

# **BEHAVIOR OF EMERGING CONTAMINANTS IN SEWAGE SLUDGE AFTER ANAEROBIC DIGESTION**

**C. Boix<sup>a</sup>, M. Ibáñez<sup>a</sup>, D. Fabregat-Safont<sup>a</sup>, E. Morales<sup>b</sup>, L. Pastor<sup>b</sup>, J.V. Sancho<sup>a</sup>, J.E. Sánchez-Ramírez<sup>b</sup>, F. Hernández<sup>a\*</sup>**

<sup>a</sup> Research Institute for Pesticides and Water, University Jaume I, Avda. Sos Baynat, E-12071, Castellón, Spain

<sup>b</sup> Depuración de Aguas del Mediterráneo, Avda. Benjamin Franklin 21 Parque Tecnológico, Paterna, Spain

\* *Corresponding author* [felix.hernandez@uji.es](mailto:felix.hernandez@uji.es), Tel +34 964 387366, Fax +34 964 387368

## **ABSTRACT**

Nowadays, there is an increasing concern over the presence of contaminants in the aquatic environment, where they can be introduced from wastewater after their incomplete removal in the treatment plants. In this work, degradation of selected emerging pollutants in the aqueous and solid phases of sewage sludge has been investigated after anaerobic digestion using two different digesters: mesophilic and thermophilic. Initially, sludge samples were screened by ultra-high-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UHPLC-QTOF MS) for identification of emerging contaminants in the samples. In a second step, a target quantitative method based on LC coupled to tandem MS was applied for selected pollutants identified in the previous screening. The behaviour of the compounds under anaerobic conditions was studied estimating the degradation efficiency and distribution of compounds between both sludge phases. Irbesartan and benzoylecgonine seemed to be notably degraded in both phases of the sludge. Venlafaxine showed a significant concentration decrease in the aqueous phase in parallel to an increase in the solid phase. The majority of the compounds showed an increase of their concentrations in both phases after the digestion. Concentrations in the solid phase were commonly higher than in the aqueous for most contaminants, indicating that they were preferentially adsorbed onto the solid particles.

## **Keywords**

Emerging contaminants; sewage sludge; anaerobic digestion; time-of-flight mass spectrometry; triple quadrupole mass spectrometry.

## 1. INTRODUCTION

Organic contaminants are receiving an increasing attention with respect to their environmental fate and toxicological properties (Evgenidou et al., 2015; Jelic et al., 2012; Petrie et al., 2014). Traditionally, research has been focused on the priority pollutants; however, in the last decade, there has been a clear trend towards the investigation of the so-called emerging contaminants. Emerging contaminants include compounds such as pharmaceuticals, veterinary drugs or drugs of abuse, that are not currently covered by existing water-quality regulations. The wide majority of these contaminants reach the environment after its consumption and excretion as unchanged compound or metabolites, and subsequent incomplete elimination in wastewater treatment plants (WWTPs) (Malmberg and Magnér, 2015). The low concentrations usually found for pharmaceuticals (typically at low ng/L levels) in treated waters are not expected to cause immediate adverse effects on human and on aquatic environment, but however not reliable data are currently available about long-term risk derived from their continuous input in the natural environment (Gracia-Lor et al., 2012; Petrovic et al., 2005). Until now, most of research has been focused on reporting concentration data for these contaminants in influent and effluent wastewater, but less attention has been paid to the sewage sludge (Gago-Ferrero et al., 2015).

Sewage sludge, also called biosolid, is the organic material resulting from the wastewater treatment plant. It is often reused in agricultural applications, principally as a fertilizer (Herrero et al., 2014; Li, 2014; Zhou et al., 2014). Although there is not current legislation on the use of biosolids on agricultural land with respect to the concentration of emerging contaminants (in part due to the scarce data reported on such analysis (Petrie et al., 2014)), it is important to perform effective removal to decrease, as much as possible, the potential risk to the environment and agriculture. In the biological treatment, bacteria degrade, metabolize or even mineralize, the dissolved and emulsified solid pollutants contained in municipal

and/or industrial wastewater (De Sena et al., 2010). Anaerobic digestion (AD) has been extensively used and proven to be an efficient biological treatment to stabilize organic substrates in sewage (Zhou et al., 2014). The bacteria commonly present in anaerobic digestion can operate in different conditions of temperature: mesophilic, with working temperature ranges from 12 to 45 °C, the most efficient being  $37\pm 2$  °C; and thermophilic, which working conditions range from 37 to 65 °C, with an ideal temperature of  $55\pm 2$  °C (Malmborg and Magnér, 2015; Zhou et al., 2014). Thermophilic digestion uses to be more efficient than mesophilic, but requires more strict temperature control (Zhou et al., 2014). The investigation of the fate and behaviour of organic pollutants in WWTPs requires a previous knowledge on the presence and potential transformation of the compounds in both, liquid and solid phases, to identify whether the removal from the liquid phase is due to biological processes or sorption in the solid sludge phase (Topuz et al., 2014).

A few papers have been recently published dealing with the analysis of emerging compounds in both liquid and solid phases, although less information exists on the solid material due to the difficulty associated to the analytical measurements in these highly-complex matrices (Topuz et al., 2014). The most applied extraction technique for the aqueous phase is solid phase extraction (SPE) using different cartridges and conditions (Bijlsma et al., 2014; Diaz et al., 2013; Paíga et al., 2015). Regarding the solid phase of the sludge, the most frequent extraction procedures are based on Pressurized Liquid Extraction (PLE) (Benítez-Villalba et al., 2013; De Sena et al., 2010; Dorival-García et al., 2013; Masiá et al., 2015; Mastroianni et al., 2013; Ternes et al., 2005), the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) (Herrero et al., 2014; Masiá et al., 2015; Peysson and Vulliet, 2013) and UltraSonic Extraction (USE) (Benítez-Villalba et al., 2013; Dorival-García et al., 2013; Gago-Ferrero et al., 2015; Ternes et al., 2005; Topuz et al., 2014; Zhou et al., 2012).

The knowledge on the behaviour and distribution of emerging contaminants in sewage sludge can be only obtained from reliable concentration data in these matrices. Due to the complexity of the samples and the low concentration levels commonly present, it is necessary to apply advanced techniques to ensure quality data. Currently, the determination of pharmaceuticals, pesticides and illicit drugs in waters and sludge is mostly based on liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS), a robust, sensitive and well-established technique for quantification of these pollutants in different matrices (Bijlsma et al., 2009; Gago-Ferrero et al., 2015; Masiá et al., 2015; Mastroianni et al., 2013; Thomas et al., 2012). Moreover, High Resolution Mass Spectrometry (HRMS) (Bijlsma et al., 2013; Boix et al., 2014; Peysson and Vulliet, 2013; Wick et al., 2011) is an advanced analytical tool for both screening and identification/elucidation, thanks to the accurate-mass full-spectrum acquisition data provided by these analyzers, both in MS and MS/MS modes.

The goal of this work was to evaluate the behavior and potential elimination of selected emerging contaminants after anaerobic digestion in a sewage sludge treatment system. For this purpose, anaerobic bacteria were used under two different conditions of temperature mesophilic and thermophilic. As sewage sludge contains both liquid and solid phases, the concentration levels and potential elimination of contaminants were evaluated in both matrices. In the first part of the study, a wide-scope screening using ultra-high-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UHPLC-QTOF MS) was carried out in the sludge, in order to identify the emerging contaminants present in the sample. In a second step, a quantitative method based on HPLC-MS/MS with triple quadrupole was used for both sludge phases, in order to estimate the removal of 13 selected compounds. In addition, the analytic distribution in aqueous and solid phase of the sewage sludge was evaluated.

