

An innovative wastewater treatment technology based on UASB and IFAS for cost-efficient macro and micropollutant removal

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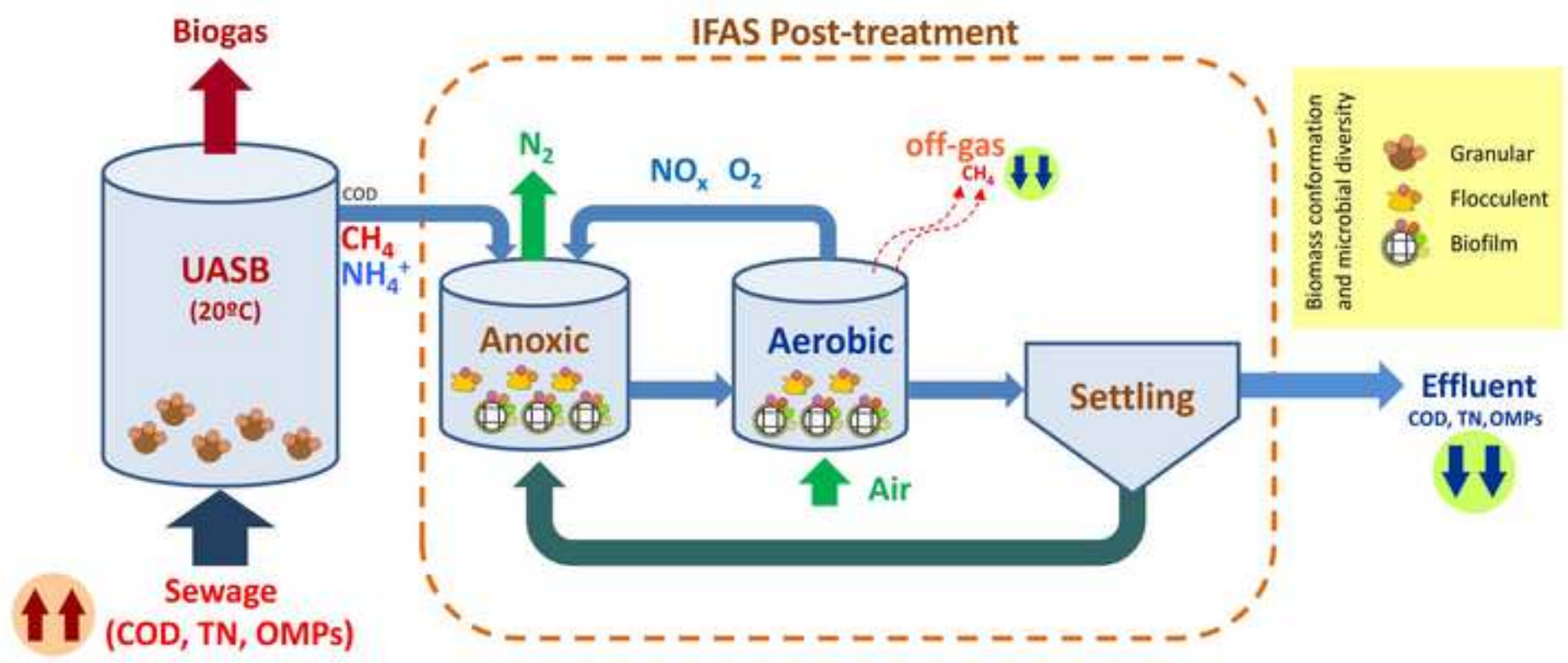
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Highlights

- The combination of redox potentials improves organic micropollutant (OMP) removal
- OMP removal is also influenced by biomass activity and conformation
- Bisphenol A and ethinylestradiol removal depends on nitrifying-denitrifying activity
- The UASB+IFAS system upgrades anaerobic treatments with lower N and GHGs emissions
- Biofilms highly enhances microbial diversity and the growth of anammox microorganisms



Abstract

An innovative process based on the combination of a UASB reactor and an IFAS system is proposed in order to combine different redox conditions and biomass conformations to promote a high microbial diversity. The objective of this configuration is to enhance the biological removal of organic micropollutants (OMPs) as well as to achieve the abatement of nitrogen by using the dissolved methane as an inexpensive electron donor. Results showed high removals of COD (93%) and dissolved methane present in the UASB effluent (up to 85%) was biodegraded by a consortium of aerobic methanotrophs and heterotrophic denitrifiers. Total nitrogen removal decreased slightly along the operation (from 44 to 33%), depending on the availability of electron donor, biomass concentration, and configuration (flocs and biofilm). A high removal was achieved in the hybrid system (>80%) for 6 of the studied OMPs. Sulfamethoxazole, trimethoprim, naproxen, and estradiol were readily biotransformed under anaerobic conditions, whereas ibuprofen or bisphenol A were removed in the anoxic-aerobic compartment. Evidence of the cometabolic biotransformation of OMPs has been found, such as the influence of nitrification activity on the removal of bisphenol A, and of the denitrification activity on ethinylestradiol removal.

An innovative wastewater treatment technology based on UASB and IFAS for cost-efficient macro and micropollutant removal

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Abstract

An innovative process based on the combination of a UASB reactor and an IFAS system is proposed in order to combine different redox conditions and biomass conformations to promote a high microbial diversity. The objective of this configuration is to enhance the biological removal of organic micropollutants (OMPs) as well as to achieve the abatement of nitrogen by using the dissolved methane as an inexpensive electron donor. Results showed high removals of COD (93%) and dissolved methane present in the UASB effluent (up to 85%) was biodegraded by a consortium of aerobic methanotrophs and heterotrophic denitrifiers. Total nitrogen removal decreased slightly along the operation (from 44 to 33%), depending on the availability of electron donor, biomass concentration, and configuration (flocules and biofilm). A high removal was achieved in the hybrid system (>80%) for 6 of the studied OMPs. Sulfamethoxazole, trimethoprim, naproxen, and estradiol were readily biotransformed under anaerobic conditions, whereas ibuprofen or bisphenol A were removed in the anoxic-aerobic compartment. Evidence of the cometabolic biotransformation of OMPs has been found, such as the influence of nitrification activity on the removal of bisphenol A, and of the denitrification activity on ethinylestradiol removal.

Keywords: Organic micropollutants, pharmaceuticals, redox conditions, methane emissions, hybrid process.

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2 **ABBREVIATIONS**
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6 AMO: ammonia monooxygenase

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8 BPA: bisphenol A

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10 COD: chemical oxygen demand

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12 COD_S: soluble chemical oxygen demand

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14 COD_T: total chemical oxygen demand

15 damo: denitrifying anaerobic methane oxidation

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17 DCF: diclofenac

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19 DO: dissolved oxygen

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21 ERY: erythromycin

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23 E1: estrone

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25 E2: β -estradiol

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27 EE2: α -ethinylestradiol

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29 FA: free ammonia

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31 FISH: fluorescence *in situ* hybridization

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33 GHG: greenhouse gas

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35 IBP: ibuprofen

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37 IFAS: integrated fixed-film activated sludge

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39 K_d: sorption coefficient

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41 MBR: membrane bioreactor

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43 MLTSS: mixed-liquor total suspended solids

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45 MLVSS: mixed-liquor volatile suspended solids

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47 NPX: naproxen

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49 OLR: organic loading rate

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51 OMPs: organic micropollutants

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53 ORP: oxidation-reduction potential

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55 R: recirculation ratio

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57 ROX: roxithromycin

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59 SMX: sulfamethoxazole

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61 TMP: trimethoprim

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63 UASB: upflow anaerobic sludge blanket
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1. INTRODUCTION

Nowadays, wastewater treatment should address not only the removal of conventional pollutants, such as nitrogen or organic matter but also the presence of organic micropollutants (OMPs), an issue of increasing concern in the society. There is a consensus in considering the effluents from conventional wastewater treatment plants as the main entrance of these substances into the aquatic environment since common treatment processes are not able to effectively remove many of them [1,2]. New advanced treatment processes, in many cases based on the combination of biological processes with physico-chemical post-treatment stages, have been developed in the last years to overcome the existing limitations [3,4,5]. High OMP removal efficiencies can be achieved by the application of biological configurations upgraded with tertiary stages with oxidants or adsorbents such as ozone or activated carbon. Nevertheless, these upgraded wastewater treatment systems increase operational expenses [6,7]. An alternative approach would be to develop enhanced biological processes for the removal of macro and micropollutants. For this purpose, recent studies have shown the interest of new biological configurations which combine different redox conditions (anaerobic, anoxic, aerobic) [8], different biomass physical conformations (granular, flocculent, biofilm) and wide microbial diversity [9].

