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Abstract:

The removal efficiency of nine pharmaceutical compounds from primary sludge was evaluated in two different operating conditions: (i) in conventional Mesophilic Anaerobic Digestion (MAD) alone and (ii) in a co-treatment process combining Mesophilic Anaerobic Digestion and a Thermophilic Aerobic Reactor (MAD-TAR). The pilot scale reactors were fed with primary sludge obtained after decantation of urban wastewater. Concerning the biodegradation of organic matter, thermophilic aeration increased solubilization and hydrolysis yields of digestion, resulting in a further 26% supplementary removal of chemical oxygen demand (COD) in MAD-TAR process compared to the conventional mesophilic anaerobic digestion. The highest removal rate of target micropollutants were observed for caffeine (CAF) and sulfamethoxazole (SMX) (>89%) with no substantial differences between both processes. Furthermore, MAD-TAR process showed a significant increase of removal efficiency for oxazepam (OXA) (73%), propranolol (PRO) (61%) and ofloxacine (OFL) (41%) and a slight increase for diclofenac (DIC) (4%) and 2 hydroxy-ibuprofen (2OH-IBP)

(5%). However, ibuprofen (IBP) and carbamazepine (CBZ) were not degraded during both processes. Anaerobic digestion affected the liquid-solid partition of most target compounds. Sorbed fraction of pharmaceutical compounds on the sludge tend to decrease after digestion, this tendency being more pronounced in the case of the MAD-TAR process due to much lower concentration of solids.

Keywords: pharmaceutical compounds; Thermophilic Aerobic Reactor; Mesophilic Anaerobic Digestion; reduction of sludge production

Nomenclature

COD	Chemical Oxygen Demand
COD_P	Particulate COD
COD_S	Soluble COD
COD_T	Total COD
DS	Digested Sludge
K_d	Solid-liquid partition coefficient
OLR	Organic Loading Rate
PS	Primary Sludge
SRT	Sludge Retention Time
TSS	Total Suspended Solids
VSS	Volatile Suspended Solids
WWTPs	Wastewater Treatment Plants

1. Introduction

Over the last twenty years, various studies have quantified concentrations and occurrences of pharmaceutical compounds in wastewater treatment plants (WWTPs). Data reported generally refers to the removal from aqueous phase without distinguishing between sorption of compounds onto sludge and/or biodegradation processes (Verlicchi et al. 2012). However, a significant fraction of pollutants can be sorbed onto the sludge, depending on the respective physicochemical properties of molecules and sludge as well as on operating conditions. The main mechanisms for the removal of organic compounds from wastewater are sorption and biodegradation. Volatilization mechanism could be neglected due to small Henry's law constants of most pharmaceutical compounds (Joss et al. 2006).

Sorption onto sludge follows two mechanisms: absorption and adsorption. On the one hand, hydrophobic interactions (absorption) occurs between the aliphatic and aromatic groups of a compound with the lipophilic cell membrane of the microorganisms or the lipid fractions of the suspended solids. On the other hand, the electrostatic interactions (adsorption) take place because of interactions of positively charged groups of chemicals with the negatively charged surfaces of the microorganisms (Verlicchi et al. 2012).

In this study, we considered nine molecules based on their variety of physicochemical properties, their prevalence in WWTP effluents, their uses for different pathologies and as a marker of human activity. These criteria led us to choose carbamazepine CBZ (anticonvulsant); diclofenac DIC, ibuprofen IBP and 2-hydroxyibuprofen 2OH-IBP (analgesics); sulfamethoxazole SMX and ofloxacin OFL (antibiotics); propranolol PRO (beta bloquer); oxazepam OXA (psycholeptic) and caffeine CAF (stimulant). Table S1 shows their therapeutic class, chemical structure, Henry's Law constant, pK_a and $LogK_{ow}$ values. It can be

55 noticed that Besse et al. (2008) included ofloxacin, propranolol, carbamazepine,
56 sulfamethoxazole and diclofenac in their preliminary risk assessment of pharmaceuticals
57 showing high Risk Quotient ratios.

58 One extended approach to evaluate the sorption of hydrophobic substances is the octanol-
59 water partition coefficient K_{OW} that indicates the lipophilicity of the sorbed compound.
60 However, some studies revealed that sorption behavior present significant deviations from
61 log K_{OW} for pharmaceuticals (Golet et al. 2003; Urase et al. 2005; Carballa et al. 2008;
62 Kümmerer 2009) indicating that polar and ionic interactions contribute to sorption behavior.
63 Overall, phase distribution of a pollutant depends on the physicochemical properties of the
64 molecule such as stereo chemical structure, pKa, solubility, polarity or log K_{OW} and the
65 external conditions, including pH, redox potential and temperature (Kümmerer 2009). Finally,
66 solid-liquid partition coefficient K_d provides distribution behavior of the compounds between
67 liquid and solid phases.