## 2. METHODS

### 2.1. Reagents and chemicals

Reference compounds of 4-aminoantipyrine (4-AA), 4-acetyl aminoantipyrine (4-AAA), 4-formyl aminoantipyrine (4-FAA), thiabendazole, venlafaxine, carbamazepine, irbesartan, valsartan, diclofenac, salicylic acid, acesulfame and fenofibric acid were purchased from Sigma-Aldrich (St Louis, MO, USA), LGC Promochem (London, UK), Toronto Research Chemicals (Ontario, Canada), Across Organics (Geel, Belgium), Bayer Hispania (Barcelona, Spain), Fort Dodge Veterinaria (Gerona, Spain), Vetoquinol Industrial (Madrid, Spain) and Aventis Pharma (Madrid, Spain). The illicit drug studied was the main metabolite of cocaine, benzoylecgonine (BE), which was obtained from Cerilliant (Round Rock, TX, USA).

Isotopically-Labeled Internal Standards (ILIS) of diclofenac-d<sub>4</sub>, valsartan-d<sub>8</sub>, thiabendazole-d<sub>6</sub>, venlafaxine-d<sub>6</sub>, irbesartan-d<sub>6</sub> and salicylic acid-d<sub>4</sub> were obtained from CDN Isotopes (Quebec, Canada). Benzoylecgonine-d<sub>3</sub> was purchased from Cerilliant as solution in methanol at a concentration of 100 mg L<sup>-1</sup>.

HPLC-grade methanol (MeOH), formic acid eluent additive for LC-MS (HCOOH, content >98%), sodium hydroxide (NaOH, >99%) and ammonium acetate (NH<sub>4</sub>Ac, reagent grade) were purchased from Scharlab (Barcelona, Spain). Leucine enkephalin was purchased from Sigma Aldrich. HPLC-grade water was obtained from deionized water passed through a Milli-Q Gradient A10 (18.2 MΩ cm) water purification system (Millipore, Bedford, MA, USA).

Oasis HLB (200 mg) cartridges, used for solid-phase extraction, were purchased from Waters (Milford, MA, USA).

## **2.2. Instrumentation**

### **2.2.1. UHPLC-QTOF MS**

An Acquity ultra-performance liquid chromatography (UPLC) system (Waters, Mildford, MA, USA) was interfaced to a QTOF mass spectrometer (QTOF Xevo G2, Waters Micromass, Manchester, UK) using an orthogonal Z-spray electrospray interface. The LC separation was performed using an Acquity UPLC BEH C18 analytical column (100 x 2.1 mm, 1.7  $\mu\text{m}$  particle size, Waters), at a flow rate of 300  $\mu\text{L min}^{-1}$ . The mobile phases used were H<sub>2</sub>O (A) and MeOH (B), both with 0.01% (v/v) HCOOH. The percentage of MeOH was linearly increased as follows: 0 min, 10%; 14 min, 90%; 16 min, 90%; and 16.01 min, 10%. The total run time was 18 min. The injection volume was 20  $\mu\text{L}$ .

For MS<sup>E</sup> experiments, two acquisition functions with different collision energies were created: the low-energy (LE) function with a collision energy of 4 eV, and the high-energy (HE) function with a collision energy ramp ranging from 15 to 40 eV, where fragmentation is promoted (Boix et al., 2014). The MS<sup>E</sup> data acquisition records accurate-mass data for the (de)protonated molecules and fragment ions while simultaneously obtaining semi-quantitative profiles from every detectable component in the sample (For further details see SI).

### **2.2.2. HPLC-MS/MS**

HPLC analysis were carried out with a Waters Alliance HT 2795 high performance liquid chromatography (HPLC) system (Waters, Mildford, MA, USA), equipped with a quaternary solvent manager and a sample manager. Chromatographic separation was performed using a Symmetry C18 analytical column (50 x 2.1 mm 3.5  $\mu\text{m}$  particle size, Waters). The mobile phases used were H<sub>2</sub>O (A) and MeOH (B), both with 0.01% HCOOH and 0.1 mM NH<sub>4</sub>Ac. The percentage of MeOH was changed linearly as follows: 0 min, 10%; 6 min, 90%; 7 min,

90%; 7.1 min, 10%. The total run time was 12 min. The flow rate was 300  $\mu\text{L min}^{-1}$ . The sample injection volume was 20  $\mu\text{L}$ .

The HPLC system was interfaced to a triple quadrupole mass spectrometer Quattro Micro API MS (Waters) equipped with an orthogonal Z-spray electrospray ionization interface (ESI) operated in positive and negative ion mode (For further details, see (Boix et al., 2016)).

### **2.3. Pilot plant description and samples collection**

An anaerobic digestion pilot plant located at Alzira WWTP (Valencia, Spain) was operated for 13 months. The process of the WWTP consisted of a pre-treatment, primary decanting, biological activated sludge process with removal of organic matter and nitrogen, and as a sludge line, an anaerobic digestion of mixed sludge. The facility had a use of biogas through a cogeneration engine.

The anaerobic digestion pilot plant consisted of two digesters with a working volume of 1.6  $\text{m}^3$  and a 1000 L fed tank (**Figure 1**), operated in two different conditions: mesophilic and thermophilic. On-line sensors (pH, organic redox potential (ORP) and temperature) and electric equipment were installed in order to automate and control the plant operation and gather data about the process. Three tubular Heater of 1 kW were installed into both reactors as heating system. A controller SC-1000 (Hach Lange) was used for the data acquisition.

The pilot plant was started up with a considerable biomass inoculum, which was taken from the anaerobic digester of Alzira WWTP (Valencia) in mesophilic conditions. It was operated at two different Sludge Residence Times (SRTs): 20 and 15 days, and under two different temperature conditions: mesophilic ( $35\pm 2$  °C) and thermophilic ( $54\pm 2$  °C). The pilot plant was fed with mixed sludge (primary and secondary sludge) from the same WWTP.



### **2.3.1. Sludge samples**

The samples were collected from October 2014 to January 2015, at both SRTs (15 and 20 days), checking that both digesters were in steady state, in this sense after approximately three different SRTs, when the variables of the system do not change over time. Influent samples were collected as 24-h composite of the feeding tank, and effluent, as grab samples of each digester. All the samples were separated in aqueous and solid phase by centrifugation. Four experiments were carried out, and in all cases the concentration of the contaminants in the sewage sludge tank (feeding tank) was compared with the digested sludge of mesophilic and thermophilic tanks. Two of them corresponded to SRT of 20 days (experiments T20.1 and T20.2), while the other two corresponded to 15 days (T15.1 and T15.2). For each experiment, 5 composite samples were prepared by sampling daily from the feeding tank along 3-4 days (influent samples). The grab samples of the digester were collected every 5 days until completing 20 or 15 days of the experiment. The contaminant concentrations were studied in aqueous and solid phase of the sludge, in order to estimate the pollutant distribution in the two phases. Samples were frozen immediately after their sampling.

### **2.3.2. Pilot plant analysis**

Influent and effluent reactor samples were analyzed three times a week in order to evaluate the performance of the biological process. The parameters analyzed were: total solids (TS), volatile solids (VS), volatile fatty acids (VFA), alkalinity (ALK), ammonium ( $\text{NH}_4\text{-N}$ ), total chemical oxygen demand (COD) and soluble chemical oxygen demand (sCOD) (**Table S1**). The parameters TS, VS, sCOD and  $\text{NH}_4\text{-N}$  were determined according to Standard Methods (APHA, 2005). And ALK and VFA concentrations were determined by titration according to the method proposed by Moosbrugger *et al.*, (1992). The biogas composition was analyzed by

gas chromatography. Volatile Solids Removal (VSR) was calculated as the fraction of volatile solids removed by balance of solids volatile in the system.

#### **2.4. Sample treatment**

Before analysis, sludge samples were thawed at room temperature, and the aqueous and solid phases separated by decantation. Solid phase was lyophilized under vacuum in order to eliminate the residual water and preserve its integrity.