OMPs biotransformation is determined by their chemical structure and clearly influenced by redox conditions [10]. For instance, sulfamethoxazole can be biotransformed at a high extent under anaerobic conditions, due to the presence of electron-withdrawing groups, such as sulfonyl, which is easily degraded under reductive conditions [11]. On the other hand, ibuprofen is a readily biotransformable OMP under aerobic conditions [12], especially favored when nitrification takes place due to its hydroxylation by the action of the enzyme ammonia monooxygenase (AMO) to produce 2-hydroxy-ibuprofen [13]. However, the branched substitutions existing on the para-position of the aromatic ring hampers its biotransformation under anaerobic redox potentials [11]. Normally, the combination of aerobic and anaerobic stages leads to the enhancement of the removal of several OMPs, such as venlafaxine or tramadol [14].

1 The use of carriers enhances the retention of slow-growing bacteria in biofilms, such as
2 nitrifiers, allowing the increase of the microbial diversity [15,16]. The presence of
3 nitrifying bacteria enhances several OMPs removal, such as the hormone estradiol,
4 whose removal is correlated with the nitrifying activity [11]. Falas et al. [17] studied the
5 removal of OMPs by biofilms and suspended biomass and measured a higher removal
6 rate per unit biomass in the biofilm for most of the OMPs, as in the case of ketoprofen
7 or gemfibrozil. Biomass conformation also influences OMP removal. In the case of
8 lipophilic OMPs, such as musk fragrances, higher sorption coefficients (K_d) were
9 observed in a membrane bioreactor (MBR) compared to a conventional activated sludge
10 (CAS) plant due to the lower biomass particle size developed in MBRs [18]. De la
11 Torre et al. [19] compared the removal of reactors operated either with suspended
12 biomass or biofilms, as well as with the combination of both. They observed the best
13 results in terms of OMP removal for the integrated fixed-film activated sludge (IFAS)-
14 MBR, which combines both suspended biomass and biofilms, while the worst results
15 were obtained for the reactor operated only with biofilms. The use of membranes and/or
16 supports allows the retention of slow-growing bacteria [8,20]. This enhances the
17 microbial diversity and the types of enzymes present in the biomass and, consequently,
18 the removal of macro- and micropollutants [10].

19 Other of the key issues in wastewater treatment is the operational cost related to energy
20 consumption. With the aim of reducing this important contribution to the overall
21 operational expenses, innovative approaches are under development. First an anaerobic
22 methanogenic step in the water line, as roughing stage, would allow the reduction of
23 aeration costs, as well as a valorization of the organic matter in terms of energy
24 production. Nevertheless, the effluent of a methanogenic stage should be treated to
25 reduce the remaining organic and nitrogen compounds, being chemical oxygen demand
26 (COD) levels still high. Part of the methane remains dissolved in the liquid phase (up to
27 25-50%) especially at temperatures below 20°C [21]. On one hand, although aerobic
28 treatment is the common post-treatment alternative, part of the dissolved methane is
29 released into the atmosphere by stripping, increasing greenhouse gas (GHG) emissions
30 of wastewater treatment [22]. On the other hand, the use of conventional nitrification-
31 denitrification is often limited by the low remaining organic matter after anaerobic
32 treatment, which limits the denitrification efficiency. Aerobic and anaerobic consortia of
33 bacteria/archaea (methanotrophs) are able to use methane as a carbon source for

1 denitrification [23]. Aerobic methanotrophs are able to convert methane into oxidized
2 species that can be employed as an organic carbon source by the denitrifying
3 heterotrophic bacteria [24]. Under anoxic conditions, denitrification coupled to
4 anaerobic methane oxidation (damo) could be carried out by either damo bacteria
5 (*Candidatus Methyloirabilis oxyfera*) or damo archaea (*Candidatus Methanoperedens*
6 nitroreducens) with nitrite or nitrate as electron acceptor, respectively [25].
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10 The aim of this study was to investigate the removal of micro- and macropollutants in a
11 combined upflow anaerobic sludge blanket (UASB)-IFAS system treating low-strength
12 wastewater. The system combines anaerobic, anoxic and aerobic redox conditions in a
13 sequence of reactors, where biomass with different conformations (granular, flocculent
14 and biofilm) is developed. The promotion of methanotrophic denitrification allowed
15 overcoming problems related to methane emissions by low temperature anaerobic
16 processes.
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24 **2. MATERIALS AND METHODS**

25 *Experimental set-up*

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28 The system combined a first UASB unit of 120 L volume, coupled to an IFAS polishing
29 stage of 56 L (Figure S1). The anaerobic reactor was inoculated with anaerobic granular
30 biomass from an internal circulation reactor treating brewery wastewater. Biomass
31 concentration in the UASB was 30 g L⁻¹ of total suspended solids (TSS). No purges
32 were carried out in the anaerobic reactor. The IFAS was started up on operating day 26.
33 The IFAS system consists of two biological compartments (anoxic, 36 L and aerobic, 20
34 L) and a secondary settler (10 L). Two types of carriers were used in order to promote
35 the growth of biofilms and retain the slow-growing bacteria: Synthetic porous foams
36 (Levapor GmbH, Germany) in the anoxic compartment and porous semi-flexible
37 carriers (Mutag Biochip, Multi Umwelttechnologie A.G., Germany) in the aerobic
38 compartment. The IFAS was started-up using an apparent volume of 20% of Levapor
39 and 7% of Biochip carriers. At the operating day 257, these volumes were raised up to
40 23 and 20%, respectively. The specific external surface of the products, were of 486 and
41 2,174 m² m⁻³, for the outer boundary of the Levapor and Biochip carriers, respectively.
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56 The system was operated during 407 days at room temperature (21 ± 1°C) with an
57 overall COD loading rate of 680 ± 190 mg L⁻¹ d⁻¹. A synthetic medium-low strength
58 wastewater was feed to the UASB composed by diluted skimmed milk as a carbon
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1 source (COD_T and COD_S of 891 ± 214 , 782 ± 204 mg L^{-1} , respectively), sodium
2 bicarbonate for maintaining alkalinity (960 mg L^{-1}) and ammonium chloride as N
3 source (18 mg N L^{-1}). HRT was 20 ± 2 h in the UASB and 9 ± 1 h in the IFAS post-
4 treatment. A total recirculation ratio (R) of 3 was applied in the IFAS stage to achieve
5 high nitrogen removal, combining both an internal and an external recirculation to the
6 anoxic compartment.
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10 *Analytical methods*

11 Mixed-liquor total and volatile suspended solids (MLTSS and MLVSS, respectively),
12 ammonium, nitrite, nitrate, total and soluble chemical oxygen demand (COD_T and
13 COD_S , respectively) were analyzed according to Standard Methods [26]. Temperature,
14 dissolved oxygen (DO) and pH were monitored with a Hach HQ40d multi-parameter
15 digital sensor. Biogas composition was measured in a gas chromatograph HP 5890
16 Series II using a column of Porapak Q 80/100 (SUPELCO). Dissolved methane
17 concentration was measured following the method described by Sánchez et al. (2016).
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26 Eleven OMPs, representative of several widely consumed pharmaceuticals and
27 chemicals with different physico-chemical behavior, which are known to be present in
28 sewage, were spiked to the synthetic wastewater: three anti-inflammatories at
29 concentrations of 10 ppb (ibuprofen IBP, naproxen NPX, diclofenac DCF), four
30 antibiotics at concentrations of 10 ppb (sulfamethoxazole SMX, trimethoprim TMP,
31 erythromycin ERY, roxithromycin ROX) and four endocrine disruptors (bisphenol
32 BPA, estrone E1, β -estradiol E2, α -ethinylestradiol EE2) at a concentration range of 1-
33 10 ppb. These values can be considered as “environmental levels” in accordance to the
34 amounts detected in sewage, an also to the limits of quantification of the analytical
35 methods applied. The removal of the selected OMPs was followed through the different
36 stages of the system in order to assess the effect of the redox conditions and the
37 different microbial communities. Samples were taken with a time delay of one hydraulic
38 retention time (HRT) in four sampling points: the influent and effluent of the UASB
39 reactor, the anoxic compartment, and final effluent. Seven sampling campaigns were
40 carried out over the operation to follow up the removal of the macro and
41 micropollutants. For the detection of OMPs, samples were prefiltered (AP3004705,
42 Millipore), preconcentrated by a solid-phase extraction (SPE) with OASIS HLB
43 cartridges. Anti-inflammatories and BPA were analysed by gas chromatography-mass
44 spectrometry (GC/MS) and antibiotics and hormones by liquid chromatography-tandem
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1 mass spectrometry (LC/MS/MS). The analytical procedure, limits of quantification and
2 recoveries were described by Alvarino et al. [4].
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6 *Molecular biology techniques*

