Cometabolic removal of organic micropollutants by enriched nitrite-dependent anaerobic methane oxidizing cultures

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Cometabolic removal of organic micropollutants by enriched nitrite-dependent anaerobic methane oxidizing cultures

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Abstract

The innovative and recently discovered n-damo process, based on anaerobic methane oxidation with nitrite, was developed in a membrane-based bioreactor and evaluated in terms of organic micropollutants (OMPs) removal. The main singularity of this study consisted in the evaluation of organic micropollutants (OMPs) removal in the biological reactor. A strategy consisting on progressively increasing the nitrogen loading rate in order to increase the specific denitrification activity was followed to check if the selected OMPs were co-metabolically biotransformed. Significant nitrite removal rate (24.1 mg N L^{-1} d^{-1}) was achieved after only 30 days of operation. A maximum specific removal of 186.3 mg N $gVSS^{-1}$ d^{-1} was obtained at the end of the operation, which is one of the highest previously reported. A successfully n-damo bacteria enrichment was achieved, being *Candidatus* Methylomirabilis the predominant bacteria during the whole operation attaining a maximum relative abundance of about 40%. The natural hormones (E1 and E2) were completely removed in the bioreactor. The specific removal rates of erythromycin (ERY), fluoxetine (FLX), roxithromycin (ROX) and sulfamethoxazole (SMX) were successfully correlated with the specific nitrite removal rates, suggesting a co-metabolic biotransformation.

Keywords: n-damo process, organic micropollutants, cometabolism, enrichment, MBR

1. INTRODUCTION

Anaerobic wastewater treatment processes have been largely applied in areas with temperate and warm climates due to their lower energy consumption, generation of an enriched-methane biogas and lower biomass production, relative to conventional aerobic systems. The generated effluents can pose an environmental problem due to their high ammonia concentration, and their significant content in dissolved methane, which is very often directly desorbed into the atmosphere (Noyola et al., 2006; Souza et al., 2011).

Conventional biological nitrogen removal process has been applied to urban wastewater in order to reduce water eutrophication in downstream water bodies. The main drawbacks of this process (as requirement of a biodegradable carbon source, high energy consumption, sludge production and greenhouse gas emissions) can be addressed with novel treatment processes, such as those based on autotrophic anaerobic ammonium oxidation (anammox), combined with nitrite/nitrate dependent anaerobic methane oxidation (n-damo), which have proven to reduce effectively nitrogen from wastewater economically and with less environmental impact (Liu et al., 2019).

The recently discovered n-damo bacteria (*Candidatus* Methylomirabilis Species: M. oxyfera; M. sinica; M. lanthanidiphila), bacteria affiliated with the NC10 phylum, have demonstrated their ability to perform both nitrogen and methane removal, by anaerobically oxidizing methane using nitrite as electron acceptor (Ettwig et al., 2010; He et al., 2016; Versantvoort et al., 2018). The slow growth of this microorganism, between 1 and 2 weeks, implies that the enrichment process of this bacteria in a mixed culture may take several months (Allegue

et al., 2018; Ettwig et al., 2009). Thus, in order to avoid problems related with biomass washout, n-damo bacteria enrichments have been successfully achieved using membrane bioreactor-based configurations (MBR). Among the first results using this type of configuration were those reported by Kampman et al. (2014) achieving a nitrite removal rate of 36 mg N L^{-1} d^{-1}. After further development, these results could be improved to nitrite removal rates of 116 mg N L^{-1} d^{-1} by Allegue et al. (2018).

N-damo microorganisms (bacteria and archaea) are currently object of interest because of their suitability to develop innovative processes in which methane and nitrogen compounds can be simultaneous removed. Additionally, previous studies showed positive results at pilot scale hybrid (anoxic-aerobic) reactors for the removal of organic micropollutants (OMPs) (Arias et al., 2018). However, until now, the n-damo based technology is not suitable for its implementation in WWTPs since it is not possible to achieve high denitrification activities (Wang et al., 2017). Thus, more research about is needed to enhance their metabolic activity as well as to analyze the possible effects on their development in the presence of other substances like OMPs.

OMPs are currently considered as an important challenge to be addressed by the new innovative technology developed, since they have been linked with several environmental risks (Tran et al., 2018). The European Union started to advance in the regulation of these contaminants in water (Sousa et al., 2018), by the establishment of a watchlist that identifies 8 compounds that should be monitored (last updated by Commission decision (EU) 2018/840 of 5 June 2018). It included in 2015 three macrolide antibiotics - erythromycin, azithromycin and clarithromycin; three hormones – estrone, estradiol and ethinylestradiol; one anti-inflammatory drug – diclofenac (DCF). Its update 2018 supposed among others the

incorporation of two additional antibiotics: amoxicillin (group of penicillins) and ciprofloxacin (group of quinolones), as well as the removal of DCF.

Among the different factors which determine OMP removal in biological reactors, the redox conditions have shown to highly influence their fate. Depending on the condition applied, different microbial populations will grow, activating different metabolic routes and enzymes and, consequently, determining the biotransformation route of the micropollutant. In general, removal efficiencies are larger in aerobic conditions compared to anoxic ones, due to the higher oxidation potential of the oxygen compared with NO_x species (Alvarino et al., 2018). The combination of different redox environments normally maximizes the final removal efficiency (Arias et al., 2018). A lack of knowledge on the OMP biotransformation in anoxic environments is still identified (Torresi et al., 2017).

OMPs are commonly present at trace levels in water bodies. Consequently, cometabolism is considered as the main biotransformation mechanism, in which the presence of a growth substrate is needed to promote the production of non-specific enzymes which can accidentally biotransform OMPs (Fischer and Majewsky, 2014; Plosz et al., 2010; Tran et al., 2013). One of the most studied cometabolic processes are linked to nitrification, in which the ammonium monooxygenase enzyme has proven to have an active role on several OMP biotransformation due to its low specificity (Fernandez-Fontaina et al., 2016). Under anoxic environments, Polesel et al. (2017) found positive correlations between the specific heterotrophic denitrifying activity and the biotransformation rate constants of different non-recalcitrant compounds. Until now, few studies about cometabolism were performed under anaerobic or anoxic environments (Gonzalez-Gil et al., 2019; Pomiès et al., 2015). As far as

the authors know, there are neither previous studies about OMP removal in processes based on the use of n-damo microorganisms.

The aim of this study was to achieve an enriched n-damo bacteria culture in an MBR-based configuration and to assess the removal efficiency of OMPs in the system. The research was focused on monitoring the reactor performance at increasing nitrogen loading rates in order to evaluate the robustness of the system in terms of conventional parameters and bacterial dynamics. Besides, the cometabolic biotransformation of OMPs was assessed by studying the correlations between the denitrification kinetics and the OMP removal rates.

2. MATERIALS AND METHODS

2.1 Reactor configuration and operation

A 10 L lab-scale MBR was used to carry out the enrichment of n-damo culture, with a working volume of 6.65 L (Fig. S1). Due to low activities of these microorganisms, complete biomass retention in the reactor was achieved by using a submerged hollow-fiber ultrafiltration membrane module (Puron), which was operated in cycles of 7 min of permeation and 0.5 min of relaxation. The surface of the membrane was 0.5 m^2 and the pore size 0.03 μm. A pressure sensor PN2069 (IFM) was used to record permeation and relaxation transmembrane pressures and an Atlas Scientific pH Probe was placed inside the reactor. The reactor was provided with a thermostatic bath to maintain the temperature at 28 ºC.