The sample treatments for solid and liquid phases of the sludge were based on the procedure described in (Boix et al., 2016). Briefly, SPE using 200 mg Oasis HLB cartridges was applied for water samples, whereas Ultrasound-Assisted Extraction (USE) was employed for solid samples.

#### **2.5. Screening by UHPLC-QTOF MS**

An initial screening was performed by UHPLC-QTOF MS to identify emerging pollutants present in the aqueous and solid phases of the sewage sludge. An in-house database of more than 600 compounds containing a large number of pharmaceuticals, illicit drugs and pesticides was used (Boix et al., 2014; Díaz et al., 2011). This database includes compound name and elemental composition of the parent compounds. Moreover, information on fragment ions and retention time (RT) was included, when available.

The identification criteria was the presence of, at least, two  $m/z$  ions ((de)protonated molecular and at least one fragment ion) at the expected retention time ( $\pm 0.2$  min RT deviation tolerance) measured at their accurate mass (mass error lower than 5 ppm) (Aguilera-Luiz et al., 2013). Commonly, the most abundant ion was the (de)protonated molecule, which was observed in the LE function, while the fragment ions were more noticeable in the HE function where the fragmentation was promoted by using higher collision energy. The

identification process was automatically performed using ChromaLynx application manager (Micromass v4.1) (Boix et al., 2013).

## **2.6. Quantitative analysis by HPLC-MS/MS.**

The quantitative method applied for the compounds selected (4-AA, 4-AAA, 4-FAA, BE, thiabendazole, venlafaxine, carbamazepine, irbesartan, valsartan, diclofenac, salicylic acid, acesulfame and fenofibric acid) was based on LC-MS/MS with triple quadrupole. It was previously developed, optimized and validated at 0.2, 2 and 20  $\mu\text{g L}^{-1}$  for the aqueous phase; and at 50, 500 and 2000  $\mu\text{g kg}^{-1}$  for the solid phase (Boix et al., 2016). Satisfactory recoveries (70-120%) and precision ( $< 20\%$ ) were obtained for the majority of the contaminants. The LOQs obtained are shown in **Table S2**.

### 3. RESULTS AND DISCUSSION

#### 3.1. Pilot plant operation

Steady-state conditions in this study lasted for 100 days approximately, and consisted of two different SRT conditions (20 and 15 days). The Organic Loading Rate (OLR) was maintained constant during each operational period. During the operation of the reactors were fed with the mixed sludge produced in the WWTP.

The start-up of mesophilic and thermophilic reactors was made using an inoculum from another mesophilic anaerobic reactor (Alzira WWTP). In this case, the conversion strategy from mesophilic to thermophilic temperatures used was a rapid and unique change from one to the other temperature (De la Rubia et al., 2013; Palatsi et al., 2009).

The digester was fed with sludge with an OLR of  $1.1 \text{ kg m}^{-3} \text{ d}^{-1}$  of VS that was progressively increased up to  $1.4 \text{ kg m}^{-3} \text{ d}^{-1}$  within the following 40 days, with the aim of controlling the production of VFA (**Table S1**). It was observed that VFA concentration increased during the first days of operation. An initial accumulation of VFA, typical of hydrolysis and fermentation of easily degradable substrates, is common during the start-up process due to the low consumption of the methanogenic organisms. The accumulation of VFA after start-up strategies in thermophilic digester can be explained by an uncoupling among the three main microbial groups (acidogens, acetogens and methanogens).

During the experiments at SRT of 20 and 15 days, samples of the influent and effluent were collected and analyzed for emerging contaminants. The results are presented and discussed below. **Figure 2** shows the evolution of the VFA concentration in the digester during the start-up and for each SRT studied. In this study, steady-state conditions were achieved after it had been passed three times the sludge retention time (SRT). The VFA / alkalinity ratio were monitored for both anaerobic systems to compare buffering capacities regarding pH

modifications. It has been reported that buffering capacity was sufficient when the VFA/alkalinity ratio was maintained around 0.3.

**Table S1** shows the average parameters measures from mesophilic and thermophilic reactors during the SRT studied in this work (20 and 15 days).

### **3.2. UHPLC-QTOF MS screening**

Firstly, a wide-scope screening of large number of emerging contaminants was applied in order to know the most relevant/abundant compounds present in the influent samples. 13 compounds were detected in the aqueous phase: 1 pesticide (thiabendazole), 1 sweetener (acesulfame), 5 pharmaceuticals (venlafaxine, carbamazepine, irbesartan, valsartan, diclofenac) and 6 metabolites (salicylic acid -metabolite of acetylsalicylic acid (Farré et al., 2007; Pérez and Barceló, 2007); fenofibric acid -metabolite of fenofibrate (Pérez and Barceló, 2007); 4-aminoantipyrine (4-AA), 4-acetyl aminoantipyrine (4-AAA) and 4-formyl aminoantipyrine (4-FAA) -metabolites of dipyron (Ergün et al., 2004; Ibáñez et al., 2012; Pérez and Barceló, 2007); and benzoylecgonine -the main metabolite of cocaine). Regarding the solid phase, 10 out of the 13 contaminants detected in the aqueous phase, were also found (**Table S3**). As an example, **Figure 3** illustrates the detection and confirmation of valsartan by LC-QTOF MS in the aqueous phase of the sludge. All these compounds were afterwards selected to study the degradation efficiency under AD.

### **3.3. Analysis of sewage sludge samples and behaviour of compounds after anaerobic digestion**

A total of 50 sewage sludge samples (50 aqueous phase and 50 solid phase samples), corresponding to the experiments T20.1, T20.2, T15.1 and T15.2 obtained in both digesters operated in mesophilic and thermophilic conditions, were analyzed by LC-MS/MS, in order to

establish the degradation efficiency of the 13 emerging pollutants selected. **Figure S1** shows the general behaviour of all the compounds studied during the anaerobic digestion.

The concentration of emerging contaminants in the environment is commonly at ng/L levels (Gracia-Lor et al., 2012; Petrovic et al., 2005). However, the compounds studied in this work were mostly at µg/L levels (**Figures 4, S2 and S3**). As an example, **Figure 4** illustrates the behaviour of BE during the whole anaerobic process in both sludge phases. The concentrations found for this compound in the feeding and digested sludge, using thermophilic and mesophilic bacteria, are shown. As it can be seen, the concentration of BE in the digested sludge was lower than in the feeding sludge, in both phases, which suggested a partial removal of this compound during this process.

In both digesters in mesophilic and thermophilic conditions, two global tendencies were found for the studied compounds. By one side, benzoylecgonine and irbesartan (**Figure 5a**) presented degradation during digestion in both aqueous and solid sludge phases. For these compounds concentration levels in the digesters were lower than in the feeding tank. Nevertheless, elimination was not complete as they were still present in effluent sludge. Similarly, the antidepressant venlafaxine (**Figure 5b**) showed a significant concentration decrease in the aqueous phase, but however concentrations increased in the solid phase. Although some degradation might have occurred, the increase in the solid phase might be explained by the adsorption of the compound in the solid residue during sludge treatment.

By the other side, most of the compounds increased their concentrations after digestion treatment. In the case of dipyrone metabolites (4-AA, 4-FAA and 4-AAA), this increase might be justified because some precursors of these metabolites, such as 4-methyl aminoantipyrine (4-MAA) or dipyrone itself, could be degraded to render the metabolites. Salicylic acid showed a huge increment in concentration during the degradation process, as shown in **Figure 5c**. It might be due to degradation of acetylsalicylic acid, precursor of

salicylic acid. The remaining analytes also showed an increase of their concentrations, possibly due to a de-conjugation of these compounds with urea, sulphate or glucuronides; or a degradation of other precursors that might be present in the samples. This fact that has been reported as possible explanation of the concentration increase for some emerging contaminants after treatment in WWTPs (Gracia-Lor et al., 2012; Gros et al., 2010; Lacey et al., 2008; Vieno et al., 2007; Zorita et al., 2009). Although, the quantitative LC-MS/MS method could not be validated for acesulfame and fenofibric acid because of the lack of an appropriate ILIS, they were monitored to estimate the global tendency of their peak areas. Acesulfame areas did not show significant variation between feeding and digested sludge, suggesting little or no degradation of this sweetener during digestion. On the other hand, fenofibric acid showed an increment of its signal in the digested sludge, which might imply the presence of a precursor in the feeding tank which was degraded to fenofibric acid during digestion. This precursor might be the pharmaceutical fenofibrate, used to reduce cholesterol levels.