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8 Microbial community was monitored by using fluorescence *in situ* hybridization (FISH)
9 and 16S rRNA gene amplicon sequencing analysis by using Illumina MiSeq. This last
10 analysis was carried out at Parque Científico de Madrid, Spain. For FISH analysis,
11 biomass samples were collected during the operation, disrupted and fixed according to
12 the procedure described by Amman et al. [27]. FISH probes and Illumina procedure are
13 described in Supplementary Material. FISH samples were collected at the operating
14 days 50, 99, 265 and 343. Illumina samples were collected at the operating days 175,
15 265 and 368 (3 samples for Levapor and 1 for Biochip biofilms).
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26 **3. RESULTS AND DISCUSSION**

27 **3.1 Reactor performance**

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29 The anaerobic stage achieved a COD_T removal of $93 \pm 2\%$ in the UASB reactor, which
30 coincides with the overall removal achieved in the combined system (Table S1). The
31 measured biogas production in the UASB was $37 \pm 9 \text{ L d}^{-1}$ with a methane percentage
32 of $75 \pm 2\%$. Most of the COD (74%) was methanized under anaerobic conditions,
33 reducing energy consumption by saving aeration and producing biogas. A fraction of
34 the produced methane remained dissolved in the effluent of the UASB, due to methane
35 solubility at low temperatures [22, 28]. Presence of dissolved methane is still a concern
36 because it increases GHG emissions. Thus, it should be post-treated in order to decrease
37 its environmental impact.
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40 Measured dissolved methane concentration in the UASB effluent was $18 \pm 3 \text{ mg L}^{-1}$
41 throughout the whole operation. Methane mass balances showed that dissolved methane
42 corresponded to 10 - 20% of the overall methane generated in the UASB reactor.
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45 The effluent from the UASB reactor contained low COD_T and COD_S values of 57 ± 2
46 mg L^{-1} of and $43 \pm 1 \text{ mg L}^{-1}$, respectively, without considering dissolved methane.
47 Ammonia concentration of $54 \pm 8 \text{ mg N L}^{-1}$ was measured. The IFAS stage contributed
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1 to achieving a higher quality effluent, being its total nitrogen concentration of 36 ± 11
2 mg L^{-1} .

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4 Considering the evolution of nitrification, two different periods of operation were
5 identified in the IFAS system: Period 1 (P1), from day 0 to 167, in which only partial
6 nitrification was observed and nitrite was the main electron acceptor under anoxic
7 conditions. At the end of P1, the development of nitrite-oxidizing bacteria (NOB) was
8 detected; and Period 2 (P2), from day 168 to 407, when nitrite oxidation to nitrate was
9 achieved and no nitrite accumulation was observed.

10
11 During P1 the reactor contained suspended biomass as well as biofilms in the anoxic
12 and aerobic compartments. MLTSS and MLVSS concentrations in the anoxic
13 compartment were 1.5 ± 0.9 and $1.3 \pm 0.8 \text{ g L}^{-1}$, respectively. DO and ORP parameters
14 were monitored, obtaining values between 0.15 and 0.31 mg L^{-1} and 0 and -150 mV,
15 respectively, and pH was 7.6 ± 0.2 . DO in the aerobic compartment was $3.2 \pm 0.9 \text{ mg L}^{-1}$.
16 At the end of P1, worse settling properties of the suspended biomass were observed,
17 which led to an almost complete washout of the suspended biomass present in the IFAS
18 system (days 146 to 167).

19
20 Along P2 suspended biomass concentration was lower than 50 mg L^{-1} and biofilm
21 growth onto the two types of carriers was observed. Thus, the system was operated as a
22 biofilm system from day 167 onwards. In the anoxic compartment, the DO and ORP
23 values ranged from 0.25 to 0.45 mg L^{-1} and 0 to -75 mV, respectively.

24
25 The quality of the effluent of the IFAS system, in terms of COD_T , was lower in P1 than
26 in P2, with values of 77 ± 55 and $52 \pm 19 \text{ mg L}^{-1}$, respectively. CODs was $24 \pm 13 \text{ mg L}^{-1}$
27 during the whole experimentation. VSS in the effluent was lower in P2 ($32 \pm 11 \text{ mg L}^{-1}$)
28 compared with P1, $50 \pm 40 \text{ mg L}^{-1}$. On the contrary, the observed TN concentration in
29 the effluent in P1 was lower than in P2, with values of 30 ± 7 and $37 \pm 7 \text{ mg L}^{-1}$,
30 respectively.

31 32 **3.2 Methane and nitrogen removal in the IFAS system.**

33
34 The UASB effluent contained $54 \pm 8 \text{ mg N L}^{-1}$ of ammonia. In P1, nitrogen ions
35 concentration present in the final effluent were $28 \pm 16 \text{ mg NH}_4^+\text{-N L}^{-1}$ and 2.5 ± 1.8
36 $\text{mg NO}_2^-\text{N L}^{-1}$. After operating day 28, TN was $30 \pm 7 \text{ mg L}^{-1}$ and TN removal
37 achieved 44%, corresponding to a nitrogen removal rate of $55 \pm 18 \text{ mg N L}^{-1} \text{ d}^{-1}$,
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1 referred to the whole IFAS system. Nitrate was hardly detected and ammonia was
2 partially oxidized to nitrite, with a nitrification rate of $158 \pm 84 \text{ mg NH}_4^+\text{-N L}^{-1} \text{ d}^{-1}$,
3 observed in the aerobic compartment. The IFAS system was not able to achieve
4 complete nitrification of the NO_2^- generated due to free ammonia (FA) accumulation of
5 $1.1 \text{ mg NH}_3\text{-N L}^{-1}$ that inhibited nitrite oxidizers [29]. Moreover, overall nitrogen
6 loading rate applied to the IFAS system was high, around $135 \text{ mg TN L}^{-1} \text{ d}^{-1}$. After
7 operating day 28, TN measured in the samples taken in the anoxic compartment were
8 $2 \pm 2 \text{ mg} \cdot \text{L}^{-1}$ higher than those observed in the final effluent. This fact indicated the
9 possible existence of simultaneous nitrification-denitrification in the biofilms of the
10 aerobic compartment.
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12 In P2, nitrite was below $0.2 \text{ mg NO}_2^-\text{-NL}^{-1}$ in the final effluent. The system could not
13 achieve full ammonia oxidation. Nitrogen ions concentration in the final effluent were
14 $22 \pm 7 \text{ mg NH}_4^+\text{-N L}^{-1}$ and $15 \pm 5 \text{ mg NO}_3^-\text{-N L}^{-1}$, with a denitrification rate of 43 ± 10
15 $\text{mg N L}^{-1} \text{ d}^{-1}$. TN fed was around $54 \pm 8 \text{ mg L}^{-1}$. Despite operating with biofilm, the
16 fraction of ammonia oxidised was completely oxidised to nitrate, achieving a
17 nitrification rate of $202 \pm 60 \text{ mg NH}_4^+\text{-N L}^{-1} \text{ d}^{-1}$. Although FA was still high, 1.6 mg
18 $\text{NH}_3\text{-N L}^{-1}$, the development of nitrifiers inside biofilms is a more adequate environment
19 than flocculent biomass to better tolerate this inhibitor [30]. Average TN concentration
20 was in $39 \pm 10 \text{ mg N L}^{-1}$ in the anoxic compartment and $36 \pm 10 \text{ mg N L}^{-1}$ in the final
21 effluent. TN removal decreased to a 33%, as result of the higher electron donor
22 requirements of nitrate denitrification and by the lower denitrification rate of this
23 nitrogen ion.
24

25 Denitrification was carried out by a heterogeneous group of bacteria comprising aerobic
26 methanotrophs and nitrate/nitrite denitrifying heterotrophs (Figure 1B). The aerobic
27 methanotrophs contributed to reducing the dissolved methane concentration. In P2 the
28 growth of anammox bacteria in biofilm was observed. The fact of operating the system
29 with biofilm in the aerobic compartment could enhance denitrification due to
30 anoxic/anaerobic environments within the internal layers of the biofilms.
31

32 Dissolved methane was biologically removed in the IFAS along P1 in a high extent
33 (85%), corresponding the remaining 15% to stripping. Around 50% of dissolved
34 methane was removed in the anoxic compartment, and some additional 35% in the
35 aerobic stage, considering k_{La} of methane and the measured dissolved methane
36 concentration in the aerobic compartment. Methane biodegradation decreased in P2,
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down to 69%, with 37% and 32% corresponding to the anoxic and aerobic compartments, respectively. This decrease in dissolved methane biodegradation is very likely related to the washout of the suspended biomass in which methane-oxidizing bacteria were abundant.

The support selected in the anoxic compartment of the IFAS, pads of Levapor, is a polyurethane foam, with high porosity (75-90%) and a size of 20x20x7 mm. This carrier was selected in order to promote the growth of anaerobic and anoxic microorganisms in the inner pores of this small rectangular prism. On the other hand, the carrier used in the aerobic compartment, Biochip, is a round form polyethylene support with a diameter of 25 mm and a thickness of 1.1 mm. The surface presents a great number of holes and cavities where microorganisms grew adhered. This support, with the higher specific external surface, was selected in order to promote the growth of a thin biofilm layer with a large fraction of aerobic microorganisms.