68 Few authors have studied solid-liquid coefficient during anaerobic digestion. Table 1
69 summarizes K_d coefficients found in literature for primary sludge. Ofloxacin and propranolol
70 have the highest expected sorption potential while ibuprofen and caffeine have the lowest one.
71 The rest of the molecules would be distributed in relatively same percentages in liquid and
72 solid phases. As we will discuss in 3.3 section, K_d is not enough to evaluate the distribution of
73 the molecules, Total Suspended Solids (TSS) concentration should be added for a complete
74 characterization. However, K_d can help to compare qualitatively the sorption of target
75 molecules in sludge when determined in the same conditions.

76 Anaerobic digestion process involves hydrolysis, acidogenesis, acetogenesis and
77 methanogenesis phases. Generally, the hydrolysis of organic matter is considered as the
78 limiting step in anaerobic digestion (Nguyen et al. 2018; Chen et al. 2020).

79 Various thermal, chemical and enzymatic processes are known to improve the removal of
80 recalcitrant organic matter (Paul et al. 2012). Among all these processes, micro-aeration has
81 drawn attention in recent years because of its positive impacts as enhancing hydrolysis,
82 dealing with overloads (Ramos and Fdz-Polanco, 2013) or enhancing biogas quality by
83 reducing the concentrations of H_2S (Díaz et al., 2010; Giordano et al., 2019). Aeration could
84 be implemented intermittently or continuously in different stages of anaerobic digestion, as
85 pre-treatment, co-treatment or post-treatment (Nguyen and Khanal, 2018).

86 As pre-treatment, Johansen and Bakke, (2006) found a positive effect on the hydrolysis of
87 proteins and carbohydrates during anaerobic digestion of primary sludge. Montalvo et al.,
88 (2016) found an increase of protein and total sugars solubilization, COD_s and methane
89 production associated to a 27% COD removal increase. Charles et al., (2009) observed
90 cellulase and protease activities after aeration pre-treatment of organic fraction of municipal
91 solids waste, confirming the hydrolytic capacities of exo-enzymes excreted by aerobic
92 bacteria. Three full-scale systems have implemented microaeration in anaerobic digestion
93 with positive effects in H_2S removal (Kobayashi et al. 2012; Jeníček et al. 2017; Giordano et
94 al. 2019).

95 As post-treatment, 47 % of COD removal in MAD and 28% in TAR was obtained in a system
96 formed by two stages MAD/TAR (MAD followed of TAR, without recirculation) (Park et al.
97 2014).

As co-treatment, micro-aeration has also shown positive effects, Xu et al., (2014) studied the effect of intermittent micro-aeration rates of anaerobic digestion of food waste. They concluded that adequate micro-aeration rate can enhanced the hydrolysis of solid organic waste and methane yield by 16.9% and 18%, respectively.

Thermophilic aerobic treatment coupled to conventional mesophilic digestion, as a co-treatment, has not been yet extendedly studied. Compare to a single mesophilic digestion, coupled process can get an additional solid removals and COD solubilisation (Dumas et al., 2010; Park et al., 2014; Fu et al., 2015). Fu et al., (2015) improved the methane yield and enhanced the hydrolysis step in the digestion of corn straw by microaeration pre-treatment at thermophilic temperature (55° C). With TAR as a co-treatment, Dumas et al., (2010) obtained a 30% supplementary increase of COD removal in lab-scale Mesophilic Anaerobic combined with a Thermophilic Aerobic Reactor (MAD-TAR) treating secondary sludge.

Anaerobic digestion is the most widely used method for treating (with valorisation) sludge produced in WWTPs (Gherghel et al., 2019) and the evaluation of the degradability of pharmaceutical molecules during digestion may be a key point to evaluate the ecotoxicological risks of digestate disposal. Table S2 summarizes the concentration of target micropollutants found in the literature during mesophilic anaerobic digestion and thermophilic anaerobic digestion (TAD). Some of molecules as IBP and OFL still had a high concentration in sludge after MAD.

The additional reduction of organic matter expected during coupled MAD-TAR digestion could be critically important as treatment of sludge represents approximately 50% of the total running cost of WWTPs (Gherghel et al., 2019). Although various studies have investigated the fate of pharmaceutical compounds in the anaerobic digestion (Carballa et al. 2007;

121 Narumiya et al. 2013; Samaras et al. 2013; Malmborg et al. 2015; Martín et al. 2015;
122 Gonzalez-Gil et al. 2016; Gonzalez-Gil et al. 2017), removal of some persistent
123 micropollutants remains contradictory, i.e. removal of CBZ was reported from 85%
124 (Malmborg et al. 2015) to <0 % (M. Narumiya et al. 2013). In addition, their behaviour under
125 thermophilic digestion has been little studied and never for a co-treatment such as MAD-TAR
126 though it gives very promising results.

127 In this work, the degradation of nine selected compounds (caffeine, ofloxacin,
128 sulfamethoxazole, propranolol, carbamazepine, oxazepam, diclofenac, ibuprofen and 2
129 hydroxy-ibuprofen) and their sorption behavior was evaluated in a pilot-scale system. A
130 QuEChERS-based method was modified to analyse targeted molecules in aqueous and total
131 daily-composite samples attempting a mass balance of the compounds in the system.
132 Conventional mesophilic anaerobic digestion (MAD) was compared to a system coupling a
133 thermophilic aerobic reactor with MAD (MAD-TAR).