The percentage of each pollutant in the aqueous and solid phases (**Figure 6**) was determined taking into account a proportion of around 1.6% of solid in the sewage sludge (therefore, water content around 98.4%). It was estimated as 2000 mL of homogenised sewage sludge sample resulted in 31.5 g of lyophilized solid residue. As **Figure 6** shows, BE, valsartan, salicylic acid, diclofenac and dipyrone metabolites showed similar compound distribution in the aqueous and solid phase. Regarding venlafaxine, irbesartan, thiabendazole, and carbamazepine, these compounds presented higher concentration in the solid (around two orders of magnitude) than in the aqueous phase. This difference could mean that these organic pollutants are preferentially adsorbed on the solid material, composed mainly by organic matter. Regarding acesulfame and fenofibric acid, they seemed to be mainly adsorbed on the solid and aqueous phase, respectively.

In relation to the hydraulic retention times, in general, no significant differences were found between the two times tested. Regarding thermophilic and mesophilic digestions, a degradation tendency could not be established for the majority of the compounds studied. Only in the case of irbesartan, bacteria presents in the digester thermophilic seemed to degrade more efficiently than in mesophilic conditions (**Figure 5a**). In thermophilic conditions also seemed to release more salicylic acid than the mesophilic digester (**Figure 5c**).



#### **4. CONCLUSIONS**

In this work, we have investigated the removal efficiency of 13 organic pollutants in the sewage sludge after two different anaerobic treatments using mesophilic and thermophilic conditions. A previous screening of the sewage sludge by UHPLC-QTOF MS allowed to identify relevant contaminants present in the samples. These compounds were subsequently selected as target analytes and analyzed by a quantitative method based on HPLC-MS/MS QqQ. Data from this study showed that most of the compounds increased their concentrations during sludge anaerobic digestion process, while only benzoylecgonine and irbesartan were significantly degraded after digestion. Moreover, no significant differences were found between the results obtain in thermophilic and mesophilic conditions. Regarding the analyte distribution, the concentration levels in the solid residue of the sludge were, in general, higher than in the aqueous phase. Consequently, future monitoring programmes of sewage sludge should be focused, preferentially, on the solid phase.

#### **ACKNOWLEDGEMENTS**

The authors are very grateful to the Ecophysiology and Biotechnology group (University Jaume I) and to The Institute of Aquaculture "Torre de la Sal" (IATS) (Consejo Superior de Investigaciones Científicas, CSIC) for using their lyophilizer systems. The financial support of Generalitat Valenciana (Prometeo II/2014/023, ISIC/2014/016) is also acknowledged.

## **Figure caption**

**Figure 1.** Anaerobic digestion pilot plant (A). General view and flow diagram (B).

**Figure 2.** Volatile fatty acids concentration (VFA) during start-up of mesophilic and thermophilic reactors.

**Figure 3.** Valsartan detection and confirmation by UHPLC-QTOF MS in the standard at 50  $\mu\text{g/L}$  (a) and aqueous sludge phase (b). Low energy (LE) and high energy (HE) spectra showed the protonated molecule and 4 fragment ions. Possible structures are proposed for each fragment ion detected.

**Figure 4.** Behavior of BE during the whole anaerobic treatments in the solid and aqueous phases.

**Figure 5.** Average concentrations in the feeding and digested tanks, in both the aqueous and solid sludge phases, for (a) irbesartan, (b) venlafaxine and (c) salicylic acid.

**Figure 6.** Mass distribution (in %) for the 13 compounds in the liquid and solid phases of the sludge before and after mesophilic and thermophilic anaerobic digestions.