3.3 Microbiological analysis

In Levapor and Biochip biofilm 27 and 22 different genera of bacteria were found, respectively, with more than 1% of relative abundance.

Experimental results indicated that nitrite oxidation took place mainly in P2. Heterotrophic denitrification is not the only biological way of nitrogen removal, so other microorganisms, as anammox, might coexist in the suspended biomass and the carriers. FISH analysis and 16S rRNA gene sequencing allowed elucidating the main biological mechanisms responsible for nitrification, as well as for removal of methane and of nitrogen.

Microbiological analysis of suspended biomass and biofilms in the aerobic compartment showed that among nitrifiers, only ammonia oxidizing bacteria were present in the system, with a small presence of nitrite oxidizers, in P1, confirming the partial nitrification in this period. In P2, *Nitrospira* spp. growth was observed, likely the responsible for the nitrite oxidation to nitrate.

Type I methanotrophs, aerobic methane oxidizers, were detected in large quantities in suspended biomass during P1, and in both type of biofilms during all the operation by FISH analysis (Figure S2). On day 368 (P2), Illumina analysis indicated that type I methanotrophs relative abundance in Levapor and Biochip biofilms were 2.10% and

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6.15%, respectively. In contrast, 0.02% of damo bacteria were only detected in the anoxic biofilm. No damo archaea were detected.

Ammonium and nitrite were available, so anammox growth operating conditions were present. They were detected by FISH analysis on day 265 in both anoxic and aerobic biofilms (Figure S2), with a relative abundance on the day of operation 368 of 3.18% in Levapor biofilm and 6.33% in Biochip biofilm, according to the results obtained by using Illumina analysis.

These data indicate that methane removal was achieved mainly by type I methanotrophs and methane removal decreased in P2 due to suspended biomass washout. Furthermore, the presence of aerobic methanotrophs in the aerobic biofilm, detected by both FISH and Illumina analysis, indicated that some biological methane removal took place in the aerobic chamber. Nitrogen removal was carried out by heterotrophic denitrifiers in P1, but in P2 anammox pathway was also contributing to nitrogen removal, present in the anoxic/anaerobic inner part of Biochip biofilms. Thus, the aforementioned lower TN observed in final effluent, could be explained among others by the anammox process, carried out by the biofilms of the aerobic compartment.

3.4 OMP removal

The removal of the selected OMPs was studied in the overall hybrid system, as well as in each compartment. Figure 2 shows the removal efficiencies obtained from mass balances applied to the both the UASB and the IFAS reactors. Sorption was not significant since the studied OMPs are not lipophilic compounds. Even though some of the studied OMPs can be partially present in the mixed liquor in its positively charged form (e.g. cationic carbamazepine) and interact electrostatically with the negatively charged surfaces of the microorganisms (known as adsorption) [31]. It was not relevant in the present study in agreement with previous studies about the long term operation of UASB reactors [8,11]. Consequently, OMPs removal was associated with biotransformation [32], as confirmed by mass flow calculations. In the anaerobic reactor, four substances were substantially removed: the antibiotics SMX, TMP, the estrogen estradiol and the anti-inflammatory NPX (> 80%). The other OMPs were only removed at a partial or low extent reaching the anoxic-aerobic IFAS (Figure 2). Their anaerobic biotransformation can be partially explained in terms of their chemical

1 structure. One of the functional groups of the antibiotic TMP is a substituted pyrimidine
2 ring which is biodegradable under anaerobic conditions [33], whereas the
3 biotransformation of SMX is related to the presence of the sulfonyl group (an electron-
4 withdrawing group) [11]. NPX can be biotransformed by o-demethylation of the
5 aromatic methoxy group [34]. The metabolite produced (o-desmethylnaproxen) is
6 recalcitrant under anaerobic conditions, although an aerobic post-treatment could further
7 improve its biotransformation [34,35]. No significant differences were observed in the
8 results during the UASB operation (Figure 2), since P1 and P2 were different only
9 concerning the anoxic-aerobic post-treatment stage. These results are in agreement with
10 previous studies carried out in UASB reactors used for treating medium-low strength
11 sewage [8,36,37].

20 Apart from the removal of COD and nitrogen, the importance of the polishing step is
21 crucial to obtain higher biotransformation efficiencies of most of the OMPs and
22 particularly of those which were not affected by the UASB reactor. Some compounds
23 such as BPA, E1, and IBP were mainly removed in the polishing stage, whereas there is
24 another group of OMPs for which the combination of the three redox conditions was
25 positive for their removal (ROX or EE2). This is an evidence of how hybrid systems
26 considering different redox environments can improve the possibilities of biological
27 reactors to enhance the biotransformation of many of the compounds present in the
28 complex mixtures of OMPs detected in sewage [10,36,38]. In fact, although higher
29 removal of OMPs is usually reported under aerobic than anoxic conditions, Torresi et
30 al., [39] showed that in absence of a cometabolic limitation, the biotransformation of
31 several OMPs can be more advantageous under anoxic than aerobic conditions.

42 Figure 3 shows the overall removal efficiencies obtained for the OMPs considering the
43 whole UASB + IFAS process among periods P1 and P2. Since the operation of the
44 UASB reactor suffered no operational changes, the differences between both periods are
45 related to the changes observed in the IFAS polishing stage. The most relevant
46 operational differences that could influence the OMP behavior were those related to
47 nitrification and biomass conformation: P1 only partial nitrification followed by
48 denitrification took place and co-existence of suspended biomass and biofilm in the
49 reactors; P2 complete nitrification and a biological process only driven by biofilm.
50 Despite the wash out of most of the suspended biomass, the OMP removal efficiencies
51 were maintained during P2. In the case of some OMPs, such as ERY, and to a lesser
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1 extent ROX and IBP, the removal efficiencies in P2 even improved. Other authors also
2 observed a higher activity in biofilm systems compared to suspended biomass in terms
3 of the removal of OMPs [40], confirming the importance of the biomass physical
4 conformation. One of the reasons indicated is the higher capacity of biofilms to protect
5 the bacteria against adverse conditions such as the presence of inhibitors [41,42].
6 Moreover, the longer sludge retention time of the attached biomass facilitates the
7 development of the slow-growing bacteria (such as nitrifiers) enhancing the microbial
8 diversity [15,19]. In this way, Alvarino et al. [8] observed a clear enhancement in the
9 removal of IBP and natural estrogens by incorporating carriers inside a bioreactor,
10 which was correlated with the increase of the nitrification rate.

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18 In the case of the natural hormones E1 and E2, removal efficiencies above 90% were
19 observed in both periods of operation. The presence of strong electron donating
20 functional groups (-OH) in their chemical structures explains their removal under
21 aerobic conditions [43]. This was very relevant in the case of E1 that was totally
22 biotransformed along the anoxic-aerobic stages. However, E2 was mainly removed in
23 the UASB (above 80%), with the remaining fraction degraded in the polishing stage
24 (Figure 2). This behavior is in accordance with Joss et al. [44] who compared the
25 removal of E1 under aerobic and anaerobic conditions and observed a higher
26 biotransformation rate with the positive redox conditions. The removal of the remaining
27 E2 in the anoxic-aerobic compartment was not affected by the shift of bacterial
28 populations observed among both periods (in P2 NOB and anammox bacteria were
29 developed). This is in accordance with Peng et al. [45] who observed that AOB-linked
30 bacteria biotransformation is dominant over the biotransformation carried out by other
31 bacteria.

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44 The removal of the synthetic hormone EE2 was below 70% throughout all periods of
45 operation (Figure 3). A positive contribution of the different redox conditions was
46 observed in the overall removal of this hormone. Unlike in the case of the natural
47 hormones, the removal of EE2 decreased slightly along the operation when the
48 polishing compartment worked as a biofilm reactor and the denitrification rate
49 decreased (P2). In fact, a linear relationship was found between the denitrification rate
50 and the EE2 removal (Figure 4), supporting its removal by cometabolism as previously
51 reported by Su et al.,[46]. They observed that the EE2 removal capacity is driven by
52 heterotrophic denitrifying activity. Moreover, heterotrophic bacteria can contribute to
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1 the removal of EE2, for instance, *Sphingobacterium* is able to biotransform EE2 to E1
2 under aerobic conditions [47].