134 ## 2. Material and Methods

135 ### 2.1. Analytical standards and reagents

136 Analytical standards of ≥98% purity of nine selected molecules were purchased from Sigma–
137 Aldrich (Saint Quentin Fallavier, France), with the exception of oxazepam and IS for
138 ofloxacin, propranolol, sulfamethoxazole, oxazepam and 2OH-ibuprofen that were furnished
139 by Alsachim (Illkirch Graffenstaden, France). All compounds were supplied in powder except
140 IS for caffeine and carbamazepine were obtained as methanolic solution at a concentration of
141 1 mg/mL and 100 µg/mL, respectively.

Tri-sodium citrate dihydrate (Na_3Cit, $2H_2O$) and disodium sulfate (Na_2SO_4) were obtained from Sigma–Aldrich. The LC-MS–grade solutions used, including methanol (MeOH), acetonitrile (ACN), formic acid and acetic acid (AA) were obtained from VWR Prolabo (Fontenay-sous-Bois, France) as well as the products ethylenediaminetetraacetic acid disodium salt dihydrate (Na_2EDTA, $2H_2O$), citric acid monohydrate (Cit, $1H_2O$), primary secondary amine (PSA) and C18 adsorbents (SUPELCO).

2.2. Anaerobic digesters

Biodegradation of the nine molecules was evaluated in two different configurations: (i) a conventional mesophilic anaerobic digestion (MAD) and (i) Mesophilic Anaerobic Digestion combined with a thermophilic aerobic reactor as a co-treatment process (MAD-TAR). The MAD was a stirred tank reactor with an effective volume of 260 L equipped with an agitator and a double jacket. The TAR, with a liquid volume of 40 L, was also equipped with an agitator and a double jacket. The aeration was operated discontinuously in the TAR compartment to provide a dissolved oxygen concentration, measured by VisiFerm™ DO optical sensor (Hamilton) between 0.2 and 0.5 mg L^{-1} with an air flowrate of 300 L h^{-1}. MAD was connected to the TAR by means of an ALBIN peristaltic recirculation pump (type ALH15). Its flow rate was fixed at 100 L h^{-1} and 15 L were recirculated from one compartment to the other three times per day corresponding to a retention time of 0.9 days in the TAR. The two compartments were heated by two independent cryostats in order to maintain the temperatures suitable for aerobic thermophilic (60 °C) and anaerobic mesophilic (35 °C) processes. For the MAD campaign, the 40 L compartment was maintained at 35°C without aeration to be in fully MAD conditions while maintaining all other operating conditions similar. Biogas composition (CH_4, CO_2 and O_2) in MAD outlet was measured online (X-Stream X2GP continuous gas analyzer).

After inoculation by the secondary sludge from Cugnaux WWTP, the digester was fed with primary sludge from our pilot-scale settling tanks. Digester was operated semi-continuously by feeding the primary sludge automatically once a day. The duration of feeding, withdrawal and recirculation from MAD to TAR were adjusted to maintain a total sludge retention time (SRT) of around 20 days and a residence time in the TAR of 0.9 day. MAD and MAD-TAR campaigns were carried out in two consecutive years, between February and April, in order to have the same type of seasonality in medication intake. The Organic Loading Rate (OLR) was 1.2 kg_{COD} m^{-3} day^{-1} during MAD and 0.9 kg_{COD} $m^{-3}day^{-1}$ during MAD-TAR campaign.

2.3. Sampling and analytical techniques

To evaluate the performances of the two studied processes, daily-composite samples of primary and digested sludge were collected regularly during steady-state periods. Sludge samples were stored at 4 °C and analyzed on the same day. In addition, aliquots of the total and dissolved fractions (obtained after 4500 g centrifugation for 15 minutes) were stored at -20 °C for micropollutants analysis. The sludge and the supernatants were characterized by Total Suspended solids (TSS), Volatile Suspended Solids (VSS) and Chemical Oxygen Demand (COD).

For quantification of targeted micropollutants, a QuEChERS-based modified method followed by UHPLC-MS-MS was performed both on total and aqueous samples. The protocol consisted in introducing 2.5 mL of real matrices in a 50 mL polypropylene (PP) Falcon tube (Falcon tubes from VWR Scientific Products). Quantification of the target analytes was performed by the internal standard approach, thus a volume of 0.2 mL of IS solution at 300 ng L^{-1} was spiked to samples. The PP tubes was left for 2 h on a shaking table to ensure the homogeneity of the sample, and more importantly the adsorption of the IS on the sludge.

189 Under slow stirring, 1.25 mL of EDTA, 1.25 mL of citrate buffer (232 g L^{-1} Na_3Cit, $2H_2O$ and
190 115.2 g L^{-1} Cit, $1H_2O$), 5 mL of acidified ACN and 4 g of Na_2SO_4 are added in tubes that are
191 immediately vortexed (Heidolph™ Multi Reax Vortex Mixer from Fisher Scientific) at
192 maximum speed for 1 min and then centrifuged at 7,100 g for 5 min. The entire organic phase
193 was transferred to a 15 mL Falcon tube containing 0.075 g of sorbents (PSA and C18 in ratio
194 1/1) and immediately vortexed at maximum speed for 1 min and then centrifuged at 7,100 g
195 for 5 min. The purified organic phase was then recovered in Pyrex tubes and evaporated under
196 reduced pressure for 7 h at 45°C using the Low BP program of the EZ-2 Envi evaporator
197 system (Genevac). A volume of 1 mL of a solution of ACN/water 95/5 (v/v) was added and
198 pyrex tubes were vortexed at maximum speed for 1 min. The liquid was filtered through a 0.2
199 µm membrane (Minisart RC 15, Sartorius, France) and transferred in vials before analysis.