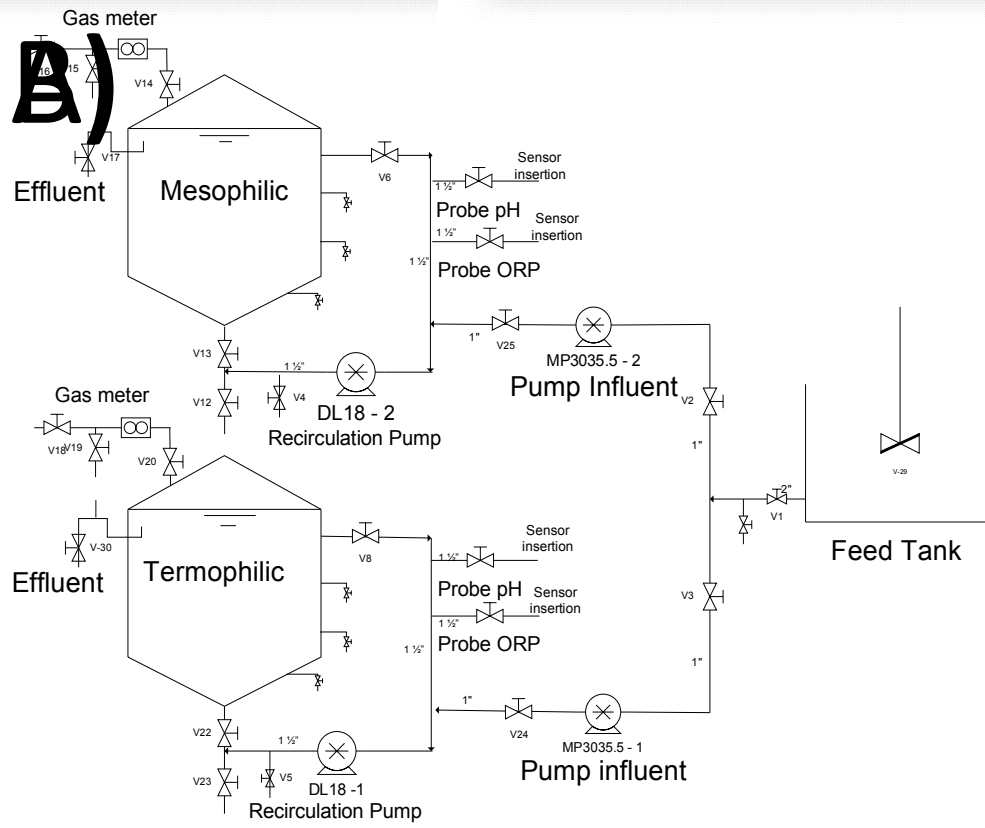
## References

- Aguilera-Luiz, M.M., Romero-González, R., Plaza-Bolaños, P., Martínez Vidal, J.L., Garrido French, A., 2013. Wide-scope analysis of veterinary drug and pesticide residues in animal feed by liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry. *Analytical and Bioanalytical Chemistry* 1–11. doi:10.1007/s00216-013-7060-5
- APHA 2005, Standard Methods for the examination of water and wastewater. Prepared and published jointly by American Public Health Association, American Water Works Association and Water Environment Federation.
- Benítez-Villalba, J.C., Zafra-Gómez, A., Dorival-García, N., Camino-Sánchez, F.J., Cantarero, S., Vilchez, J.L., 2013. Ultra-performance liquid chromatography MS/MS method for the determination of parabens in compost from sewage sludge: comparison of the efficiency of two extraction techniques. *Journal of separation science* 36, 2635–45. doi:10.1002/jssc.201300318
- Bijlsma, L., Beltrán, E., Boix, C., Sancho, J. V, Hernández, F., 2014. Improvements in analytical methodology for the determination of frequently consumed illicit drugs in urban wastewater. *Analytical and bioanalytical chemistry* 406, 4261–72. doi:10.1007/s00216-014-7818-4
- Bijlsma, L., Emke, E., Hernández, F., de Voogt, P., 2013. Performance of the linear ion trap Orbitrap mass analyzer for qualitative and quantitative analysis of drugs of abuse and relevant metabolites in sewage water. *Analytica Chimica Acta*. doi:10.1016/j.aca.2013.01.010
- Bijlsma, L., Sancho, J. V, Pitarch, E., Ibáñez, M., Hernández, F., 2009. Simultaneous ultra-high-pressure liquid chromatography-tandem mass spectrometry determination of amphetamine and amphetamine-like stimulants, cocaine and its metabolites, and a cannabis metabolite in surface water and urban wastewater. *Journal of Chromatography A* 1216, 3078–3089.
- Boix, C., Ibáñez, M., Sancho, J. V, León, N., Yusá, V., Hernández, F., 2014. Qualitative screening of 116 veterinary drugs in feed by liquid chromatography-high resolution mass spectrometry: potential application to quantitative analysis. *Food chemistry* 160, 313–20. doi:10.1016/j.foodchem.2014.03.086
- Boix, C., Ibáñez, M., Sancho, J. V, Niessen, W.M.A., Hernández, F., 2013. Investigating the presence of omeprazole in waters by liquid chromatography coupled to low and high resolution mass spectrometry: degradation experiments. *Journal of mass spectrometry : JMS* 48, 1091–100. doi:10.1002/jms.3260
- Boix, C., Ibáñez, M., Fabregat-Safont, D., Morales, E., Pastor, L., Sancho, J.V., Sánchez-Ramírez, J.E., Hernández, F. 2016. Analytical methodologies based on LC-MS/MS for monitoring selected emerging compounds in liquid and solid phases of the sewage sludge. *MethodsX* 3, 333–342. doi: 10.1016/j.mex.2016.04.010
- De la Rubia, M.A., Riau, V., Raposo, F., Borja, R., 2013. Thermophilic anaerobic digestion of sewage sludge: focus on the influence of the start-up. A review. *Critical reviews in biotechnology* 33, 448–60. doi:10.3109/07388551.2012.726962
- De Sena, R.F., Tambosi, J.L., Moreira, R.F.P.M., José, H.J., Gebhardt, W., Schröder, H.F., 2010. Evaluation of sample processing methods for the polar contaminant analysis of sewage sludge using liquid chromatography - Mass spectrometry (LC/MS). *Química Nova* 33, 1194–1198.

- Díaz, R., Ibáñez, M., Sancho, J. V, Hernández, F., 2013. Qualitative validation of a liquid chromatography-quadrupole-time of flight mass spectrometry screening method for organic pollutants in waters. *Journal of Chromatography A* 1276, 47–57. doi:10.1016/j.chroma.2012.12.030
- Díaz, R., Ibáñez, M., Sancho, J. V, Hernández, F., 2011. Building an empirical mass spectra library for screening of organic pollutants by ultra-high-pressure liquid chromatography/hybrid quadrupole time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry* 25, 355–369. doi:10.1002/rcm.4860
- Dorival-García, N., Zafra-Gómez, A., Camino-Sánchez, F.J., Navalón, A., Vílchez, J.L., 2013. Analysis of quinolone antibiotic derivatives in sewage sludge samples by liquid chromatography-tandem mass spectrometry: comparison of the efficiency of three extraction techniques. *Talanta* 106, 104–18. doi:10.1016/j.talanta.2012.11.080
- Ergün, H., Frattarelli, D.A.C., Aranda, J. V, 2004. Characterization of the role of physicochemical factors on the hydrolysis of dipyrone. *Journal of pharmaceutical and biomedical analysis* 35, 479–87. doi:10.1016/j.jpba.2004.02.004
- Evgenidou, E.N., Konstantinou, I.K., Lambropoulou, D.A., 2015. Occurrence and removal of transformation products of PPCPs and illicit drugs in wastewaters: A review. *Science of The Total Environment* 505, 905–926. doi:10.1016/j.scitotenv.2014.10.021
- Farré, M., Petrovic, M., Barceló, D., 2007. Recently developed GC/MS and LC/MS methods for determining NSAIDs in water samples. *Analytical and bioanalytical chemistry* 387, 1203–14. doi:10.1007/s00216-006-0936-x
- Gago-Ferrero, P., Borova, V., Dasenaki, M.E., Thomaidis, N.S., 2015. Simultaneous determination of 148 pharmaceuticals and illicit drugs in sewage sludge based on ultrasound-assisted extraction and liquid chromatography-tandem mass spectrometry. *Analytical and bioanalytical chemistry* 407, 4287–97. doi:10.1007/s00216-015-8540-6
- Gracia-Lor, E., Sancho, J. V, Serrano, R., Hernández, F., 2012. Occurrence and removal of pharmaceuticals in wastewater treatment plants at the Spanish Mediterranean area of Valencia. *Chemosphere* 87, 453–462. doi:10.1016/j.chemosphere.2011.12.025
- Gros, M., Petrovic, M., Ginebreda, A., Barceló, D., 2010. Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. *Environment international* 36, 15–26. doi:10.1016/j.envint.2009.09.002
- Herrero, P., Borrull, F., Pocurull, E., Marcé, R.M., 2014. A quick, easy, cheap, effective, rugged and safe extraction method followed by liquid chromatography-(Orbitrap) high resolution mass spectrometry to determine benzotriazole, benzothiazole and benzenesulfonamide derivatives in sewage sludge. *Journal of Chromatography A* 1339, 34–41. doi:10.1016/j.chroma.2014.02.081
- Ibáñez, M., Gracia-Lor, E., Sancho, J. V, Hernández, F., 2012. Importance of MS selectivity and chromatographic separation in LC-MS/MS-based methods when investigating pharmaceutical metabolites in water. Dipyrone as a case of study. *Journal of mass spectrometry : JMS* 47, 1040–6. doi:10.1002/jms.3050
- Jelic, A., Fatone, F., Di Fabio, S., Petrovic, M., Cecchi, F., Barcelo, D., 2012. Tracing pharmaceuticals in a municipal plant for integrated wastewater and organic solid waste treatment. *The Science of the total environment* 433, 352–61. doi:10.1016/j.scitotenv.2012.06.059
- Lacey, C., McMahon, G., Bones, J., Barron, L., Morrissey, A., Tobin, J.M., 2008. An LC-MS method for the determination of pharmaceutical compounds in wastewater treatment