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4 A recalcitrant behavior of the anti-inflammatory IBP was observed under anaerobic
5 conditions, while it was readily biotransformed under aerobic conditions (Figure 2).
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7 Previous studies showed the influence of nitrification in IBP removal [8,48]. This is a
8 result of the availability of secondary and tertiary carbons in linear alkyl chains in its
9 chemical structure to be hydroxylated by AMO, as well as to the action of NOB [13]. In
10 the reactor, a biotransformation enhancement was observed in P2 for IBP (Figure 3),
11 that might be related to the presence of NOB bacteria when the total nitrification was
12 achieved. In fact, Fernandez-Fontaina et al. [13] determined higher biotransformation
13 rates of IBP when the NOB activity was the predominant in the nitrification. The
14 contribution of other bacteria in IBP removal, such as heterotrophic bacteria, has to be
15 considered, even though these bacteria influence to less extent IBP biotransformation
16 than autotrophic bacteria [13,48].
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26 The removal of the antibiotics ERY and ROX was clearly enhanced throughout the
27 second period of operation (Figure 3). In fact, the behavior of ERY along P1 was quite
28 recalcitrant (removals below 25%), whereas in P2 it was almost completely removed
29 (higher than 95%). ROX also suffered a moderate removal increase along P2. In this
30 case, the development of NOB leading to total nitrification and the growth of anammox
31 bacteria could be factors explaining these observations. In fact, Torresi et al. [49]
32 observed a positive correlation of ERY removal rate and the microbial diversity.
33 Alvarino et al. [8] reported a direct correlation between the ammonium degradation rate
34 and the removal of both antibiotics. Additionally, the influence of the anammox activity
35 on ERY removal in nitrification-anammox reactors was also reported [50].
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45 As shown in Figure 3, DCF was the only compound that exhibited higher removal
46 efficiency in P1 than in P2. The explanation seems to be related to the presence of
47 nitrite in the aerobic compartment (Figure 5a), since only partial nitrification/denitrification
48 was taking place. In fact, a correlation was observed between the nitrite concentration
49 and DCF removal (Figure 5a). Osorio et al. [51] studied the removal of DCF in a
50 conventional nitrification-denitrification process and detected the metabolites nitroso-
51 DCF and 5-nitro-diclofenac in the presence of nitrite. Nitroso-DCF is produced by the
52 nitrosation of the nitrogen atom present in the molecule of DCF, whereas the formation
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2 of 5-nitro-diclofenac is related to the transfer of a nitro group by an electron-deficient
3 group on the aromatic ring of the phenylacetic acid [52].

4 The fate of the endocrine disruptor BPA shows that it is recalcitrant under anaerobic
5 conditions but readily biotransformed during the anoxic-aerobic stage in both periods of
6 operation (Figures 2 and 3). Its degradation can be related to the nitrification rate in the
7 polishing step [53,54]. In the case of the BPA removal efficiency, a correlation with the
8 nitrate concentration in the aerobic chamber was observed (Figure 5b), which evidences
9 the role of the nitrite oxidation bacteria (NOB) in this transformation. Even though, the
10 BPA removal efficiencies were quite stable during the whole operation of the hybrid
11 system (Figure 3). This indicated that also other bacteria (e.g. heterotrophic bacteria)
12 have played a role in its biotransformation [55].
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23 4. CONCLUSIONS

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26 In the context of developing more sustainable processes for low-strength wastewater
27 treatment, anaerobic digestion based processes are becoming more attractive. The
28 innovative process proposed in this work, based in the combination of an anaerobic step
29 (UASB reactor) and an IFAS system, is especially suited to minimize problems derived
30 from this approach, such as nitrogen and methane emissions, as well as to achieve high
31 removal efficiencies for a wide range of OMPs. In this system, high removals of COD
32 were achieved (93%), whereas nitrogen was removed by a consortia of heterotrophic
33 denitrifiers, methane oxidation, and anammox microorganisms. Methanotrophs present
34 in suspended biomass and biofilms in the anoxic-aerobic compartments were
35 responsible for the reduction of dissolved methane up to 85%. The anaerobic stage
36 enhanced the removal of several OMPs, such as the antibiotics TMP and SMX with
37 respect to the conventional anoxic-aerobic processes. The combination of several
38 biomass conformations enhanced the microbiology of the reactor, mainly under anoxic
39 conditions. The growth of anammox bacteria in the biofilm influenced positively the
40 removal of the antibiotic ERY. A correlation between the presence of nitrite in the
41 aerobic compartment and the removal of DCF was observed, while the removal of BPA
42 was slightly dependent on the nitrite oxidation. The biomass washout episode did not
43 have a significant effect on OMP removal (showing the crucial role of biofilm),
44 although methanotrophs were clearly affected. The maintenance of biomass
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1 concentrations rich in these organisms would be a tool to optimize the system in terms
2 of nitrogen and methane abatement.
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7
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22 **REFERENCES**

- 23
24
25
26 [1] Liu, H.Q., Lam, J.C., Li, W.W., Yu, H.Q., Lam, P.K., Spatial distribution and
27 removal performance of pharmaceuticals in municipal wastewater treatment plants in
28 China, *Sci. Total Environ.* 586 (2017) 1162-1169.
29
30
31 [2] Fernández, M., Fernández, M., Laca, A., Díaz, M., Seasonal occurrence and removal
32 of pharmaceutical products in municipal wastewater, *J. Environ. Chem. Eng.* 2 (2014)
33 495–502.
34
35
36
37 [3] Beijer, K., Björlenius, B., Shaik, S., Lindberg, R.H., Brunström, B., Brandt, I.,
38 Removal of pharmaceuticals and unspecified contaminants in sewage treatment
39 effluents by activated carbon filtration and ozonation: Evaluation using biomarker
40 responses and chemical analysis, *Chemosphere* 176 (2017) 342-351.
41
42
43 [4] Alvarino, T., Komesli, O., Suarez, S., Lema, J.M., Omil, F., The potential of the
44 innovative SeMPAC process for enhancing the removal of recalcitrant organic
45 micropollutants. *J. Hazard. Mater.* 308 (2016) 29-36.
46
47
48 [5] Altmann, J., Ruhl, A.S., Zietzschmann, F., Jekel, M., Direct comparison of ozonation
49 and adsorption onto powdered activated carbon for micropollutant removal in advanced
50 wastewater treatment, *Water Res.* 55 (2014) 185-193.
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65
- [6] De Gisi, S., Lofrano, G., Grassi, M., Notarnicola, M., Characteristics and adsorption capacities of low-cost sorbents for wastewater treatment: A review, *Sust. Mater. Technol.* 9 (2016) 10–40.
- [7] Nielsen, U., Hastrup, C., Klausen, M.M., Pedersen, B.M., Kristensen, G.H., Jansen, J.L.C., Bak, S.N., Tuerk, J., Removal of APIs and bacteria from hospital wastewater by MBR plus O₃, O₃+ H₂O₂, PAC or ClO₂, *WaterSci. Technol.* 67 (2013) 854–862.
- [8] Alvarino, T., Suarez, S., Garrido, J.M., Lema, J.M., Omil, A UASB reactor coupled to a hybrid aerobic MBR as innovative plant configuration to enhance the removal of organic micropollutants, *Chemosphere* 144 (2016) 452-458.
- [9] Escolà Casas, M., Chhetri, R.K., Ooi, G., Hansen, K.M., Litty, K., Christensson, M., Kragelund, C., Andersen, H.R., Bester, K., Biodegradation of pharmaceuticals in hospital wastewater by a hybrid biofilm and activated sludge system (Hybas), *Sci. Total Environ.* 530-531 (2015) 383-392.
- [10] Alvarino, T., Suarez, S., Lema, J.M., Omil, F., Understanding the sorption and biotransformation of organic micropollutants in innovative biological wastewater treatment technologies, *Sci. Total Environ.* 615 (2018) 297-306.
- [11] Alvarino, T., Suarez, S., Lema, J.M., Omil, F., Understanding the removal mechanisms of PPCPs and the influence of main technological parameters in anaerobic UASB and aerobic CAS reactors, *J. Hazard. Mater.* 278 (2014) 506-513.
- [12] Kassotaki, E., Pijuan, M., Joss, A., Borrego, C.M., Rodriguez-Roda, I., Buttiglieri, G., Unraveling the potential of a combined nitrification-anammox biomass towards the biodegradation of pharmaceutically active compounds, *Sci. Total Environ.* 624 (2018) 722–731.
- [13] Fernandez-Fontaina, E., Gomes, I.B., Aga, D.S., Omil, F., Lema, J.M., Carballa, M., Biotransformation of pharmaceuticals under nitrification, nitrification and heterotrophic conditions. *Sci. Total Environ.* 541 (2016) 1439-1447.
- [14] Falås, P., Wick, A., Castronovo, S., Habermacher, J., Ternes, T.A., Joss, A., Tracing the limits of organic micropollutant removal in biological wastewater treatment, *Water Res.* 95 (2016) 240-249.
- [15] Jiang, Q., Ngo, H.H., Nghiem, L.D., Hai, F.I., Price, W.E., Zhang, J., Liang, S., Deng, L., Guo, W., Effect of hydraulic retention time on the performance of a hybrid

1 moving bed biofilm reactor-membrane bioreactor system for micropollutants removal
2 from municipal wastewater, *Bioresour. Technol.* 247 (2018) 1228-1232.