200 Liquid chromatography was carried out using an Ultimate 3000 UHPLC System from Dionex
201 (France). Sample aliquots (10µL) were injected onto an ACQUITY UPLC HSS (High
202 Strength Silica) T3 (100 mm x 2.1 mm, 1.8µm) column from Waters. Column oven
203 temperature was maintained at 35°C and flow rate was 0.6 mL min^{-1}. For the positive
204 ionization, the mobile phase was composed of solvent 1 (HCOOH 0.1% in pure water) and
205 solvent 2 (HCOOH 0.1% in ACN) with the gradient as follows: 0–0.5 min 95/5, 0.5–7 min
206 95/5 to 0/100, 7–8 min 0/100, 8–8.2 min 0/100 to 95/5 and 8.2–9.5 min 95/5 (ratios expressed
207 as 1/2 ratios). For the negative ionization, the solvents 1 and 2 are pure water and ACN,
208 respectively. Detection was achieved with an Applied Biosystems Sciex QTRAP® hybrid
209 linear ion-trap triple quadrupole mass spectrometer (Foster City, USA) equipped with a
210 Turbolon-Spray Interface. The instrument was operated in ElectroSpray (ESI) positive (+) or
211 negative (-) in Multiple Reaction Monitoring (MRM) mode (dwell time, 80 ms). Details of
212 analytical conditions are presented in supplementary information (Table S3). Performance

213 characteristics of the method were evaluated by accuracy and precision on total fraction of
214 primary sludge and digestate. Method accuracy (estimated by means of relative recovery (RR)
215 experiments) and precision (expressed as intra-day repeatability in terms of relative standard
216 deviation (RSD)) were studied by spiking samples at different concentrations according to the
217 concentration levels found in real samples. RR of the analytes ranged within 90%–120% and
218 the intra-day RSD values were found to be lower than 20% indicating a satisfactory accuracy
219 and precision. For all matrixes (primary sludge and digestate, total and liquid fractions), limit
220 of detection (LD) was calculated according to the EPA's method by considering 3 times the
221 standard deviation determined by analyzing 8 blank samples or spiked samples at low levels
222 when it is necessary. Therefore, it is a robust and reliable method for complex and simple
223 matrices which makes it possible to study the performances of the MAD and MAD-TAR
224 processes. For each campaign, 13 samples were taken and analyzed in duplicate by adding
225 internal standards for each targeted molecule targeted prior to extraction. For each samples
226 and analytes, standard deviations of less than 10% were obtained.

227 **2.4. Calculations**

228 *Mass balances and pharmaceuticals removal*

229 Because of the discontinuous feed and withdrawal (one per day) and the variation of
230 concentrations in primary sludge, all the data were cumulated for calculations. When a
231 stationary state is reached, a straight line must be obtained when representing $\Sigma m_{outlet} = f$
232 (Σm_{inlet}). The obtained slope allowed to calculate the removal efficiency of the compound
233 $(\eta_{removal} = (1\text{-slope}) \times 100)$. This method was applied for VSS, COD and micropollutants.

Statistical analysis was carried out following (Sperling et al., 2020) recommendations. Linearity of the regression was confirmed for all parameters by ensuring that the linear model was satisfying the assumptions of linearity, independence, normality of residuals and homogeneity of variances. Furthermore, calculations of the 95% confidence interval of the slope of linear model obtained for MAD and MAD-TAR campaign allowed evaluating if performances were significantly different (not overlap of the confidence interval). This analysis was performed with R software.

Distribution of target molecules between solid and liquid phases of sludge

Solid-liquid partition coefficient K_d (L kg_{TSS}^{-1}) of target molecules in sludge and supernatants of each sample was calculated as:

$$K_{d,i} = \frac{C_{i,sorbed}}{C_{i,liquid}} = \frac{\left(C_{i,total} - C_{i,supernatant} \cdot \frac{V_{supernatant}}{V_{total}}\right) / \ TSS}{C_{i,supernatant} \cdot \frac{V_{supernatant}}{V_{total}}} \qquad \text{eq. 1}$$

Where $C_{i,sorbed}$ and $C_{i,liquid}$ are the solid (µg kg_{TSS}^{-1}) and liquid (µg L^{-1}) concentrations of molecule *i*, respectively. $C_{i,total}$ is the total concentration of molecule i in the sludge (µg·L^{-1}) and $C_{i,supernatant}$ is the supernatant concentration of molecule i (µg·L^{-1}) in the liquid phase after centrifugation of the sludge. $V_{supernatant}$ / V_{total} is the ratio of the supernatant and total volumes after centrifugation.