- plant influent and effluent samples 75, 1089–1097. doi:10.1016/j.talanta.2008.01.011
- Li, W.C., 2014. Occurrence, sources, and fate of pharmaceuticals in aquatic environment and soil. *Environmental pollution* (Barking, Essex: 1987) 187, 193–201. doi:10.1016/j.envpol.2014.01.015
- Malmborg, J., Magnér, J., 2015. Pharmaceutical residues in sewage sludge: effect of sanitization and anaerobic digestion. *Journal of environmental management* 153, 1–10. doi:10.1016/j.jenvman.2015.01.041
- Masiá, A., Vásquez, K., Campo, J., Picó, Y., 2015. Assessment of two extraction methods to determine pesticides in soils, sediments and sludges. Application to the Túria River Basin. *Journal of Chromatography A* 1378, 19–31. doi:10.1016/j.chroma.2014.11.079
- Mastroianni, N., Postigo, C., de Alda, M.L., Barcelo, D., 2013. Illicit and abused drugs in sewage sludge: method optimization and occurrence. *Journal of chromatography. A* 1322, 29–37. doi:10.1016/j.chroma.2013.10.078
- Moosbrugger, R.E., Wentzel, M.C., Ekama, G.A. Marais, G.vR., 1992. Simple titration procedures to determine H<sub>2</sub>CO<sub>3</sub>\* alkalinity and short-chain fatty acids in aqueous solutions containing known concentrations of ammonium, phosphate and sulphide weak acid/bases. WRC Report No. TT 57/92, UCT Research Report W 74.
- Paíga, P., Lolić, A., Hellebuyck, F., Santos, L.H.M.L.M., Correia, M., Delerue-Matos, C., 2015. Development of a SPE-UHPLC-MS/MS methodology for the determination of non-steroidal anti-inflammatory and analgesic pharmaceuticals in seawater. *Journal of pharmaceutical and biomedical analysis* 106, 61–70. doi:10.1016/j.jpba.2014.06.017
- Palatsi, J., Laurenzi, M., Andrés, M. V., Flotats, X., Nielsen, H.B., Angelidaki, I., 2009. Strategies for recovering inhibition caused by long chain fatty acids on anaerobic thermophilic biogas reactors. *Bioresource technology* 100, 4588–96. doi:10.1016/j.biortech.2009.04.046
- Pérez, S., Barceló, D., 2007. Application of advanced MS techniques to analysis and identification of human and microbial metabolites of pharmaceuticals in the aquatic environment. *TrAC Trends in Analytical Chemistry* 26, 494–514. doi:10.1016/j.trac.2007.05.004
- Petrie, B., Barden, R., Kasprzyk-Hordern, B., 2014. A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring. *Water research*. doi:10.1016/j.watres.2014.08.053
- Petrovic, M., Hernando, M.D., Diaz-Cruz, M.S., Barcelo, D., 2005. Liquid chromatography-tandem mass spectrometry for the analysis of pharmaceutical residues in environmental samples: a review. *Journal of Chromatography A* 1067, 1–14. doi:http://dx.doi.org/10.1016/j.chroma.2004.10.110
- Peysson, W., Vulliet, E., 2013. Determination of 136 pharmaceuticals and hormones in sewage sludge using quick, easy, cheap, effective, rugged and safe extraction followed by analysis with liquid chromatography-time-of-flight-mass spectrometry. *Journal of chromatography. A* 1290, 46–61. doi:10.1016/j.chroma.2013.03.057
- Ternes, T.A., Bonerz, M., Herrmann, N., Löffler, D., Keller, E., Lacida, B.B., Alder, A.C., 2005. Determination of pharmaceuticals, iodinated contrast media and musk fragrances in sludge by LC tandem MS and GC/MS. *Journal of Chromatography A* 1067, 213–223. doi:10.1016/j.chroma.2004.10.096
- Thomas, K. V., Bijlsma, L., Castiglioni, S., Covaci, A., Emke, E., Grabic, R., Hernández, F.,

- Karolak, S., Kasprzyk-Hordern, B., Lindberg, R.H., Lopez de Alda, M., Meierjohann, A., Ort, C., Pico, Y., Quintana, J.B., Reid, M., Rieckermann, J., Terzic, S., van Nuijs, A.L.N., de Voogt, P., 2012. Comparing illicit drug use in 19 European cities through sewage analysis. *Science of the Total Environment* 432, 432–439. doi:10.1016/j.scitotenv.2012.06.069
- Topuz, E., Sari, S., Ozdemir, G., Aydin, E., Pehlivanoglu-Mantas, E., Okutman Tas, D., 2014. Optimization of diclofenac quantification from wastewater treatment plant sludge by ultrasonication assisted extraction. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences* 958, 48–54. doi:10.1016/j.jchromb.2014.02.047
- Vieno, N., Tuhkanen, T., Kronberg, L., 2007. Elimination of pharmaceuticals in sewage treatment plants in Finland 41, 1001–1012. doi:10.1016/j.watres.2006.12.017
- Wick, A., Wagner, M., Ternes, T.A., 2011. Elucidation of the transformation pathway of the opium alkaloid codeine in biological wastewater treatment. *Environmental Science and Technology* 45, 3374–3385. doi:10.1021/es103489x
- Zhou, H., Zhou, J., Wang, M., Wang, X., Zhang, Q., Zhang, Q., Zhan, Y., 2014. Removal of typical pharmaceutically active compounds in sewage sludge using mesophilic and thermophilic anaerobic digestion processes. *International Journal of Environmental Science and Technology* 12, 2169–2178. doi:10.1007/s13762-014-0627-7
- Zhou, L.-J., Ying, G.-G., Liu, S., Zhao, J.-L., Chen, F., Zhang, R.-Q., Peng, F.-Q., Zhang, Q.-Q., 2012. Simultaneous determination of human and veterinary antibiotics in various environmental matrices by rapid resolution liquid chromatography-electrospray ionization tandem mass spectrometry. *Journal of chromatography. A* 1244, 123–38. doi:10.1016/j.chroma.2012.04.076
- Zorita, S., Mårtensson, L., Mathiasson, L., 2009. Occurrence and removal of pharmaceuticals in a municipal sewage treatment system in the south of Sweden 407, 2760–2770. doi:10.1016/j.scitotenv.2008.12.030



**Figure 1**

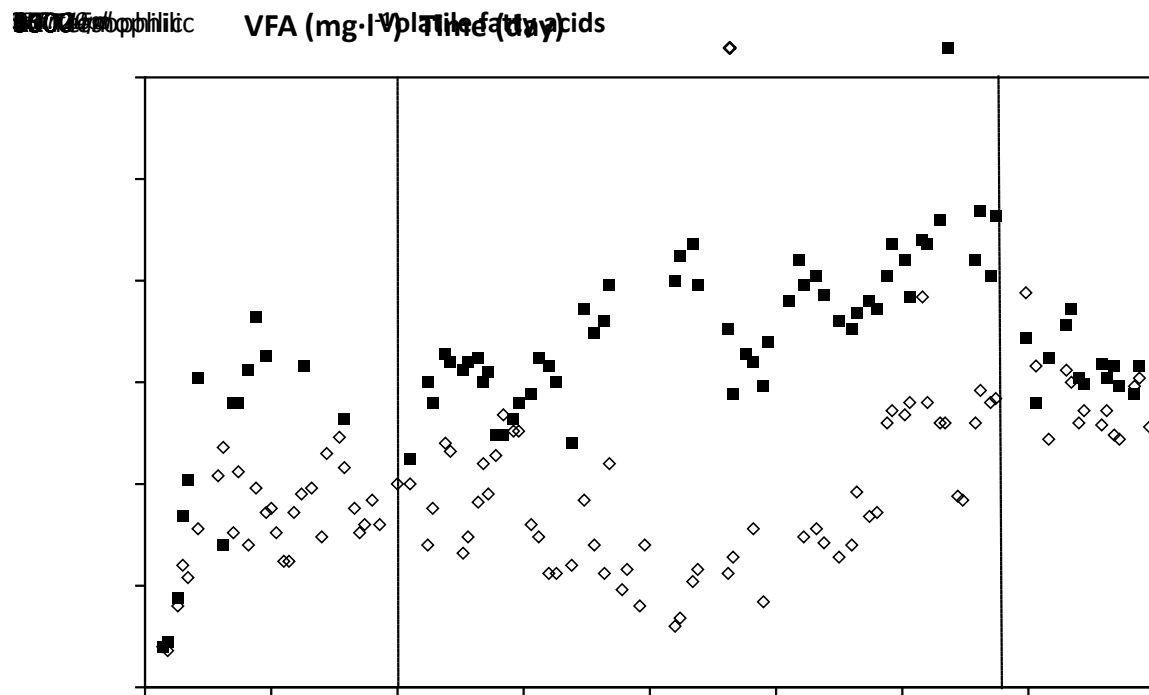
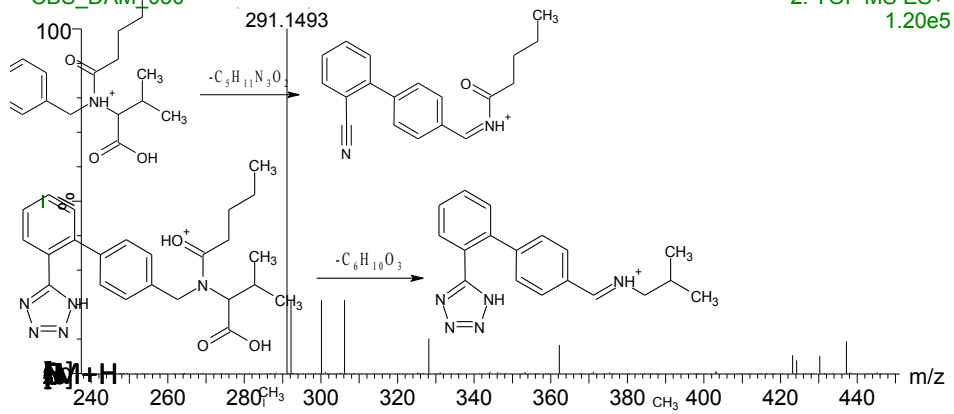