The inoculum used was taken from a denitrifying pre-anoxic MBR treating an UASB effluent, in which dissolved methane was used as carbon source for denitrification (Silva-Teira et al., 2017). This biomass was composed of a complex microbial population and characterized by a low presence of n-damo bacteria. A total suspended solids concentration

131 of 0.5 g L^{-1} was used as inoculum. The system was fed with a medium containing 9 mg L^{-1}
132 of NH_4Cl, 100 mg L^{-1} of $NaHCO_3$ and $NaNO_2$ as nitrite source according to biomass activity
133 capacity and requirements for this study. The addition of ammonium nitrogen was decided
134 according to previous studies suggesting that there is a need of supplying an additional
135 nitrogen source to promote n-damo bacteria growth, as they are unable to extract it from their
136 denitrification pathway (Wang et al., 2019). Macronutrients and trace compounds used in the
137 enrichment were the proposed by Allegue et al. (2018), and the same feeding strategy was
138 performed. Aluminum foil feeding bags, which are totally impermeable to oxygen, were used
139 and feeding was deoxygenated to avoid the presence of oxygen by using nitrogen gas during
140 10 min. A programmable PLC Micro 820 (Allen-Bradley) connected to a computer was used
141 to control the operation of the system. Apart from the liquid feed, 9 L d^{-1} of a gas mixture,
142 composed of 95% of CH_4 and 5% of CO_2, were supplied to the system by using a mass-flow
143 controller 4800 Series (Brooks). CO_2 provided buffer capacity to the system as well as the
144 sodium bicarbonate added in the liquid feed. A 5.5 L min^{-1} recirculation gas from the top of
145 the reactor to the bottom using a mini laboratory blower N 86 KT. 18 (Laboport) made
146 available methane and CO_2 in excess in order to avoid electron donor or buffer limitation,
147 attain complete mixing and prevent membrane fouling.

148 Twelve OMPs known to be present in sewage, were spiked continuously to the synthetic
149 feed: three neurodrugs (fluoxetine FLX, carbamazepine CBZ, diazepam DZP), four
150 antibiotics (erythromycin ERY, roxithromycin ROX, sulfamethoxazole SMX, trimethoprim
151 TMP), three endocrine disruptors (estrone E1, β-estradiol E2, 17 α-ethinylestradiol EE2), and
152 two anti-inflammatories (ibuprofen IBP and naproxen NPX). They were spiked on day 35 at
153 a concentration of 1 ppb, to simulate environmental concentrations. Sampling was limited to

154 the liquid phase (feed and permeate), because of the low biomass concentration inside the
155 reactor and the limited affinity of the selected OMPs to sorb onto solids.

156 **2.2 Analytical methods**

157 Temperature, pH, nitrogen species, total suspended solids (TSS), volatile suspended solids
158 (VSS) and dissolved organic carbon (DOC) were measured according to Standard Methods
159 (Rice et al., 2012). The dissolved oxygen (DO) concentration in the feeding bags was
160 recorded by using a multi-parameter meter (Hach HQ40d) connected to a luminescent optical
161 probe (IntelliCAL LDO101). Gas flow was measured by using a Milli GasCounter MGC-1
162 V3.3 PMMA (Ritter) and its composition was measured in a gas chromatograph (HP 5890
163 Series II) with a Porapack Q 80/100 2 m × 1.8" (SUPELCO) column.

164 Microbial community was characterized by withdrawing a homogeneous biomass sample
165 from the reactor, fixing in 4% v/v paraformaldehyde for fluorescence *in situ* hybridization
166 (FISH) and keeping biomass samples frozen for DNA extraction. FISH was performed as
167 described by Regueiro et al. (2012), with specific probes for n-damo bacteria, damo archaea
168 and anammox bacteria. Sequencing of the 16s rRNA gene was carried out according to the
169 protocol described by Allegue et al. (2018). Raw sequences processing was carried out with
170 USEARCH v11.1. Clustering into Operational Taxonomic Units (OTUs) was performed with
171 the UPARSE algorithm taking into account a 97% of sequence similarity.
172 OMPs analysis samples were collected in amber glass bottles, prefiltered (AP3004705,
173 Millipore), stored at 4 ºC and pre-concentrated by using solid phase extraction (SPE) in 3 mL
174 OASIS HLB cartridges. Pre-concentrated samples were analyzed by LC-MS-MS for
175 antibiotics, hormones and neurodrugs, while anti-inflammatories were analyzed by GC-MS.

Analytical procedure, equipment, quantification limits and recoveries were described by Alvarino et al. (2015).

3. RESULTS AND DISCUSSION

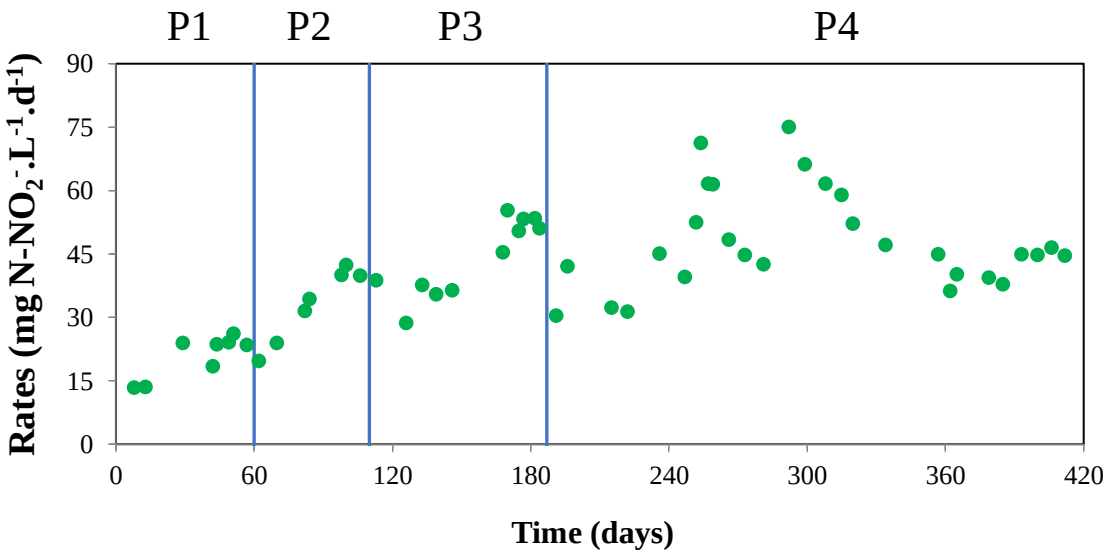
3.1 MBR performance

The n-damo MBR has been operated for 412 days at 28.3 ± 0.5 ºC, with a hydraulic retention time (HRT) of 23.76 ± 0.96 h. The nitrite loading rate (NLR) was increased according to the nitrite removal rates achieved (NRR), maintaining always a low value of residual nitrite in the system to control the MBR activity. Four different periods can be observed, according to activity increase and OMPs sampling campaigns (Fig. 1).

Period 1 (days 0-57) corresponded to the start-up of the reactor. A significant NRR of 13.4 mg N L^{-1} d^{-1} was achieved after 8 d of operation which was progressively increased to 24 mg N L^{-1} d^{-1} until the day 29. NRR was maintained in 24.3 ± 1.3 mg N L^{-1} d^{-1} from day 44 to 57, during the OMPs sampling campaign. Depletion of oxygen and using impermeable-to-oxygen feeding bags, as indicated Allegue et al. (2018), led to a faster start-up regarding other studies (Bhattacharjee et al., 2016; Ma et al., 2017). In Period 2 (days 58-106) NRR was increased and maintained at 40.8 ± 1.4 mg N L^{-1} d^{-1} from day 98 to day 106. The same strategy was followed in Period 3 (days 107-184), where activity was increased up to 52.6 ± 1.3 mg N L^{-1} d^{-1} from day 177 to day 184. Other studies reported similar removal rates using a membrane bioreactor for n-damo enrichment like Kampman et al. (2014) who achieved 36-40 mg N-NO_2^- L^{-1} d^{-1} or in sequence batch reactors like Hu et al. (2019) who achieved a maximum rate of 58.61 mg N L^{-1} d^{-1} .