The amount of compound *i* in the liquid phase in function of log K_d value for the range of TSS sludge concentrations was assessed as:

$$100 - \frac{C_{i,sorbed}}{C_{i,total}} = \frac{K_{d,i} \cdot TSS}{1 + K_{d,i} \cdot TSS} \qquad \text{eq. 2}$$

Oxygen consumption in TAR reactor.

In order to evaluate the fate of organic matter (i.e. removal in aerobic or anaerobic conditions), the oxygen uptake rate (OUR) in the TAR was calculated from the decrease of the dissolved oxygen concentration (C_{O2}) during non-aerated periods.

$$OUR = -\frac{dC_{O2}}{dt} \quad \text{eq. 3}$$

3. Results

3.1. Start-up and operation

Although the feeding showed fluctuations, as can be expected when working with real influent, the VSS and COD_t concentrations after digestion remained constant with standard deviation below 4%. Considering comparable operating conditions (see Table 2), higher elimination of solid organic matter was achieved in the hybrid MAD-TAR system (around 76% for VSS) compared to the mesophilic one (around 47%) confirming lab results obtained a few years ago (Dumas et al. 2010).

During MAD-TAR campaign, COD balances were performed to estimate the fate of transformed COD. Collected grab samples at the inlet and outlet of the TAR compartment allowed estimating that 26.6% of the total removed COD was eliminated in this thermophilic compartment. The additional COD removal compared to MAD campaign being eliminated in the aerated compartment, no additional methane recovery in the MAD compartment was allowed. Furthermore, the average OUR in the TAR was 7.5 ± 0.8 $mgO_2\ L^{-1}\ h^{-1}$ that corresponds only to 3.9% of the total removed COD that was degraded in aerobic conditions. Even if volatilization of certain substances such as volatile fatty acids could occur, this observation tends to show that the thermophilic condition could have more effect than the aeration. Two main mechanisms could be associated with this temperature effect. On the one

hand, the microbial activity could be directly positively affected. Indeed, several authors reported a significant increase in hydrolysis rate in thermophilic compared to mesophilic conditions (Siegrist et al. 2002; Lynd et al. 2002). Ge et al. (2011) even demonstrated that this rate strongly follow the Arrhenius relationship, with nearly a doubled value when increasing the temperature by 20°C. On the other hand, temperature may play a role in physical phenomena, in particular on mass transfer and solubility even if their effect on the rate of microbial utilization remains to confirm (Lynd et al. 2002).

3.2. Fate of pharmaceutical compounds during anaerobic digestion

The total concentration (µg L^{-1}) of the nine selected compounds is illustrated in Figure 1 for both primary sludge and digestate from MAD (Figure 1a) and MAD-TAR (Figure 1b) processes. All the target molecules were detected and quantified in the primary sludge (see Table S4 for limit of detection). Even if some variations in the concentrations have been observed between the two experimental campaigns (-23% in average during the MAD-TAR experiment) the order of magnitude were the same, allowing for a relevant comparison of performances between the two operating conditions. The highest concentrations of targeted compounds were observed for OFL and CAF (more than 40 µg L^{-1}) while medium to low concentrations were reported for SMX, CBZ, PRO, DIC, OXA, IBP and 2OH-IBP, around 1.0-10.0 µg L^{-1}.

The comparison of removal efficiencies in MAD and MAD-TAR are reported in Figure 2. Compounds placed above the dotted line show better removal efficiency by the innovative process (MAD-TAR) compared to conventional process (MAD).

The highest removal yields were obtained for CAF and SMX (>89%) with slight increase in MAD-TAR campaign for CAF, but with no significant differences between both processes for

299 SMX. The removal of several other target compounds was significantly improved by MAD-
300 TAR process. Moderate removal ranging between 54% and 75% were observed for OFL and
301 OXA respectively in MAD-TAR compared to only 9% and 49% in MAD. Moreover, while
302 PRO was not removed at all during MAD process, a rate of 69% was achieved in MAD-TAR.
303 DIC and 2OH-IBP presented low removal during MAD-TAR (6-10%) while they were
304 accumulated in MAD. Finally, a negative removal rate was observed for IBP and CBZ in both
305 processes, MAD (-30% and -71%, respectively) and MAD-TAR (-35% and -91%),
306 underlining that concentrations were higher in the digestate than in primary sludge.

307 In MAD conditions, similar removal efficiencies were reported in the literature for OFL, PRO
308 (Narumiya et al. 2013), CAF (Narumiya et al. 2013) and SMX (Carballa et al. 2007;
309 Narumiya et al. 2013; Alvarino et al. 2014; Falas et al. 2016; Lakshminarasimman et al.
310 2018).

311 Ibuprofen is generally well removed under aerobic conditions (Alvarino et al. 2018) but the
312 removal under anaerobic conditions remained controversial. Carballa et al. (2007); Martín et
313 al. (2012); Samaras et al. (2013) showed a moderately-high removal, but recent studies found
314 a recalcitrant behaviour after anaerobic processes (Alvarino et al. 2014; Phan et al. 2018) as
315 underlined in our results.