Figure 2

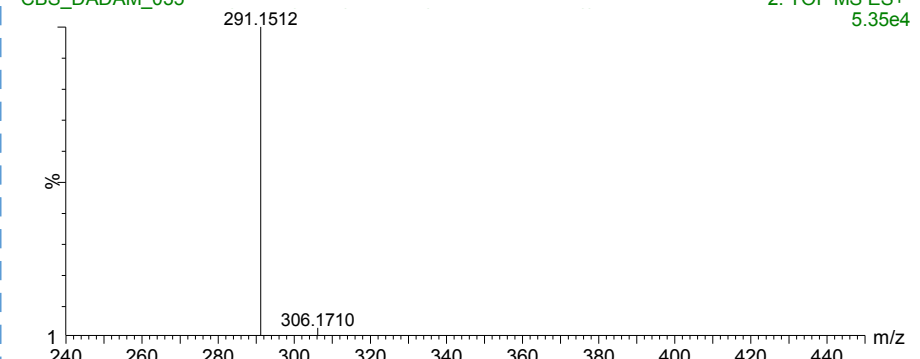


St 50ppb DAM  
CBS\_DAM\_030



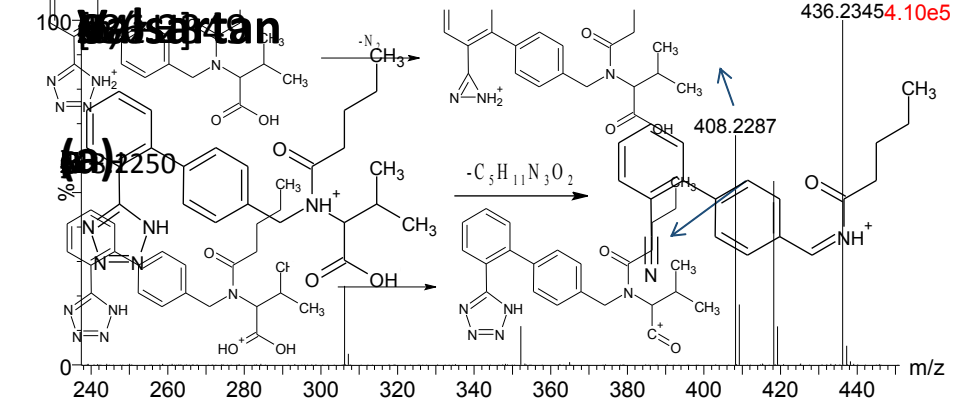
2: TOF MS ES+  
1.20e5

St 50ppb DAM  
H2O SPE\_(B)  
CBS\_DADAM\_035



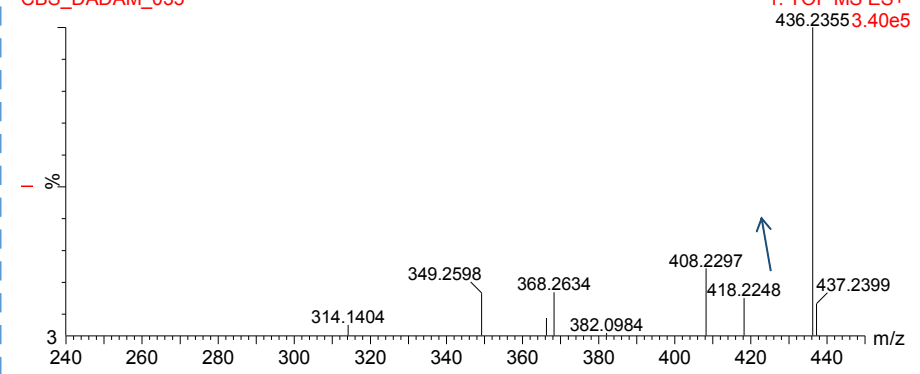
2: TOF MS ES+  
5.35e4

CBS\_DAM\_030



1: TOF MS ES+  
436.23454.10e5

CBS\_DADAM\_035