Period 4 (days 185-412) was characterized by reactor unstability which appears to be quite frequent in these systems, when applying higher nitrite loading rates (Kampman et al., 2014). Besides, in the beginning of period 4 there was a decrease in the reactor activity (from 52.6 to 31-32 mg N L^{-1} d^{-1}) due to technical problems with the blower and the seal of the MBR. This implied that some air entered the reactor, severely affecting the n-damo performance, which is extremely sensitive to oxygen exposure (Luesken et al., 2012). We suggest that part of the nitrogen removal rate during this unstable period of operation could be attributed to conventional heterotrophic denitrifiers present in the reactor biomass, that could use the decay n-damo biomass products to denitrify the NO_2 in the reactor, as previously reported in Allegue et al. (2018). This resulted in the increase of NRR with maximum values achieved between days 250 and 320 (Fig. 1). After solving the problem, denitrification activity became stable at the end of Period 4 at a NRR of 39.1 ± 1.2 mg N L^{-1} d^{-1}, similar to that of Period 2.

In Periods 1, 2 and 3 no biomass growth was observed, being the measured TSS and VSS concentration around 0.53 g TSS L^{-1} and 0.41 g VSS $L^{-1,}$ respectively. This was expected according to the very low growth rates of this type of microorganisms, whose doubling time was estimated in 11.5 days (Allegue et al., 2018). However, an important fraction of the biomass was observed to be attached to the reactor walls, which made the accurate quantification of solids complicated, as reported in previous studies (Kampman et al., 2014). At the beginning of period 4, biomass concentration decreased to concentrations about 0.25 g TSS L^{-1} and 0.21 g VSS L^{-1}, probably due to biomass decay, which was then kept constant from day 357 onwards.



218

219 **Figure 1**. Volumetric nitrite consumption rate according to the different periods.

220 OMPs sampling campaigns were made at the end of each of the four operating periods when
221 stable conditions were maintained, with a specific nitrite removal rates of 54.6 ± 4.9 , 94.8 ±
222 3.2 , 131.5 ± 3.3 and 186.3 ± 5.5 mg N g VSS^{-1} d^{-1} and a pH of 6.8, 7.2, 7.5 and 7.4, in Period
223 1, 2, 3 and 4, respectively.

224 The ammonia fed to the culture during the whole operation was low in order to prevent the
225 proliferation of anammox bacteria. Ammonia consumption was lower than 1 mg N L^{-1} d^{-1} in
226 the first 146 days. After this period, ammonia consumption varied between 2 and 4 mg N L^{-}
227 ^{1} d^{-1}. However, the ammonia addition in this study did not cause any observable effect on the
228 development of the n-damo community.

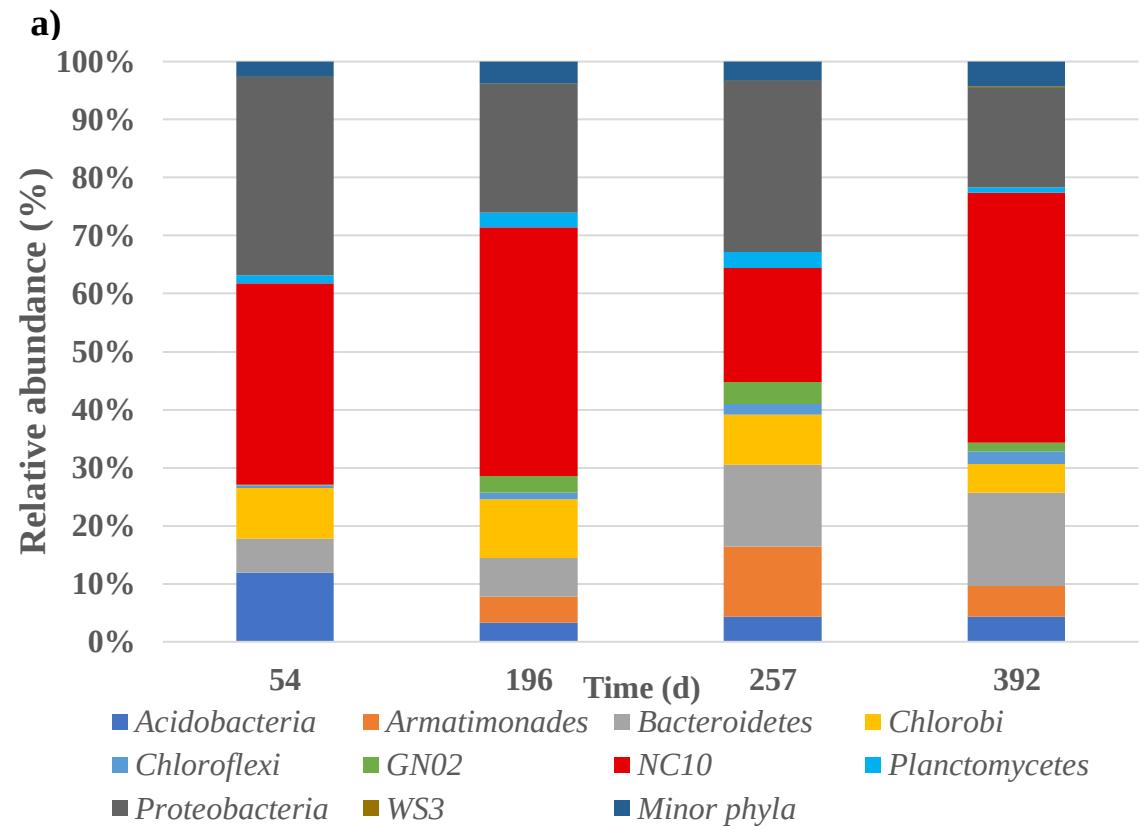
229 ***3.2 Microbial community dynamics***

230 Once a significant n-damo activity was achieved in the system (NRR of 13.4 mg N L^{-1} d^{-1}
231 were achieved after 8 days of operation), the microbial community dynamics were monitored
232 by FISH and 16s RNA gene sequencing. FISH probes were used on days 29 and 54 of P1

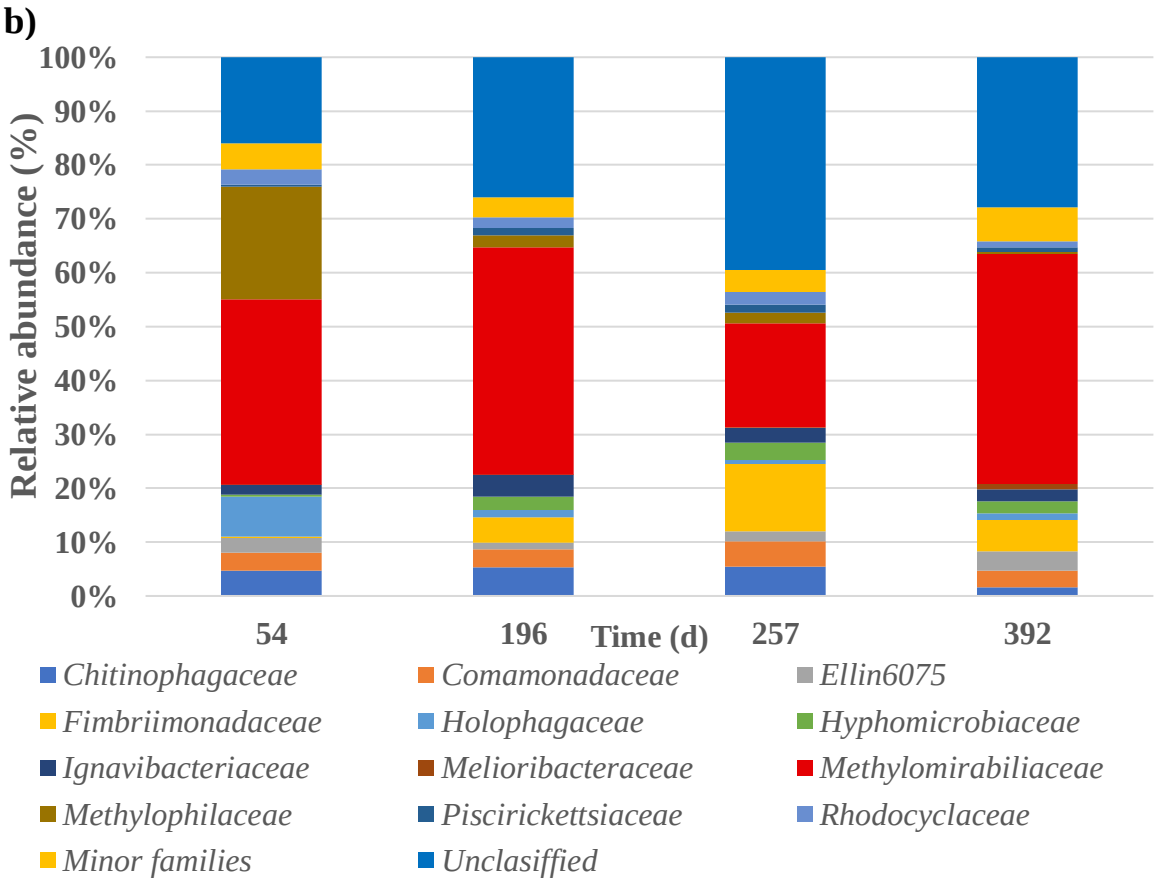
233 and 196 of P3 to check the presence of n-damo bacteria, n-damo archea and anammox. On
234 day 29, n-damo bacteria were detected (Fig. S2), confirming the fast start-up of the MBR.
235 The culture enrichment was further confirmed by the increasing abundance of n-damo
236 bacteria observed from day 54 onwards. Besides, FISH analyses confirmed the absence of n-
237 damo archaea and anammox expected from the nitrogen mass balances.