3
4 [16] Di Trapani, D., Di Bella, G., Mannina, G., Torregrossa, M., Viviani, G.,
5 Comparison between moving bed-membrane bioreactor (MB-MBR) and membrane
6 bioreactor (MBR) systems: influence of wastewater salinity variation,
7 *BioresourTechnol.* 162 (2014) 60-69.

8
9
10
11 [17] Falås, P., Baillon-Dhumez, A., Andersen, H.R., Ledin, A., la CourJansen, J.,
12 Suspended biofilm carrier and activated sludge removal of acidic pharmaceuticals,
13 *Water Res.* 46 (2012) 1167-1175.

14
15
16
17 [18] Reif, R., Besancon, A., Corre, K.L., Jefferson, B., Lema, J.M., Omil, F.,
18 Comparison of PPCPs removal on a parallel-operated MBR and AS system and
19 evaluation of effluent post-treatment on vertical flow reedbeds, *WaterSci. Technol.*
20 63(2011) 2411-2417.

21
22
23
24 [19] de la Torre, T., Alonso, E., Santos, J.L., Rodríguez, C., Gómez, M.A., Malfeito,
25 J.J., Trace organics removal using three membrane bioreactor configurations: MBR,
26 IFAS-MBR and MBMBR, *WaterSci. Technol.* 71 (2015) 761-768.

27
28
29
30 [20] Clara, M., Kreuzinger, N., Strenn, B., Gans, O., Kroiss, H., The solids retention
31 time – a suitable design parameter to evaluate the capacity of wastewater treatment
32 plants to remove micropollutants, *Water Res.* 39(2005) 97-106.

33
34
35
36 [21] Noyola, A., Morgan, J.M., Lopez, J.E., Treatment of Biogas Produced in
37 Anaerobic Reactors for Domestic Wastewater: Odor Control and Energy/Resource
38 Recovery, *Rev. Environ. Sci. Biotechnol.* 5 (2006) 93-114.

39
40
41
42 [22] Sánchez, A., Rodríguez-Hernández, L., Buntner, D., Esteban-García, A. L., Tejero,
43 I. and Garrido, J. M., Denitrification coupled with methane oxidation in a membrane
44 bioreactor after methanogenic pre-treatment of wastewater, *J. Chem. Technol.*
45 *Biotechnol.* 91 (2016) 2950–2958

46
47
48
49 [23] Ettwig, K.F., Butler, M.K., Le Paslier, D., Pelletier, E., Mangenot, S., Kuypers,
50 M.M., Schreiber F., Dutilh, B.E., Zedelius, J., de Beer, D., Gloerich, J., Wessels, H.J.,
51 van Alen, T., Luesken, F., Wu, M.L., van de Pas-Schoonen, K.T., Op den Camp, H.J.,
52 Janssen-Megens, E.M., Francoijs, K.J., Stunnenberg, H., Weissenbach, J., Jetten, M.S.
53 Strous, M., Nitrite-driven anaerobic methane oxidation by oxygenic bacteria, *Nature* 464
54 (2010) 543-548.

- 1
2
3
4
5
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58
59
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61
62
63
64
65
- [24] Zhu, J., Wang, Q., Yuan, M., Tan, G.Y.A., Sun, F., Wang, C., Wu, W., Lee, P.H., Microbiology and potential applications of aerobic methane oxidation coupled to denitrification (AME-D) process: A review. *Water Res.* 90 (2016) 203–215.
- [25] Chen, Xueming, JianhuaGuo, Guo-Jun Xie, Zhiguo Yuan, and Bing-Jie Ni. (2016) “Achieving Complete Nitrogen Removal by Coupling Nitrification-Anammox and Methane-Dependent Denitrification: A Model-Based Study.” *Biotechnology and Bioengineering* 113 (5) 1035–45.
- [26] APHA-AWWA-WPCF, *Methods for the Examination of Water and Wastewater*, twentieth ed. American Public Health Association/American Water Works Association/Water Environment Federation, 1999, Washington DC, USA.
- [27] Amann, R., Ludwig, W., Schleifer, K. H., Phylogenetic identification and in-situ detection of individual microbial-cells without cultivation, *Microbiol. Rev.* 59 (1995) 143-169.
- [28] Noyola, A., Morgan-Sagastume, J.M., López-Hernández, J.E., Treatment of biogás produced in anaerobic reactors for domestic wastewater: Odor control and energy/resource recovery, *Rev. Environ. Sci. Biotechnol.* 5 (2006) 93–114.
- [29] Anthonisen, A.C., Loehr, R.C., Prakasam, T.B., Srinath, E.G., Inhibition of nitrification by ammonia and nitrous acid. *J. Water Pollut. Control Fed.* 48 (1976) 835–852.
- [30] Villaverde, S., Fdz-Polanco, F., García, P.A., Nitrifying biofilm acclimation to free ammonia in submerged biofilters. Start-up influence, *Water Res.* 34 (2000) 602-610.
- [31] Torresi, E., Polesel, F., Bester, K., Christensson, M., Smets, B.F., Trapp, S., Andersen, H.R., Gy, B., . Diffusion and sorption of organic micropollutants in biofilms with varying thicknesses. *Water Res.* 123 (2017) 388-400.
- [32] Suárez, S., Carballa, M., Omil, F., Lema, J.M., How are pharmaceutical and personal care products (PPCPs) removed from urban wastewaters? *Rev. Environ. Sci. Biotechnol.* 7 (2008) 125-138.
- [33] Adrian, N.R., Suflita, Ü.M., Anaerobic biodegradation of halogenated and nonhalogenated N-, S-, and O-heterocyclic compounds in aquiferslurries, *Environ.Toxicol. Chem.* 13 (1994) 1551-1557.

- 1
2
3
4
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56
57
58
59
60
61
62
63
64
65
- [34] Ghattas, A.K., Fischer, F., Wick, A., Ternes, T.A., Anaerobic biodegradation of (emerging) organic contaminants in the aquatic environment, *Water Res.* 116 (2017) 268-295.
- [35] Lahti, M., Oikari, A., Microbial transformation of pharmaceuticals naproxen, bisoprolol and diclofenac in aerobic and anaerobic environments, *Arch. Environ. Contam. Toxicol.* 61 (2019)202-210.
- [36] de Wilt, A., He, Y., Sutton, N., Langenhoff, A., Rijnaarts, H., Sorption and biodegradation of sixpharmaceutically active compounds under four different redox conditions, *Chemosphere* 193 (2018) 811-819.
- [37] de Graaff, M.S., Vieno, N.M., Kujawa-Roeleveld, K., Zeeman, G., Temmink , H., Buisman, C.J.N., Fate of hormones and pharmaceuticals during combined anaerobic treatment and nitrogen removal by partial nitrification-anammox in vacuum collected blackwater. *Water Res.* 45(2011) 375-383.
- [38] Falås, P., Wick, A., Castronovo, S., Habermacher, J., Ternes, T.A., Joss, A., Tracing the limits of organic micropollutant removal in biological wastewater treatment. *Water Res.* 95 (2016) 240-249.
- [39] Torresi, E., Gülay, A. , Polesel, F., Jensen, M.M., Christensson, M., Smets, B.F., Plósz, B.G., Reactor staging influences microbial community composition and diversity of denitrifying MBBRs- Implications on pharmaceutical removal. *Water Res.* 138 (2018) 333-345.
- [40] Falås, P., Longrée, P., la Cour Jansen, J., Siegrist, H., Hollender, J., Joss, A., Micropollutant removal by attached and suspended growth in a hybrid biofilm-activated sludge process, *Water Res.* 47(2013) 4498-4506.
- [41] Ooi, G.T.H., Tang, K., Chhetri, R.K., Kaarsholm, K.M.S., Sundmark, K., Kragelund, C., Litty, K., Christensen, A., Lindholst, S., Sund, C., Christensson, M., Bester, K., Andersen, H.R., Biodegradation of pharmaceuticals in a pilot-scale staged Moving Bed Biofilm Reactors (MBBRs). 15th International Conference on Environmental Science and Technology, Rhodes, Greece, 2017.
- [42] Butler, C.S., Boltz, J.P., Biofilm Processes and Control in Water and Wastewater Treatment, *Earth Systems Environ. Sci.* 3 (2014) 90–107.
- [43] Grandclément, C., Seyssiecq, I., Piram, A., Wong-Wah-Chung, P., Vanot, G., Tiliacos, N., Roche, N., Doumenq, P., From the conventional biological wastewater

1 treatment to hybrid processes, the evaluation of organic micropollutant removal: A
2 review, *Water Res.* 111 (2017) 297-317.

3
4 [44] Joss, A. , Ternes, H.A., Richle, P., Siegrist, H., Removal of estrogens in municipal
5 wastewater treatment under aerobic and anaerobic conditions: consequences for plant
6 optimization, *Environ. Sci. Technol.* 38(2004) 3047-3055.