316 DIC is generally persistent with low removal efficiencies (Alvarino et al. 2014; Ghattas et al.
317 2017; Phan et al. 2018; L. Gonzalez-Gil et al. 2019) whereas, moderate removal was showed
318 in some lab-scale data (Carballa et al. 2007; Malmborg et al. 2015). CBZ is also recalcitrant in
319 wastewater treatments, low or negligible removal was reported in the studies of (Ghattas et
320 al. 2017; Alvarino et al. 2018; Lakshminarasimman et al. 2018; Phan et al. 2018; Kent et al.
321 2019).

322 Redox conditions played an important role conditioning the development of microbial
323 populations and thus on biodegradation pathways thanks to the complementary hydrolytic
324 capacities of external enzymes excreted by thermophilic aerobic bacteria. Some molecules are
325 only degraded under specific aerobic, anaerobic or both conditions. Stadler et al. (2015) and
326 Alvarino et al. (2018) studied the influence of redox potential in the removal of organic
327 compounds, showing that in the case of SMX the microaerobic conditions increased their
328 removal.

329 Phan et al. (2018) reported that molecules containing only electron donating functional groups
330 (-NH_2, -OH, -CH_3, -OCH_3…) in their structure are more susceptible to biodegradation, as is
331 the case of SMX. In anaerobic digestion. Ghattas et al. (2017) proposed a degradation
332 pathway via reduction of the electron withdrawing sulfonyl group. In the case of IBP, this
333 molecule contains three electron donating functional groups and it should be biotransformed
334 but it remains recalcitrant after anaerobic digestion, maybe because their substitutions in their
335 aromatic ring. In the case of DIC, a possible pathway could be the reductive dichlorination
336 (Phan et al. 2018) or a biodegradation catalysed by decarboxylases or acetate kinases
337 (Gonzalez-Gil et al. 2019). Concerning SMX, they reported that most probably pathway was
338 the abiotic reduction of the Ne-O bond mediated by microbial reaction. About DIC, they
339 proposed that their biodegradation may be catalysed by decarboxylases or acetate kinases
340 (Gonzalez-Gil et al. 2019).

341 Microaeration on anaerobic digestion affects microbial communities by increasing the
342 diversity and activity of hydrolytic and fermentative microorganisms (Nguyen et al. 2018;
343 Chen et al. 2020). Comparing microaeration to strict anaerobic condition, an increase of
344 phylum Firmicutes (class Clostridia), which is related to hydrolysis step was found. In
345 addition, microaeration also affects the methane-producing archaea presented in anaerobic

346 digestion: Methanosarcina, slightly aerotolerant, and Methanobacterium both doubled (Fu et
347 al., 2016). Cometabolism is considered the main mechanism of pharmaceutical degradation
348 during biological wastewater treatments (Gonzalez-Gil et al., 2017). Further research is
349 necessary to understand the degradation pathway attributed to co-metabolism during hybrid
350 digestion process and the additional degradation of some targeted micropollutants during
351 MAD-TAR.

352 Therefore, MAD-TAR process allows the exposure of compounds to different redox and
353 temperature conditions improving significantly the removal efficiency of OXA, PRO and
354 OFL and slightly DIC and 2OH-IBP concomitantly with the improvement of overall organic
355 matter degradation. These are very positive results considering the ecotoxicological risks of
356 OXA, PRO and OFL as underlined by Besse et al. (2008). However, IBP and CBZ even show
357 more negative efficiencies and the mechanisms involved need further investigations. The
358 negative removal or apparent production of some molecules have already been reported in the
359 literature, explained by the transformation back of their metabolites to their parent
360 compounds. Indeed, parent chemicals are often excreted from the human body with a number
361 of associated metabolites. In the case of IBP, it is excreted as the unchanged drug in 1% and
362 76% as metabolites (Petrie et al. 2015). There is a general lack of data about metabolite
363 concentrations and biotransformation during wastewater treatments but some authors reported
364 higher metabolites concentrations than parent compound in urban wastewater for IBP
365 (Brezinova et al. 2018). In the case of CBZ, the negative removal or apparent production was
366 also reported (Narumiya et al. 2013; Falas et al. 2016). He et al. (2019) reported the
367 quantification of some phase I and phase II metabolites of CBZ showing that CBZ N-
368 glucuronide (CBZ-Glu) concentration (59.4 ng L^{-1}) was higher than CBZ (53.8 ng L^{-1}) in
369 wastewater influent from Kyoto WWTP (Japan). In their effluent, CBZ concentration

increased by 15% and CBZ-Glu decreased by 19%. They attributed the increase of CBZ to the deconjugation of CBZ-Glu. Further research is needed to confirm this hypothesis in IBP and CBZ as some metabolites may be biologically active and have similar pharmacological activity than parent compound (Besse et al. 2008).

3.3. Partitioning pharmaceutical compounds

Considering the possible relation between sorption on solids and inaccessibility for biodegradation, distribution of compounds was studied based on measured concentration in total and soluble phases, as described in section 2.4. From eq. 1, it is obvious that a single value of log K_d is not enough to evaluate the partition of the compounds between solid and liquid phases and that this partitioning constant should be always given together with the TSS concentration. Indeed, in the case of primary sludge of MAD campaign, which presented the highest TSS concentration, a log K_d value below 1.73 and 0.78 indicates that the percentage of compound in the liquid phase is above 50% and 90%, respectively (Figure 3a). At the opposite, when considering the lowest concentrated sludge, i.e. the digestate during MAD-TAR campaign log Kd values below 2.31 and 1.36 indicate a percentage above 50% and 90% respectively (Figure 3b).