238 In addition to FISH analysis, 16s RNA gene sequences were analyzed at four different days
239 of the MBR operation, in order to have a complete information of the microbial composition:
240 days 54 (end of P1), 196 (end of P3), 257 and 392 (unstable and stable operation in P4,
241 respectively) . Additionally, quantitative information on the n-damo bacteria enrichment was
242 sought. Table S1 shows the number of bacterial sequences read and the bacterial taxonomic
243 units (OTUs) and singletons identified in each sample. The taxonomic allocation varied from
244 81.70% when classifying OTUs at phyla level to 32.63% at family level. Archaea
245 microorganisms were not analyzed by this method, due to its absence in the FISH analyses.

246 OTUs were distributed among 37 different phyla in which the most abundant ones (>1%)
247 represent the 96.6% ± 0.74% of total OTUs distributed in this taxonomic level (Fig. 2). The
248 NC10 phylum, the one associated with n-damo bacteria (Ettwig et al., 2010), was the
249 predominant in 3 samples followed by the *Proteobacteria* phylum. In the third sample, due
250 to the operational problems and the entrance of air in the system mentioned in the section
251 3.1., the relative abundance of NC10 phylum decreased sharply (from around 40% to 20%).
252 As it is mentioned in other studies, n-damo bacteria is very sensitive to oxygen (Luesken et
253 al., 2012), so this may be the reason for the decrease in the n-damo abundance. Thus, being
254 in contact with air not only affected the MBR performance in terms of nitrogen removal, but
255 also modified the microbial composition.


a)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Relative abundance (%)
54
196
257
392
Time (d)
Acidobacteria
Armatimonades
Bacteroidetes
Chlorobi
Chloroflexi
GN02
NC10
Planctomycetes
Proteobacteria
WS3
Minor phyla

256


b)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Relative abundance (%)
54
196
257
392
Time (d)
Chitinophagaceae
Comamonadaceae
Ellin6075
Fimbriimonadaceae
Holophagaceae
Hyphomicrobiaceae
Ignavibacteriaceae
Melioribacteraceae
Methylomirabiliaceae
Methylophilaceae
Piscirickettsiaceae
Rhodocyclaceae
Minor families
Unclasiffied

257

Figure 2. Microbial communities present in MBR through the whole operation. a) Bacteria phyla and b) Bacteria families with relative abundances over 1%.

Species were distributed in more than 100 families in which *Methylomirabiliaceae*, the family of *Candidatus* Methylomirabilis oxyfera associated with the NC10 phylum, was the most abundant one in all samples. A significant relative abundance (around 20%) was detected in the first sample of *Methylophylaceae* family, a known methylotroph, probably coming from the inoculum. However, their presence in the MBR is reduced in the next samples, being his relative abundance in the last point around 0.26%. As in other studies (Fu et al., 2017; Wang et al., 2019), there was a presence of other conventional denitrifying heterotrophs, like *Comamonaceae* and *Hyphomicrobiaceae*. Their relative abundance was higher at day 257, 4.73% and 3.26% respectively, in which the mixing liquor was exposed to air. As it was mentioned in the previous section, they probably use biomass decay products to perform the conventional denitrification (Allegue et al., 2018). Nevertheless, these bacterial families never constituted the major groups in the MBR community (less than 4% in both cases). What is clear from the 16s RNA gene sequences analysis is the negative impact of the oxygen entrance to the reactor on n-damo bacteria enrichment, stating as crucial the maintenance of an anoxic environment in the system.

Due to the oxygen depletion in the feeding bags, the presence of aerobic methanotrophs, like the family *Methylococcaceae*, only represented the 0.15% in the first sample and less than 0.02% in the rest of them. In the same line, *Brocadiaceae* family only represented the 0.06 % of the total microbial community, indicating that despite the addition of a low quantity of ammonium in the feeding bags, the presence of anammox bacteria in the whole culture was negligible, since the species belonging to this family (*Candidatus* Brocadia) are one of the

most common organisms found in the enrichments of wastewater treatment technologies (Kuenen, 2008). Although Luesken et al. (2011) proposed that anammox bacteria have higher affinity for NO_2^- than n-damo bacteria, its minor presence in the whole culture of the reactor suggests a residual contribution to denitrification .

According to the FISH analyses, the operational results in terms of nitrogen removal and the 16s RNA gene sequence (Illumina), an enrichment of the culture in n-damo bacteria was clearly achieved. The relative abundance values of the NC10 phylum were similar that those reported in Allegue et al. (2018), in a very similar enrichment procedure and system. Due to the low abundance of other denitrifying groups, like anammox or heterotrophic denitrifying bacteria, it is assumed that n-damo bacteria is responsible for almost all the nitrogen removed in the MBR.

All the changes in the bacterial community occurred when the MBR performance suffered operational issues; there was no evidence that the presence of the OMPs affected the microbial community.

3.3 OMPs results

The OMPs removal efficiency was studied in the system at the end of each operational period (Section 3.1) to determine their removal efficiency for the different specific activities achieved in the reactor (Fig. 3) and maintained stable at least for 10-15 days. Sorption was not considered as a relevant removal mechanism in this study because the studied OMPs are not lipophilic, except for FLX (whose particular behavior is discussed in section 3.4). Additionally, the concentration of VSS during the MBR performance was very low (< 0.4 g $VSS \cdot L^{-1}$) supporting the statement of low contribution of sorption to OMP removal.

According to the reported values of the Henry coefficients for the selected OMPs, neither volatilization supposed a relevant removal mechanism (Suárez et al., 2008). Consequently, the OMP removal efficiencies determined in this work were attributed to biotransformation. Accordingly, the selected OMPs can be grouped in the following three categories: recalcitrant compounds (removal efficiency < 20%) such as TMP, CBZ, DZP, EE2, IBP, NPX and DCF; highly biotransformable substances (> 90%) including E1 and E2; and compounds moderately biotransformed in the reactor (20-80%) as ERY, SMX, ROX and FLX.