1: TOF MS ES+  
436.23553.40e5

Figure 3

### BE elimination during T20 experiment

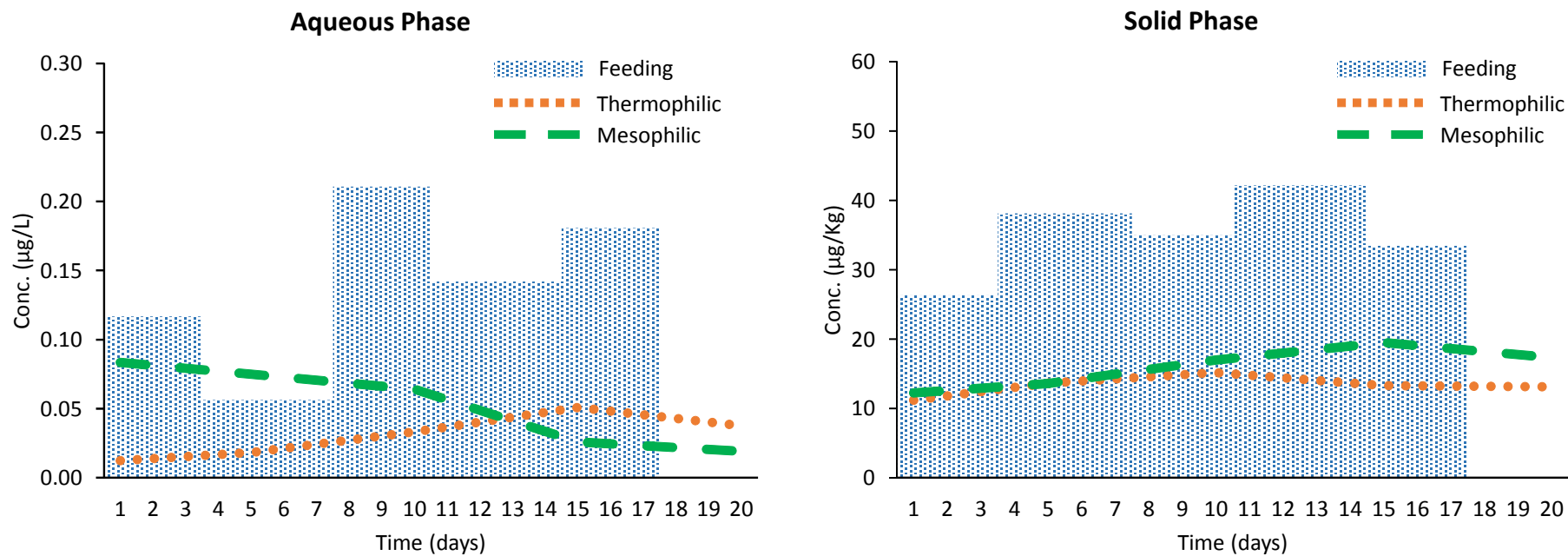


Figure 4

(g)  $\frac{1}{2} \ln \left( \frac{1 + \cos(\theta)}{1 - \cos(\theta)} \right)$

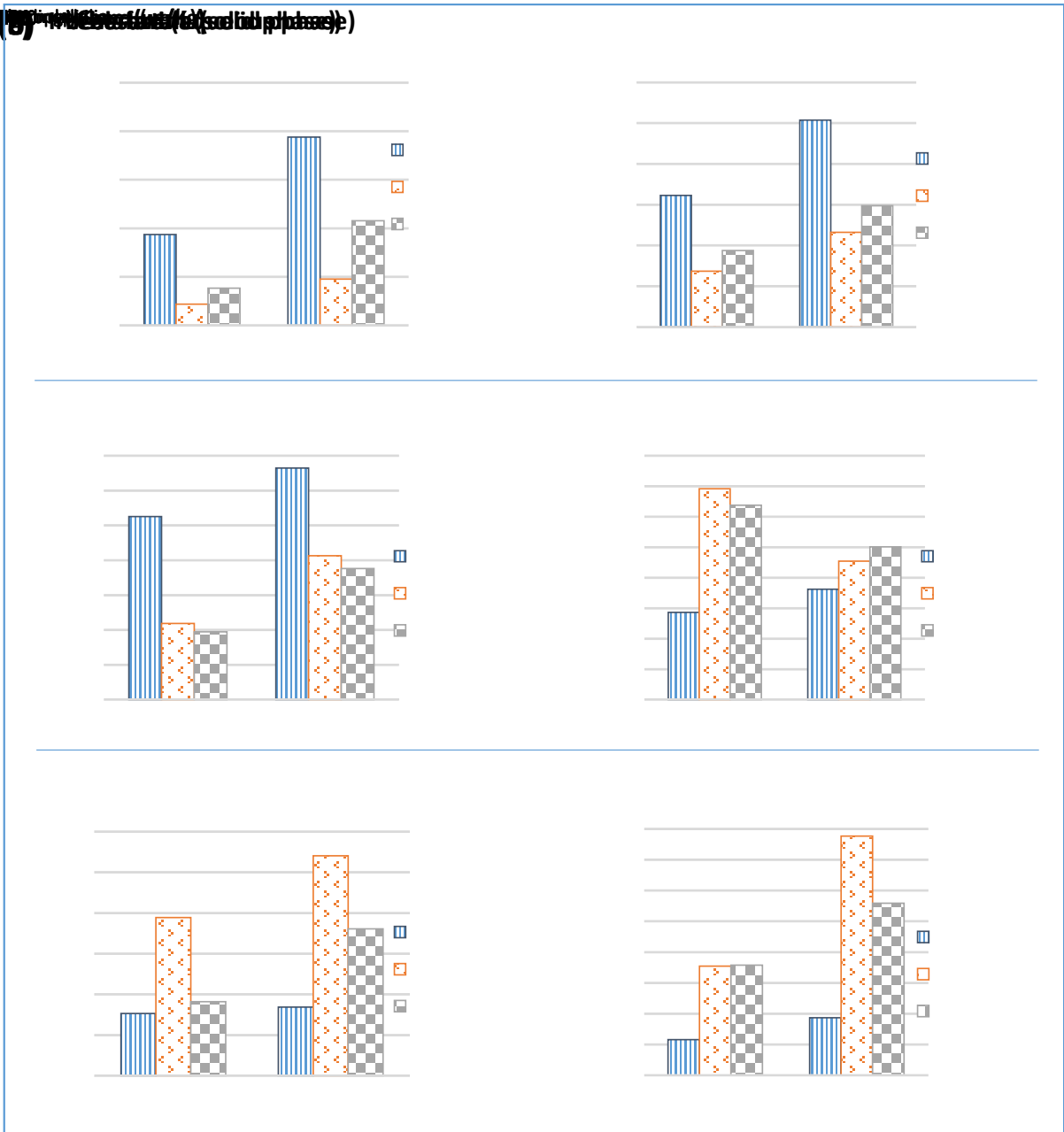


Figure 5



Figure 6

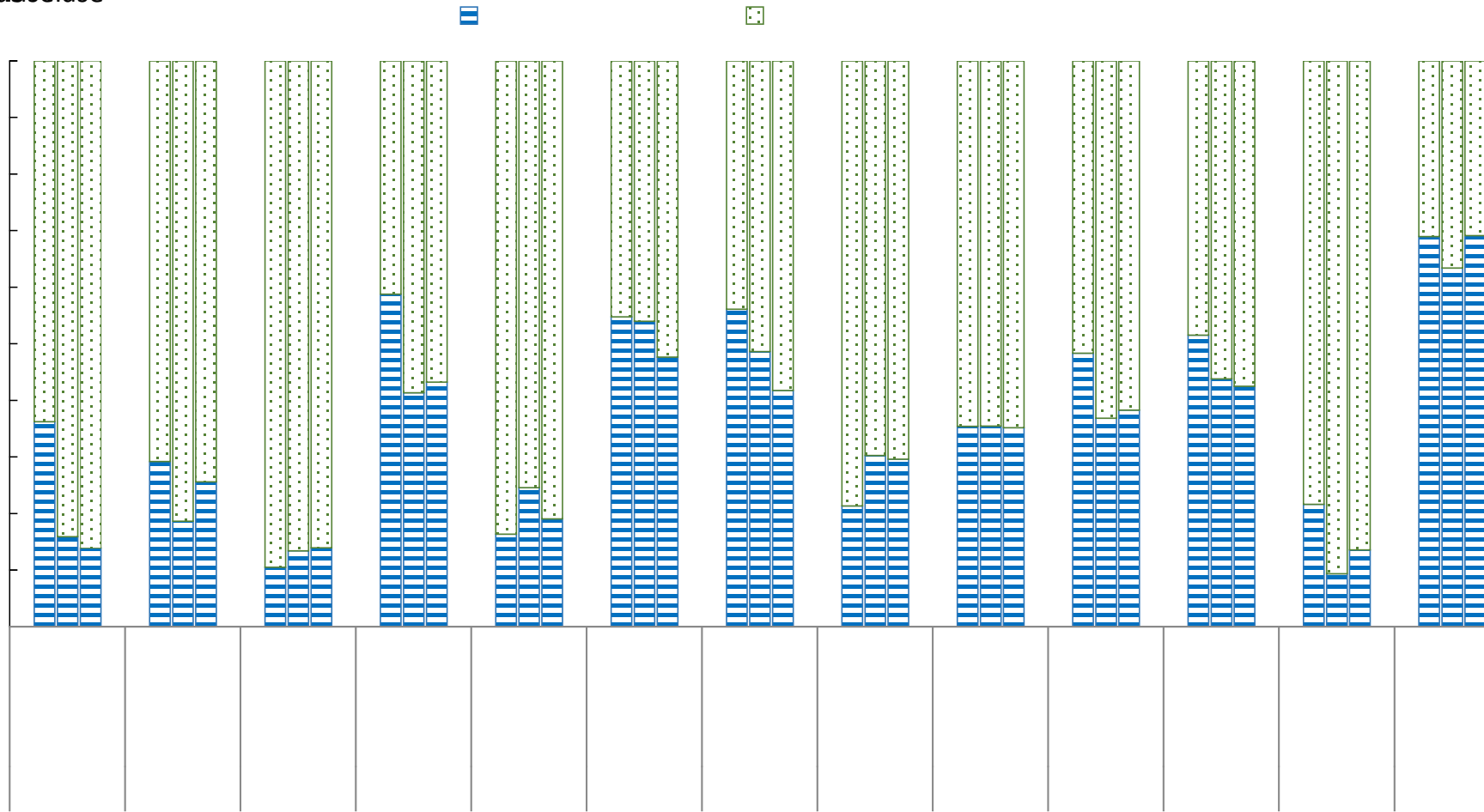


Figure 6