Thus, even if the partition coefficients were not greatly modified during digestion, the significant decrease in TSS in the MAD-TAR campaign leads to higher fractions in the liquid (Figure 3a) compared to the MAD campaign (Figure 3b).

The distribution of targeted compounds between the particulate and liquid phases are presented in Figure 4. Primary sludge presented similar sorption behavior for the two campaigns, OFL and PRO showed the highest sorption (>90%) followed by OXA (71%). IBP, SMX, DIC and CBZ range of 35-63%. Finally, a low sorption is found for 2OH-IBP

393 (20% and 24 %, MAD and MAD-TAR respectively %) and CAF (21 and 34 %, MAD and
394 MAD-TAR respectively).

395 The liquid fraction of SMX, 2OH-IBP and PRO increased slightly after digestion in MAD (3-
396 5%) and moderately in MAD-TAR (11-19%); CBZ and OXA increased moderately in MAD
397 (7%-13%) and significantly in MAD-TAR (27-39%); IBP and DIC increased significantly in
398 MAD (23%-34%) and in MAD-TAR (22-28%). Only CAF presented a contradictory
399 behaviour, liquid fraction increased in MAD-TAR (16%) and decreased in MAD (-8%). Only
400 OFL showed no significant change in sorption after digestion, 0 and 2% in MAD and MAD-
401 TAR, respectively.

402 As indicated in Table S5, for a same affinity constant, the fraction in liquid after digestion is
403 significantly different in MAD and MAD-TAR due to drastic changes in TSS. Indeed, the
404 suspended solid concentration dropped from 18.5±3.4 to 11.7±0.5 g L^{-1} during the MAD
405 experiment and from 15.4 ± 2.1 to 4.8 ± 0.7 g L^{-1} during the MAD-TAR campaign.

406 The difference in phase distribution after digestion can partly be attributed to the different pH
407 value of primary and digested sludge because of the presence of compounds containing
408 functional groups which can be protonated and deprotonated (Ternes et al. 2004). In function
409 of pH, the compound can be neutral, cationic, anionic or zwitterionic, consequently its
410 physical, chemical and biological properties may be affected (Verlicchi et al. 2012) by
411 inducing positively charged compounds, that are likely to interact with the negatively charged
412 surface of sludge, or at the contrary anions inducing hydrophilic behaviour (Alvarino et al.
413 2018).

414 Among the compounds mostly present in liquid phase after digestion (pH 7.5 in MAD - 7.4 in
415 MAD-TAR), IBP, 2OH-IBP and DIC have a carboxylic acid group (pKa = 4.15 - 4.63). For a

pH higher than pKa value, the fraction of anionic species increased. Initial pH of the primary sludge was 6.1– 6.2, after digestion of the organic matter, the pH increased resulting in an increase of hydrophilicity of the compounds, in line with our results. For instance, in the case of IBP, the liquid fraction in primary sludge was 65% (MAD) and 59% (MAD-TAR) and it increased to 87% (MAD) and 92% (MAD-TAR) after digestion. Similar increase of liquid partition was found in DIC and 2OH-IBP. The same partitioning behaviour was also expected in SMX (pKa 1.8 and 5.6). At the range of pH in anaerobic digestion, the fraction of anionic species should also increase due to the dissociation pathway of sulfonamides and consequently an increase of hydrophilicity is likely to occur. Ma et al. (2015) confirmed in their study of oxidation of SMX that the dissociation of SMX increases with increasing pH. Consequently, deprotonated compounds were more readily to oxidation. In our study, partition behaviour of SMX remained almost constant before and after digestion (liquid fraction slightly increased 5-11%).

Among the compounds present mostly in solid phase, OFL has a zwitterion structure with a basic amino group (pKa 9.28) and an acid carboxylic group (pKa 5.97). Between pH 6.0 and 9.3, cationic (protonated amino group), anionic (deprotonated carboxyl group) and zwitterion species would be present. At pH of digestion (7.4-7.5), zwitterions should be dominant. In accordance with Narumiya et al. 2013, due to the increase of zwitterionic species, the hydrophobicity of OFL would also increase; this fact may explain the tendency to sorb. In our study, sorption behaviour of OFL did not significantly change and remained at high level with more than 99% of the molecule located in the particulate compartment. OXA is uncharged under normal pH conditions (Svahn et al. 2015), it has also a zwitterion structure (pKa 1.55, 10.9). Between pH 6 and 11 zwitterion and anionic species would be predominant. With increasing pH during digestion, anionic species would also increase, resulting in higher

hydrophilicity, in agreement with our results: the liquid fraction of OXA in primary sludge was 28-29% (MAD and MAD-TAR) and it increased to 65-67% (MAD and MAD-TAR) after digestion.