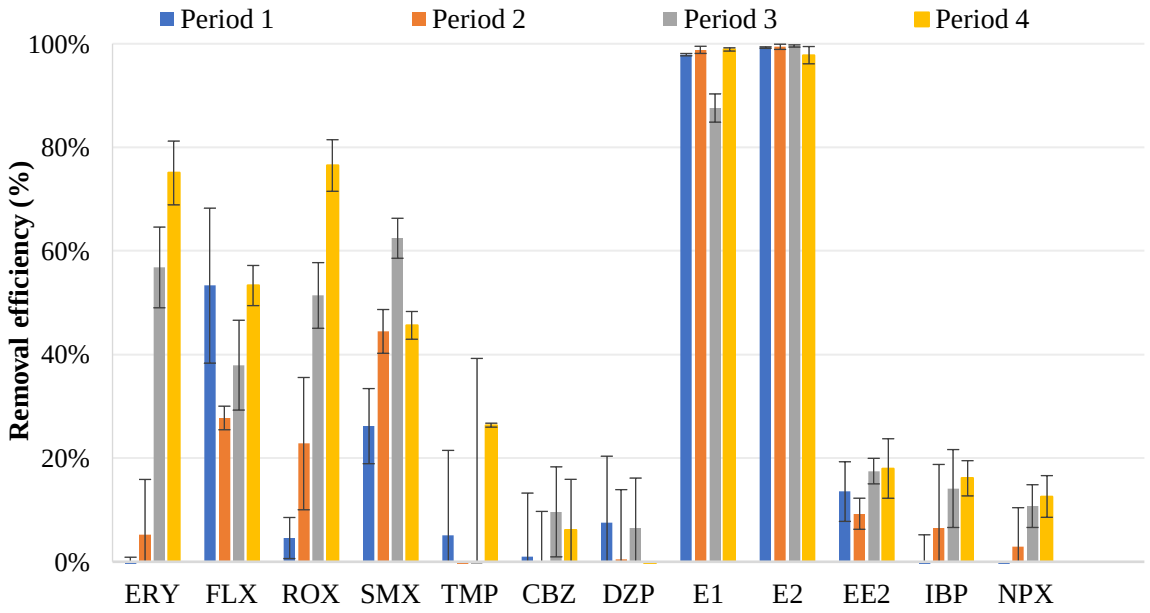


Figure 3. Removal efficiencies of the selected OMPs in each stage.

As expected, the removal efficiency of CBZ and DZP barely reached 10% in any of the 4 sampling campaigns. Their recalcitrant behavior in the three redox environments was previously pointed out in several studies (Alvarino et al., 2018; Suarez et al., 2010). Regarding IBP, its biotransformation degree was around 15% in the last two stages. In this work, IBP proved to be much more recalcitrant in anoxic environments compared to removal efficiencies reported in aerobic reactors, with conventional heterotrophic bacteria

(Fernandez-Fontaina et al., 2016). However, this low biotransformation degree in anoxic conditions is in accordance with by previous batch experiments using a synthetic media with acetate and nitrate (Kassotaki et al., 2018) or fed with pre-clarified wastewater with an external nitrate addition (Torresi et al., 2017). Thus, we can confirm that IBP biodegradation is highly influenced by redox conditions. According to Falås et al. (2013), the removal of this compound is strongly influenced by the physical conformation of the biomass, achieving better results with biomass attached to carriers than suspended in the mixed liquor.

The high removal of natural hormones as well as the recalcitrant behavior of the EE2, was observed previously in a denitrifying activated sludge reactor (Suarez et al., 2010). Nevertheless, the removal efficiencies obtained in this study for natural hormones are higher (almost 100% of biodegradation versus 72%) than those reported by Suarez et al. (2010). No effect was observed in the removal of natural and synthetic hormones during the reactor operation. Despite having a similar chemical structure, a steric impediment was suggested in other studies to explain the different removal efficiencies achieved for the hormones E2 and EE2 (Czajka and Londry, 2006).

In the case of the moderately biodegradable compounds (ERY, ROX, SMX and FLX) the removal efficiency observed during the reactor operation varied strongly (< 10% up to > 70%). This behavior will be further discussed in section 3.4. In the anoxic chamber of a pilot plant which combined the three redox conditions, Arias et al., (2018) found a 30% removal efficiency for ROX and ERY, comparable with the removal found in the aerobic compartment. Moreover, Burke et al. (2014) observed a preferential biodegradation of ROX under anoxic compared to aerobic conditions. In the case of SMX, its removal is commonly associated with anaerobic conditions (Alvarino et al., 2018; Arias et al., 2018). Nevertheless,

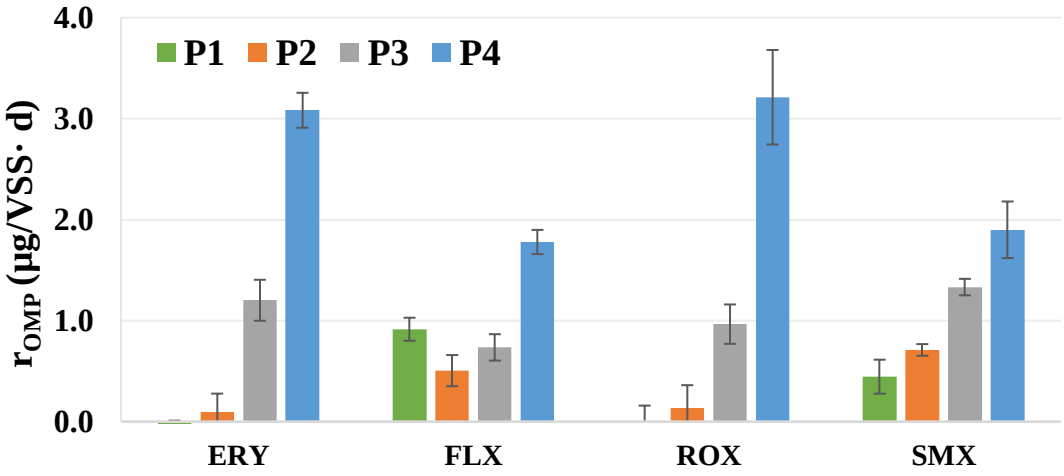
342 significant biotransformation rates were reported in anoxic denitrifying conditions in batch
343 tests, in agreement with the results obtained in this research (Kassotaki et al., 2018; Polesel
344 et al., 2017; Torresi et al., 2017). According to Torresi et al. (2017), when the primary
345 substrate of the microbial consortia is available, the removal rate of SMX is enhanced,
346 suggesting that the main biotransformation mechanism of this compound is cometabolism.
347 Concerning FLX, some authors suggested that its removal is mainly attributed to sorption in
348 anoxic environments (Alvarino et al., 2018; Pomiès et al., 2015). This could explain the high
349 removal efficiency observed for FLX already in P1 (Fig. 3) as further discussed in the next
350 section. On the other hand, Suarez et al. (2010) reported a high removal of FLX in an anoxic
351 denitrifying reactor with a strong influence of the SRT on its biodegradation. Thus, the
352 removal of this compound could be enhanced with time in the reactor as no purges of biomass
353 were carried out throughout its operation.

354 ***3.4 Cometabolic biotransformation of antibiotics***

355 The behavior of FLX, ROX, ERY and SMX could suggest removal by cometabolism,
356 according to the increasing trend in their removal efficiencies along the different operational
357 periods. Since the OMPs concentration fed to the bioreactor was the same during the whole
358 operation, if the biodegradation rate of one compound increased in parallel to the n-damo
359 primary metabolism we suggest that this pollutant was cometabolically biotranformed. In
360 order to analyze this, the specific removal rates of the primary substrate (nitrite) and the
361 OMPs were plotted in Fig 4. For this purpose, the data from the OMP concentrations in the
362 feed and in the permeate, the applied HRT and the measured VSS concentration in the reactor
363 were considered for each period of operation. The denitrifying specific activity in each stage
364 was specified in the section 3.1.

365

a)



366

b)

	Line equation	R^2
ERY	y: 0.0344x - 3.3274	0.9999
FLX	y: 0.0152x - 1.1011	0.9598
ROX	y: 0.0361x - 3.5831	0.9885
SMX	y: 0.0133x - 0.5420	0.9686

367

368 **Figure 4**. (a) Specific micropollutant removal rate for ERY, FLX, ROX and SMX at
369 different specific biomass activity. (b) Linear regression equation and correlation
370 coefficient (R^2) for each compound (y is the specific removal rate of each compound and x
371 is the specific removal rate for nitrite) and considering the data for periods P2-P4.