7
8
9 [45] Peng, L., Dai, X., Liu, Y., Sun, J., Song, S., Ni, B.J., Model-based assessment of
10 estrogen removal by nitrifying activated sludge, *Chemosphere* 197 (2018) 430-437.

11
12 [46] Su, L., Aga, D., Chandran, K., Khunjar, W.O., Factors impacting biotransformation
13 kinetics of trace organic compounds in lab-scale activated sludge systems performing
14 nitrification and denitrification. *J. Hazard. Mater.* 282 (2005) 116-24.

15
16 [47] Haiyan, R., Shulan, J., uddin Ahmad, N., Dao, W., Chengwu, C., Degradation
17 characteristics and metabolic pathway of 17 α -ethinylestradiol by *Sphingobacterium* sp.
18 *JCR5*, *Chemosphere* 66 (2007) 340–346.

19
20 [48] Tran, H.T., Urase, T., Kusakabe, O., The characteristics of enriched nitrifier culture
21 in the degradation of selected pharmaceutically active compounds, *J. Hazard. Mater.*
22 171 (2009) 1051-1057.

23
24 [49] Torresi, E., Fowler, S.J., Polesel, F., Bester, K., Andersen, H.R., Smets, B.F.,
25 Plósz, B.G., Christensson, M., Biofilm Thickness Influences Biodiversity in Nitrifying
26 MBBRs-Implications on Micropollutant Removal. *Environ. Sci. Technol.* 50 (2016)
27 9279-88.

28
29 [50] Alvarino, T., Suarez, S., Katsou, E., Vazquez-Padin, J., Lema, J.M., Omil, F.,
30 Removal of PPCPs from the sludge supernatant in a one stage nitrification/anammox
31 process. *Water Res.* 68 (2015) 701-709.

32
33 [51] Osorio, V., Sanchís, J., Abad, J.L., Ginebreda, A., Farré, M., Pérez, S., Barceló, D.,
34 Investigating the formation and toxicity of nitrogen transformation products of
35 diclofenac and sulfamethoxazole in wastewater treatment plants, *J. Hazard. Mater.* 309
36 (2016) 157-164.

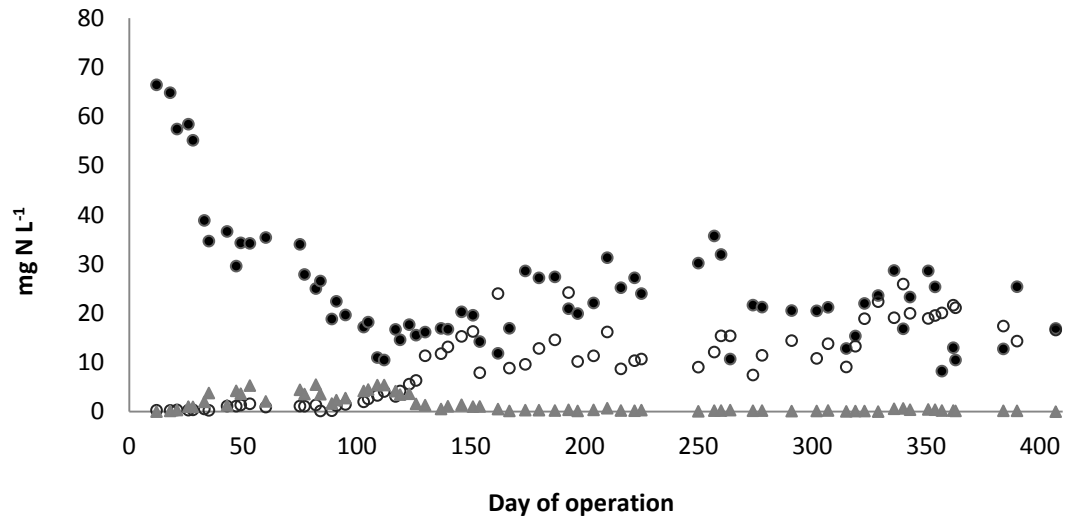
37
38 [52] Osorio, V., Imbert-Bouchard, M., Zonja, B., Abad, J.L., Pérez, S., Barceló, D.,
39 Simultaneous determination of diclofenac, its human metabolites and microbial
40 nitration/nitrosation transformation products in wastewaters by liquid
41 chromatography/quadrupole-linear ion trap mass spectrometry, *J. Chromatogr. A* 1347
42 (2014) 63–71.

1 [53] Yang, S., Hai, F.I., Price, W.E., McDonald, J., Khan, S.J., Nghiem, L.D.,
2 Occurrence of trace organic contaminants in wastewater sludge and their removals by
3 anaerobic digestion, *Bioresour. Technol.* 210 (2016) 153–159.
4

5 [54] Guerra, P., Kim, M., Teslic, S., Alaei, M., Smyth, S.A., Bisphenol-A removal in
6 various wastewater treatment processes: operational conditions, mass balance, and
7 optimization, *J EnvironManage.* 152 (2015) 192-200.
8
9

10 [55] Zielińska, M., Cydzik-Kwiatkowska, A., Bernat, K., Bułkowska, K., Wojnowska-
11 Baryła, I., Removal of bisphenol A (BPA) in a nitrifying system with immobilized
12 biomass. *Bioresour. Technol.* 171 (2014) 305-313.
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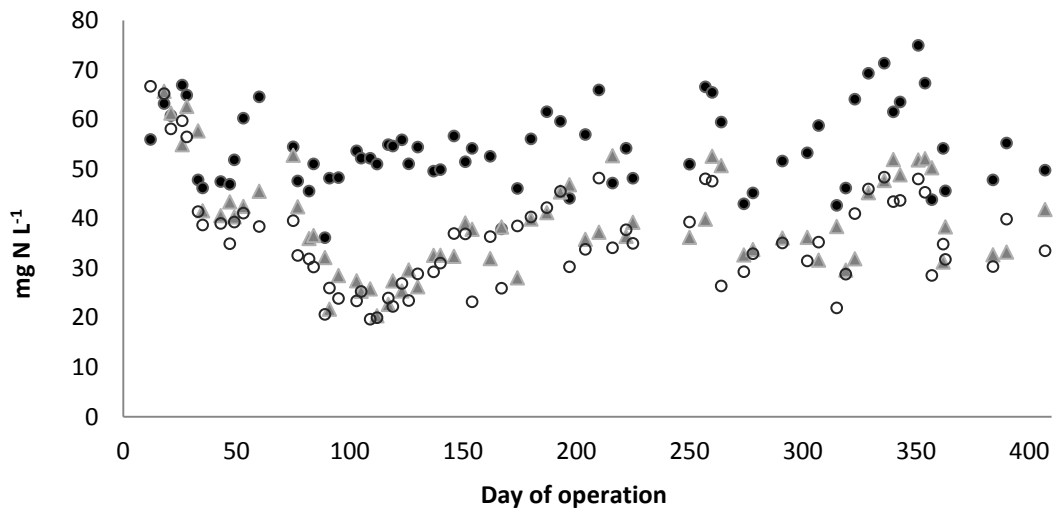


Figure 1. Evolution of A) the nitrogen ions concentration in the final effluent: N-NH_4^+ (\bullet), N-NO_2^- (\blacktriangle) and N-NO_3^- (\circ); and B) the comparison of the TN in the three stages: anaerobic (\bullet), anoxic (\blacktriangle) and aerobic (\circ) stages.

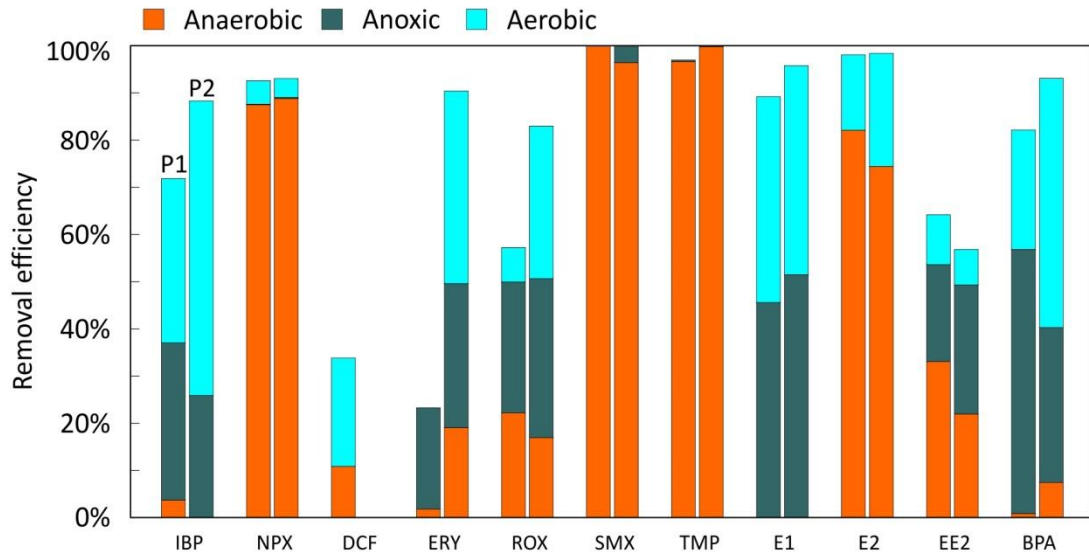


Figure 2. OMPs removal efficiencies attained in each compartment: anaerobic, anoxic and aerobic along both periods of operation (P1 and P2).

