	**11.80 ± 0.2**	**-**	**-**

Ci-LAS: carbon number of linear alkyl

Table IV.8. *LAS removal from the dried matter of sludge at different conditions (%)*

	//HRT (d)	26	22	18	12	8
Thermophilic	C10-LAS	57.80 ± 3.5	56.43 ± 4.7	**-54.61** ± 6.2	**-50.33** ± 6.2	**-34.58** ± 4.1
	C11-LAS	86.11 ± 6.3	81.21 ± 5.6	87.51 ± 4.6	85.00 ± 5.4	86.06 ± 9.2
	C12-LAS	88.59 ± 4.5	87.66 ± 6.1	90.68 ± 8.1	88.56 ± 3.8	80.80 ± 5.5
	C13-LAS	96.92 ± 6.3	95.54 ± 5.5	97.84 ± 7.7	96.23 ± 4.6	86.66 ± 6.1
	LAS	86.27 ± 9.6	84.46 ± 7.2	72.01 ± 8.3	69.18 ± 5.7	67.31 ± 9.5
Mesophilic	C10-LAS	5.51 ± 0.3	12.87 ± 0.6	11.24 ± 0.6	-	-
	C11-LAS	**-5.65** ± 0.6	9.01 ± 0.0	10.54 ± 0.0	-	-
	C12-LAS	28.39 ± 3.2	21.13 ± 2.1	4.55 ± 0.0	-	-
	C13-LAS	35.69 ± 2.1	18.07 ± 1.6	11.35 ± 0.2	-	-
	LAS	17.65 ± 2.2	15.78 ± 1.2	9.58 ± 0.5	-	-