372

373 The linear correlations in Fig. 4b showed a clear relationship between the or specific primary
374 substrate removal rate and the specific removal rate of ERY, SMX, ROX and FLX between
375 P2 and P4. This implies that best results in terms of OMPs biotransformation kinetics were
376 achieved in P4, when the specific denitrification capacity of the n-damo bacteria was the
377 highest. Even in the case of SMX, for which a lower removal efficiency (45.6%) was
378 measured in P4 (Fig. 3), the specific removal rate in this period was higher than in the

previous operational periods, supporting cometabolic features. Between the first and the second sampling campaign, there were no significant differences in the specific removal rate of the OMPs, with the exception of FLX. If cometabolism is the main biodegradation mechanism, some authors suggest that enough growth substrates are necessary to stimulate the creation of the relevant enzymes for the degradation of the OMPs (Tran et al., 2013). This may have occurred in the first sampling period (P1), when the nitrite concentration fed to the system was the lowest of the total reactor operation (20 mg N L^{-1} d^{-1} of NRL). So, for that reason the results obtained in the period 1 were not included when considering the cometabolic data analysis (Fig. 4).

In the case of FLX, the higher specific removal rate achieved in P1 compared to the other OMPs may be due to its sorption onto the sludge in the MBR. Considering a Kd of 1000 L $kgTSS^{-1}$ (Alvarino et al., 2016) for this compound, the removal would be almost completely associated to such mechanism (concentration in feed: 1 ppb, 0.51 gTSS L^{-1} in P1) since its global removal efficiency was around 50% (Fig. 3). The sorption coefficient of this compound may be slightly higher in anoxic environments, however the order of magnitude is the same and sorption is more dependent on the physico-chemical charactheristics of the micropollutants and the biomass conformation than to redox environment (Alvarino et al., 2018). Pomiès et al. (2015) exposed that FLX removal in anoxic conditions was mainly due to sorption, with little influence of biodegradation. However, once sorption equilibrium is established, normally after several days of contact between the sludge and the OMPs, biotransformation becomes the main removal mechanism (Yang et al., 2011). Thus, from P1 onwards, the removal of FLX could be attributed fully to biodegradation, due to the

401 negligible biomass growth during the whole reactor operation and only values from P2 to P4
402 have been considering for the correlation with the specific nitrite removal of the reactor.

403 In previous studies, the removal of SMX has been associated with the activity of aerobic
404 autotrophic and heterotrophic bacteria. Its biotransformation rate was positively correlated
405 with the presence of organic matter as main substrate (Alvarino et al., 2018; Fernandez-
406 Fontaina et al., 2016). However, in our study no correlation has been found between the
407 presence of the primary substrate (nitrite) with the microcontaminants removal rate. Polesel
408 et al. (2017) reported a clear correlation between the specific denitrification rate and the
409 biotransformation constant of SMX and ERY (the correlation coefficient reported is greater
410 than 0.95) in batch assays with heterotrophic denitrification bacteria, in agreement with our
411 results. Their denitrification range varied between 10 and 50 mg N $gTSS^{-1}$ d^{-1}, consequently
412 focusing on lower levels than the present research (50-190 mg N $gVSS^{-1}$ d^{-1}). In Fig. 4b)
413 different slopes were determined for the OMP studied, showing that ERY and ROX were
414 more influenced by the cometabolic effect than SMX and FLX. This may indicate that
415 cometabolism is more dependent to the specific denitrification rates, as it was previously
416 reported (Polesel et al., 2017; Torresi et al., 2018), than to the presence of high concentration
417 of the primary substrate as in P2 and P4 the NRR was almost the same (around 40 mg N L^{-1}
418 d^{-1}) but the specific nitrite removal rate in such periods was different.

419 It is worth to note that most results on OMP removal under anoxic conditions have been
420 published for conventional denitrification processes. Since the nitrogen removal metabolism
421 of the n-damo process is different from conventional denitrification (Fig. 5c), the behavior
422 of OMP in those processes could be different. In conventional denitrification, the nitrate is
423 progressively reduced to nitrite, nitric oxide, nitrous oxide and, finally, to nitrogen gas.

Specially the nitrite reduction pathway is different, due to the presence of and NO dismutase (NOD) which performs the last step of the denitrification and provides O_2 for the methane oxidation (Fig. 5a) (Wu et al., 2011). The common enzyme between both processes, the copper nitrite reductase (EC 1.7.2.1) (NIR), was correlated with the biotransformation rate constant of some compounds like SMX, ERY or TMP and the abundance of the gene encoding for these enzyme (Torresi et al., 2018). However, there are other enzymes in the methane oxidation pathway like monooxygenases (particulate methane monooxygenase (*pMMO*) (EC 1.14.18.3)) or dehydrogenases (methanol dehydrogenase (MDH) (EC 1.1.2.7)) which can be other candidates to biotransform OMPs, as they are known to participate in some pollutant degradations such as toluene or xylene (Jindrová et al., 2002). Further research needs to be done to elucidate which enzymes in the n-damo metabolism are responsible for these biotransformations.

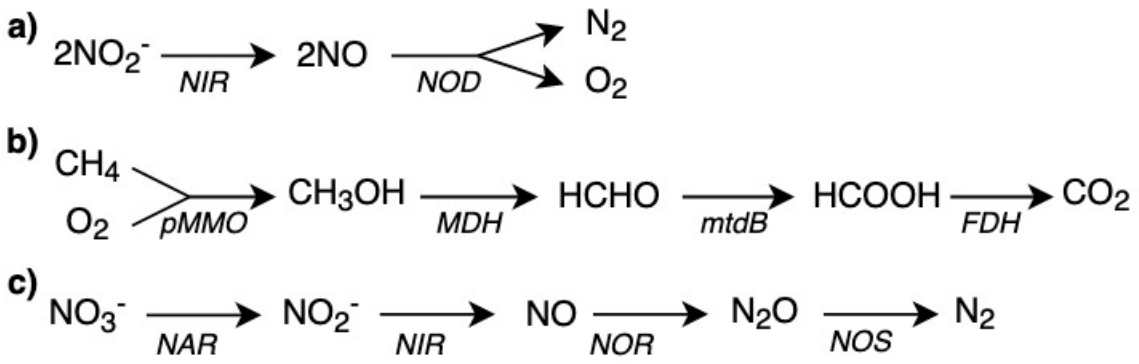


Figure 5. Candidatus M. oxyfera metabolic pathways. (a) Nitrite pathway, (b) Methane pathway. (c) Conventional heterothrophic denitrification chain. Abbreviations: NIR; nitrite reductase (EC 1.7.2.1); NOD, nitric oxide dismutase; pMMO, particulate methane mono-oxygenase (EC 1.14.18.3); MDH, methanol dehydrogenase (EC 1.1.2.7) ; mtdB, methylene-H4MPT dehydrogenase (EC 1.5.1.5); FDH, fromate dehydrogenase (EC 1.17.1.10); NAR, nitrite reductases (EC 1.7.1.1); NOR, nitric oxide reductase (EC 1.7.5.2); NOS, nitrous oxide reductase (EC 1.7.2.4).

4. CONCLUSIONS

This research provided new knowledge about the capability of n-damo cultures to biotransform OMPs. A successful n-damo enrichment was achieved along 412 d of operation in a MBR. Limiting the quantity of oxygen entering the MBR, by sealing efficiently the reactor and deoxygenating the feeding bags, was revealed as a crucial factor in order to achieve a fast n-damo bacteria enrichment and denitrification activity (24 mg N L^{-1} d^{-1} in around 30 days). The system also showed its robustness, being able to recover the previous denitrifying activity (40 mg N L^{-1} d^{-1}), after an inhibitory period. The 16S rRNA gene sequencing results showed that *Methylomirabiliaceae* family dominated the microbial community with a relative abundance of 40 % during stable reactor operation. The OMPs could be classified according to the achieved removal efficiencies in the reactor as: recalcitrant, completely biodegradable and moderately biodegradable. For the moderately biodegradable OMPs (ERY, ROX, SMX and FLX), a positive correlation between the n-damo bacteria denitrification kinetics and the OMP specific biotransformation rates has been found. This suggests that the main mechanism driving such biotransformation was cometabolism.