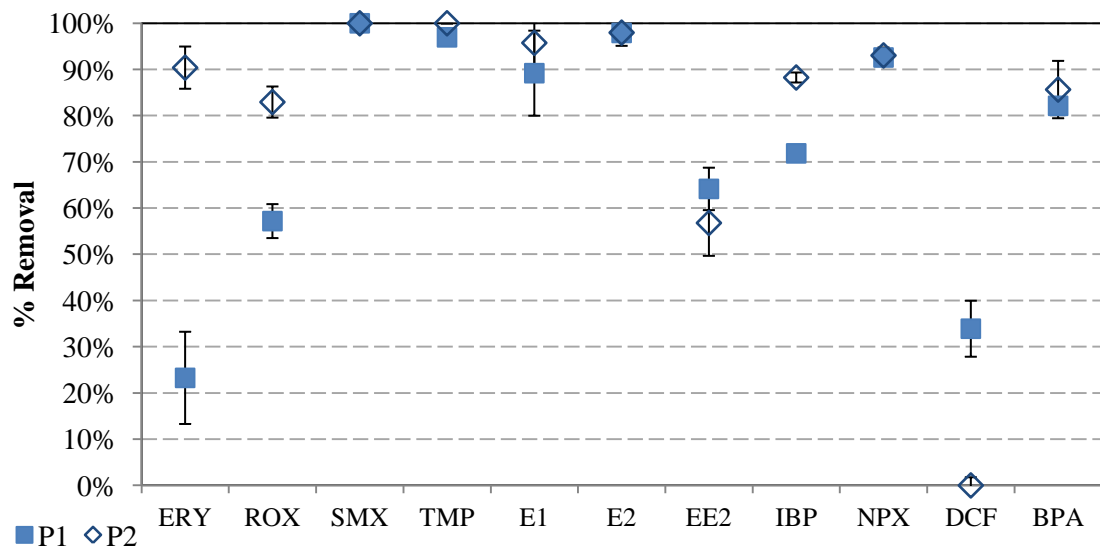


Figure 3. Comparison among both periods of operation in terms of OMPs removal attained in the overall system.

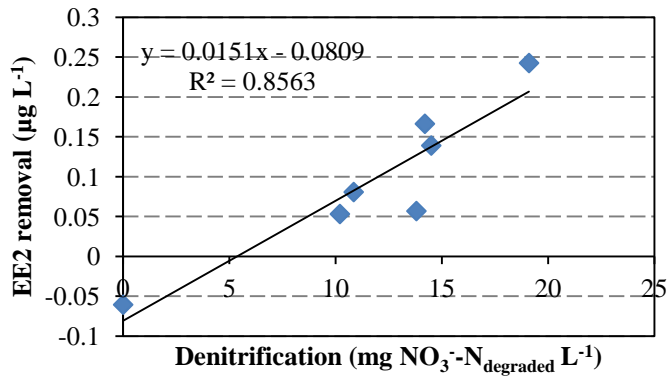


Figure 4. Influence of the denitrification rate in the removal of the hormone EE2.

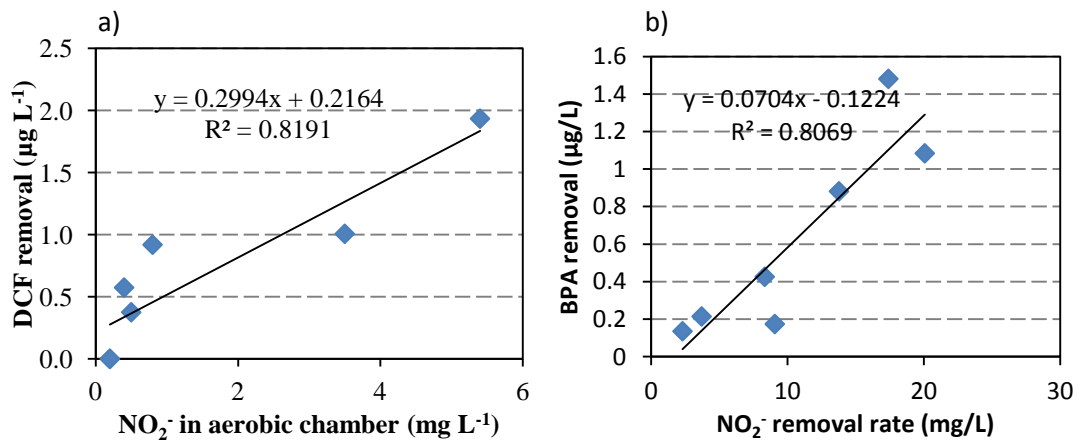


Figure 5. Influence of presence of nitrite in the aerobic compartment in the removal of DCF (a) and the nitrite oxidation in the removal of BPA (b).

Materials and Methods

Molecular biology techniques

Ntspa712 (most members of phylum Nitrospirae), Nso1225 (Ammonio-oxidizing- β -Proteobacteria), NIT3 (Nitrobacter spp.), Amx368 (all anammox bacteria), MG705 and MG84 (Type I methanotrophs), DBACT193 and DBACT1027 (damo bacteria) and DARCH872 (damo archaea) were the probes selected for FISH analysis. All the details of each probe (formamide percentage, sequence and target organism) can be found in the probe-Base database (<http://www.microbial-ecology.net/probebase/>). Fluorescence signals were recorded with an acquisition system (Coolsnap, Roper Scientific Photometrics) coupled with an Axioskop2 epifluorescence microscope (Zeiss, Germany).

For the sequencing of the 16S rRNA gene, the protocol described by Regueiro et al. [1] was followed with few modifications. In brief, after extracting the bulk DNA, the V3V4 and V2V3 regions of the 16S rRNA gene were amplified for Bacteria [2] and Archaea [3] domains respectively, and sequenced in an Illumina MiSeq platform. Raw sequences were filtered to remove low-quality reads and then clustered into Operational Taxonomic Units (OTU) at 97% of sequence similarity using QIIME v1.9.1 [4]. Community diversity was analyzed to measure the compositional complexity of reactor microbiome. Richness was evaluated by the estimated the number of species (S) and the Gini-Simpson index (HGS) while community evenness was measured with the Simpson evenness index (E). The community similarity was determined by Bray-Curtis dissimilarities and explored by non-metric multidimensional scaling (NMDS). All statistical analyses were performed in R [5]. Finally, a co-occurring microbial network was built for the abundant OTUs (relative abundances over 0.1%) with CoNet v.1.1.1 [6].

[1] L. Regueiro, P. Veiga, M. Figueroa, J. Alonso-Gutierrez, A.J.M. Stams, J.M. Lema, M. Carballa, Relationship between microbial activity and microbial community structure in six full-scale anaerobic digesters, *Microbiol. Res.* 167 (2012) 581–589.

- [2] A. Klindworth, E. Pruesse, T. Schweer, J. Peplies, C. Quast, M. Horn, F.O. Glöckner, Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies, *Nucleic Acids Res.* 41 (2013).
- [3] P. Cruaud, A. Vigneron, C. Lucchetti-Miganeh, P.E. Ciron, A. Godfroy, M.A. Cambon-Bonavita, Influence of DNA extraction method, 16S rRNA targeted hypervariable regions, and sample origin on microbial diversity detected by 454 pyrosequencing in marine chemosynthetic ecosystems, *Appl. Environ. Microbiol.* 80 (2014) 4626–4639.
- [4] J.G. Caporaso, J. Kuczynski, J. Stombaugh, K. Bittinger, F.D. Bushman, E.K. Costello, N. Fierer, A.G. Peña, J.K. Goodrich, J.I. Gordon, G.A. Huttley, S.T. Kelley, D. Knights, J.E. Koenig, R.E. Ley, C.A. Lozupone, D. McDonald, B.D. Muegge, M. Pirrung, J. Reeder, J.R. Sevinsky, P.J. Turnbaugh, W.A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, R. Knight, QIIME allows analysis of high-throughput community sequencing data, *Nat. Methods* 7 (2010) 335–336.
- [5] R. Core Team, *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna, Austria, 2016.
- [6] K. Faust, J.F. Sathirapongsasuti, J. Izard, N. Segata, D. Gevers, J. Raes, C. Huttenhower, Microbial co-occurrence relationships in the human microbiome, *PLoS Comput. Biol.* 8 (2012) e1002606.