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References

Allegue, T., Arias, A., Fernandez-Gonzalez, N., Omil, F., Garrido, J.M., 2018. Enrichment of nitrite-dependent anaerobic methane oxidizing bacteria in a membrane bioreactor. Chem. Eng. J. 347, 721–730. https://doi.org/10.1016/j.cej.2018.04.134

Alvarino, T., Suárez, S., Garrido, M., Lema, J.M., Omil, F., 2016. A UASB reactor coupled to a hybrid aerobic MBR as innovative plant configuration to enhance the removal of organic micropollutants. Chemosphere 144, 452–458. https://doi.org/10.1016/j.chemosphere.2015.09.016

Alvarino, T., Suarez, S., Katsou, E., Vazquez-Padin, J., Lema, J.M., Omil, F., 2015. Removal of PPCPs from the sludge supernatant in a one stage nitritation/anammox process. Water Res. 68, 701–709. https://doi.org/10.1016/j.watres.2014.10.055

Alvarino, T., Suarez, S., Lema, J., Omil, F., 2018. Understanding the sorption and biotransformation of organic micropollutants in innovative biological wastewater treatment technologies. Sci. Total Environ. 615, 297–306. https://doi.org/10.1016/j.scitotenv.2017.09.278

Arias, A., Alvarino, T., Allegue, T., Suárez, S., Garrido, J.M., Omil, F., 2018. An innovative wastewater treatment technology based on UASB and IFAS for cost-efficient macro and micropollutant removal. J. Hazard. Mater. 359, 113–120. https://doi.org/10.1016/j.jhazmat.2018.07.042

Bhattacharjee, A.S., Motlagh, A.M., Jetten, M.S.M., Goel, R., 2016. Methane dependent denitrification- from ecosystem to laboratory-scale enrichment for engineering applications. Water Res. 99, 244–252. https://doi.org/10.1016/j.watres.2016.04.070

492 Burke, V., Richter, D., Hass, U., Duennbier, U., Greskowiak, J., Massmann, G., 2014.
493 Redox-dependent removal of 27 organic trace pollutants: Compilation of results from
494 tank aeration experiments. Environ. Earth Sci. 71, 3685–3695.
495 https://doi.org/10.1007/s12665-013-2762-8
496 Czajka, C.P., Londry, K.L., 2006. Anaerobic biotransformation of estrogens. Sci. Total
497 Environ. 367, 932–941. https://doi.org/10.1016/j.scitotenv.2006.01.021
498 Ettwig, K.F., Butler, M.K., Le Paslier, D., Pelletier, E., Mangenot, S., Kuypers, M.M.M.,
499 Schreiber, F., Dutilh, B.E., Zedelius, J., De Beer, D., Gloerich, J., Wessels, H.J.C.T.,
500 Van Alen, T., Luesken, F., Wu, M.L., Van De Pas-Schoonen, K.T., Op Den Camp,
501 H.J.M., Janssen-Megens, E.M., Francoijs, K.J., Stunnenberg, H., Weissenbach, J.,
502 Jetten, M.S.M., Strous, M., 2010. Nitrite-driven anaerobic methane oxidation by
503 oxygenic bacteria. Nature 464, 543–548. https://doi.org/10.1038/nature08883
504 Ettwig, K.F., Van Alen, T., Van De Pas-Schoonen, K.T., Jetten, M.S.M., Strous, M., 2009.
505 Enrichment and molecular detection of denitrifying methanotrophic bacteria of the
506 NC10 phylum. Appl. Environ. Microbiol. 75, 3656–3662.
507 https://doi.org/10.1128/AEM.00067-09
508 Falås, P., Longrée, P., La Cour Jansen, J., Siegrist, H., Hollender, J., Joss, A., 2013.
509 Micropollutant removal by attached and suspended growth in a hybrid biofilm-
510 activated sludge process. Water Res. 47, 4498–4506.
511 https://doi.org/10.1016/j.watres.2013.05.010
512 Fernandez-Fontaina, E., Gomes, I.B., Aga, D.S., Omil, F., Lema, J.M., Carballa, M., 2016.
513 Biotransformation of pharmaceuticals under nitrification, nitratation and heterotrophic
514 conditions. Sci. Total Environ. 541, 1439–1447.
515 https://doi.org/10.1016/j.scitotenv.2015.10.010

516 Fischer, K., Majewsky, M., 2014. Cometabolic degradation of organic wastewater
517 micropollutants by activated sludge and sludge-inherent microorganisms. Appl.
518 Microbiol. Biotechnol. 98, 6583–6597. https://doi.org/10.1007/s00253-014-5826-0
519 Fu, L., Ding, J., Lu, Y., Ding, Z., Zeng, R.J., 2017. Nitrogen source effects on the
520 denitrifying anaerobic methane oxidation culture and anaerobic ammonium oxidation
521 bacteria enrichment process. Appl. Microbiol. Biotechnol. 101, 3895–3906.
522 https://doi.org/10.1007/s00253-017-8163-2
523 Gonzalez-Gil, L., Krah, D., Ghattas, A.K., Carballa, M., Wick, A., Helmholz, L., Lema,
524 J.M., Ternes, T.A., 2019. Biotransformation of organic micropollutants by anaerobic
525 sludge enzymes. Water Res. 152, 202–214.
526 https://doi.org/10.1016/j.watres.2018.12.064
527 He, Z., Cai, C., Wang, J., Xu, X., Zheng, P., Jetten, M.S.M., Hu, B., 2016. A novel
528 denitrifying methanotroph of the NC10 phylum and its microcolony. Sci. Rep. 6, 1–
529 10. https://doi.org/10.1038/srep32241
530 Hu, Z., Ru, D., Wang, Y., Zhang, J., Jiang, L., Xu, X., Nie, L., 2019. Optimization of a
531 nitrite-dependent anaerobic methane oxidation (n-damo) process by enhancing
532 methane availability. Bioresour. Technol. 275, 101–108.
533 https://doi.org/10.1016/j.biortech.2018.12.035
534 Jindrová, E., Chocová, M., Demnerová, K., Brenner, V., 2002. Bacterial Aerobic
535 Degradation of Benzene, Toluene, Ethylbenzene and Xylene. Folia Microbiol. (Praha).
536 47, 83–93. https://doi.org/10.1007/BF02817664
537 Kampman, C., Temmink, H., Hendrickx, T.L.G., Zeeman, G., Buisman, C.J.N., 2014.
538 Enrichment of denitrifying methanotrophic bacteria from municipal wastewater sludge
539 in a membrane bioreactor at 20°C. J. Hazard. Mater. 274, 428–435.

https://doi.org/10.1016/j.jhazmat.2014.04.031

Kassotaki, E., Pijuan, M., Joss, A., Borrego, C.M., Rodriguez-Roda, I., Buttiglieri, G., 2018. Unraveling the potential of a combined nitritation-anammox biomass towards the biodegradation of pharmaceutically active compounds. Sci. Total Environ. 624, 722–731. https://doi.org/10.1016/j.scitotenv.2017.12.116

Kuenen, J.G., 2008. Anammox bacteria from discovery. Nature 6, 320–326. https://doi.org/10.1038/nrmicro1857

Liu, T., Hu, S., Guo, J., 2019. Enhancing mainstream nitrogen removal by employing nitrate/nitrite-dependent anaerobic methane oxidation processes. Crit. Rev. Biotechnol. 39, 732–745. https://doi.org/10.1080/07388551.2019.1598333

Luesken, F.A., Sánchez, J., van Alen, T.A., Sanabria, J., Op denCamp, H.J.M., Jetten, M.S.M., Kartal, B., 2011. Simultaneous nitrite-dependent anaerobic methane and ammonium oxidation processes. Appl. Environ. Microbiol. 77, 6802–6807. https://doi.org/10.1128/AEM.05539-11

Luesken, F.A., Wu, M.L., Op den Camp, H.J.M., Keltjens, J.T., Stunnenberg, H., Francoijs, K.J., Strous, M., Jetten, M.S.M., 2012. Effect of oxygen on the anaerobic methanotroph “Candidatus Methylomirabilis oxyfera”: Kinetic and transcriptional analysis. Environ. Microbiol. 14, 1024–1034. https://doi.org/10.1111/j.1462-2920.2011.02682.x

Ma, R., Hu, Z., Zhang, J., Ma, H., Jiang, L., Ru, D., 2017. Reduction of greenhouse gases emissions during anoxic wastewater treatment by strengthening nitrite-dependent anaerobic methane oxidation process. Bioresour. Technol. 235, 211–218. https://doi.org/10.1016/j.biortech.2017.03.094

Noyola, A., Morgan-Sagastume, J.M., López-Hernández, J.E., 2006. Treatment of biogas

produced in anaerobic reactors for domestic wastewater: Odor control and energy/resource recovery. Rev. Environ. Sci. Biotechnol. 5, 93–114. https://doi.org/10.1007/s11157-005-2754-6

Plosz, B.G., Leknes, H., Thomas, K. V, 2010. Impacts of competitive inhibition, parent compound formation and partitioning behaviour on antibiotic micro-pollutants removal in activated sludge. Environ. Sci. Technol. 44, 734–742.

Polesel, F., Torresi, E., Loreggian, L., Casas, M.E., Christensson, M., Bester, K., Plósz, B.G., 2017. Removal of pharmaceuticals in pre-denitrifying MBBR – Influence of organic substrate availability in single- and three-stage configurations. Water Res. 123, 408–419. https://doi.org/10.1016/j.watres.2017.06.068

Pomiès, M., Choubert, J.M., Wisniewski, C., Miège, C., Budzinski, H., Coquery, M., 2015. Lab-scale experimental strategy for determining micropollutant partition coefficient and biodegradation constants in activated sludge. Environ. Sci. Pollut. Res. 22, 4383–4395. https://doi.org/10.1007/s11356-014-3646-5

Regueiro, L., Veiga, P., Figueroa, M., Alonso-Gutierrez, J., Stams, A.J.M., Lema, J.M., Carballa, M., 2012. Relationship between microbial activity and microbial community structure in six full-scale anaerobic digesters. Microbiol. Res. 167, 581–589. https://doi.org/10.1016/J.MICRES.2012.06.002

Rice, E.W. (ed), Rice, E.W., Bridgewater, L., Association, A.P.H., Association, A.W.W., Federation, W.E., 2012. Standard methods for the examination of water and wastewater.

Silva-Teira, A., Sánchez, A., Buntner, D., Rodríguez-Hernández, L., Garrido, J.M., 2017. Removal of dissolved methane and nitrogen from anaerobically treated effluents at low temperature by MBR post-treatment. Chem. Eng. J. 326, 970–979.

588 https://doi.org/10.1016/j.cej.2017.06.047
589 Sousa, J.C.G., Ribeiro, A.R., Barbosa, M.O., Pereira, M.F.R., Silva, A.M.T., 2018. A
590 review on environmental monitoring of water organic pollutants identified by EU
591 guidelines. J. Hazard. Mater. 344, 146–162.
592 https://doi.org/10.1016/j.jhazmat.2017.09.058
593 Souza, C.L., Chernicharo, C.A.L., Aquino, S.F., 2011. Quantification of dissolved methane
594 in UASB reactors treating domestic wastewater under different operating conditions.
595 Water Sci. Technol. 64, 2259–2264. https://doi.org/10.2166/wst.2011.695
596 Suárez, S., Carballa, M., Omil, F., Lema, J.M., 2008. How are pharmaceutical and personal
597 care products (PPCPs) removed from urban wastewaters? Rev. Environ. Sci.
598 Biotechnol. 7, 125–138. https://doi.org/10.1007/s11157-008-9130-2
599 Suarez, S., Lema, J.M., Omil, F., 2010. Removal of Pharmaceutical and Personal Care
600 Products (PPCPs) under nitrifying and denitrifying conditions. Water Res. 44, 3214–
601 3224. https://doi.org/10.1016/j.watres.2010.02.040
602 Torresi, E., Escolà Casas, M., Polesel, F., Plósz, B.G., Christensson, M., Bester, K., 2017.
603 Impact of external carbon dose on the removal of micropollutants using methanol and
604 ethanol in post-denitrifying Moving Bed Biofilm Reactors. Water Res. 108, 95–105.
605 https://doi.org/10.1016/j.watres.2016.10.068
606 Torresi, E., Gülay, A., Polesel, F., Jensen, M.M., Christensson, M., Smets, B.F., Plósz,
607 B.G., 2018. Reactor staging influences microbial community composition and
608 diversity of denitrifying MBBRs- Implications on pharmaceutical removal. Water Res.
609 138, 333–345. https://doi.org/10.1016/j.watres.2018.03.014
610 Tran, N.H., Reinhard, M., Gin, K.Y.H., 2018. Occurrence and fate of emerging
611 contaminants in municipal wastewater treatment plants from different geographical

612 regions-a review. Water Res. 133, 182–207.
613 https://doi.org/10.1016/j.watres.2017.12.029
614 Tran, N.H., Urase, T., Ngo, H.H., Hu, J., Ong, S.L., 2013. Insight into metabolic and
615 cometabolic activities of autotrophic and heterotrophic microorganisms in the
616 biodegradation of emerging trace organic contaminants. Bioresour. Technol. 146,
617 721–731. https://doi.org/10.1016/j.biortech.2013.07.083
618 Versantvoort, W., Guerrero-Cruz, S., Speth, D.R., Frank, J., Gambelli, L., Cremers, G., van
619 Alen, T., Jetten, M.S.M., Kartal, B., Op den Camp, H.J.M., Reimann, J., 2018.
620 Comparative genomics of Candidatus Methylomirabilis species and description of Ca.
621 Methylomirabilis lanthanidiphila. Front. Microbiol. 9, 1–10.
622 https://doi.org/10.3389/fmicb.2018.01672
623 Wang, D., Wang, Y., Liu, Y., Ngo, H.H., Lian, Y., Zhao, J., Chen, F., Yang, Q., Zeng, G.,
624 Li, X., 2017. Is denitrifying anaerobic methane oxidation-centered technologies a
625 solution for the sustainable operation of wastewater treatment Plants? Bioresour.
626 Technol. 234, 456–465. https://doi.org/10.1016/j.biortech.2017.02.059
627 Wang, J., Hua, M., Li, Y., Ma, F., Zheng, P., Hu, B., 2019. Achieving high nitrogen
628 removal efficiency by optimizing nitrite-dependent anaerobic methane oxidation
629 process with growth factors. Water Res. 161, 35–42.
630 https://doi.org/10.1016/j.watres.2019.05.101
631 Wu, M.L., Ettwig, K.F., Jetten, M.S.M., Strous, M., Keltjens, J.T., Van Niftrik, L., 2011. A
632 new intra-aerobic metabolism in the nitrite-dependent anaerobic methane-oxidizing
633 bacterium Candidatus “Methylomirabilis oxyfera.” Biochem. Soc. Trans. 39, 243–248.
634 https://doi.org/10.1042/BST0390243
635 Yang, S.F., Lin, C.F., Yu-Chen Lin, A., Andy Hong, P.K., 2011. Sorption and

636 biodegradation of sulfonamide antibiotics by activated sludge: Experimental
637 assessment using batch data obtained under aerobic conditions. Water Res. 45, 3389–
638 3397. https://doi.org/10.1016/j.watres.2011.03.052
639
640