CAF (pKa 10.40) and PRO (pKa 9.42) are cationic species when pH is lower than pKa value due to their protonated amino group. During the digestion, pH increased and the fraction of cationic species would decrease in favour to their neutral conjugates. The increase in the neutral fraction would decrease the hydrophilicity of the molecules and hence liquid fraction would decrease (Narumiya et al. 2013). We found this behaviour for CAF during MAD campaign, where the liquid fraction decreased from 79% in primary sludge to 70% after digestion. However, liquid fraction of CAF increased after digestion in MAD-TAR campaign. In the case of PRO, there were not a significant increase of hydrophilicity in MAD campaign (liquid fraction only increased 3% after digestion) but the liquid fraction increased from 10% in primary sludge to 22% after digestion during MAD-TAR experiment. Consequently, a parameter other than pH influenced their solid-liquid partition during MAD-TAR campaign.

CBZ (pKa -3.8, 13.9) is a neutral compound and hence sorption should be governed by hydrophobic interactions (Hai et al. 2018) without influence of pH (Urase et al. 2005; M. Carballa et al. 2008). Svahn et al. (2015) also suggested that hydrophobic adsorption would predominate because of the high apolar to polar surface ratio of the CBZ. According to our results, liquid fraction of CBZ increased after digestion in MAD and MAD-TAR process and hence a parameter other than pH should influence CBZ partitioning behaviour during anaerobic digestion

When considering the compounds that suffered a biodegradation during digestion, results tend to show an improvement of biodegradation in MAD-TAR that may be associated with the

463 increase of the fraction in the liquid phase in the case of DIC, OXA and 2-OH IBP (Figure 5).
464 The increase of the removal of OFL and PRO may be linked to the increase of solid reduction
465 in MAD-TAR because of the fact that these molecules are largely sorbed in solids. Indeed, the
466 concentration of solids in MAD-TAR decrease down to 4.9 mg L^{-1} compared to 11.7 mg L^{-1}
467 in MAD campaign resulting in an increased availability of compounds in the liquid phase.
468 SMX and CAF are widely removed in MAD and MAD-TAR campaigns, so a clear link
469 between liquid fraction and removal cannot be established. Furthermore, liquid fraction of
470 IBP decreased and CBZ increased during MAD-TAR campaign and both components showed
471 more negative efficiencies during this campaign. Thus, bioavailability cannot be the only
472 mechanism driving the biodegradation and reversibility of the biological reactions has to be
473 considered.

474 **Conclusions**

475 The removal efficiency of nine pharmaceutical compounds in conventional Mesophilic
476 Anaerobic Digestion (MAD) was compared with Mesophilic Anaerobic Digestion combined
477 with a Thermophilic Aerobic Reactor (MAD-TAR). In addition to a significant improvement
478 in the degradation of solid organic matter (76% vs 47% for MAD-TAR and MAD
479 respectively), interesting results were also obtained on the targeted compounds. Moderate
480 (DIC, 2OH-IBP) to high (OXA, PRO, OFL) improvement of removal was observed in MAD-
481 TAR while removal higher than 90% was found for CAF and SMX whatever the process.
482 Even if the mechanisms implied in the fate of IBP and CBZ need further investigation, hybrid
483 process MAD-TAR that combines two redox conditions (anaerobic and micro aeration)
484 presents a feasible alternative to improve not only the removal a wide number of pollutants,
485 but also overall organic matter degradation.

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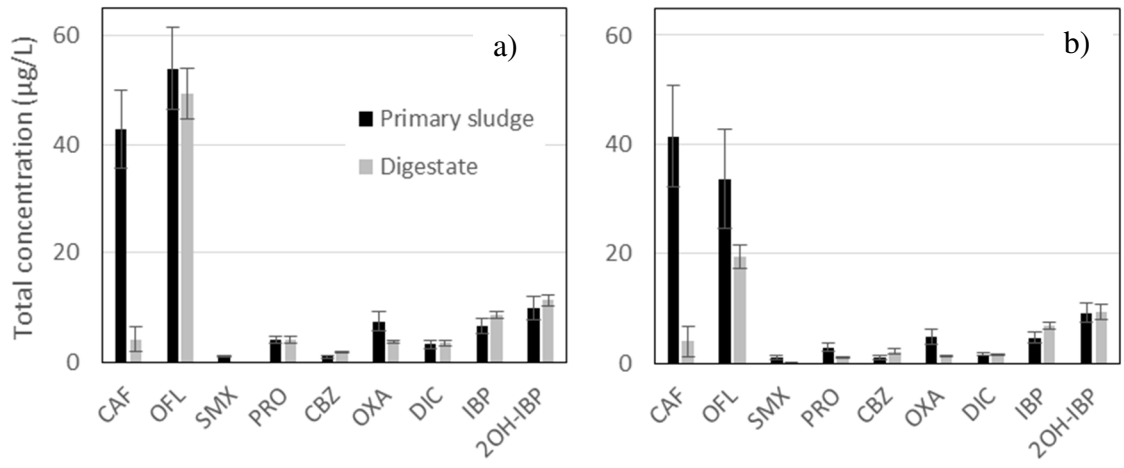


Figure 1. Concentration of targeted compounds in primary sludge and digestate in a) MAD (n=13); b) MAD-TAR (n=13)

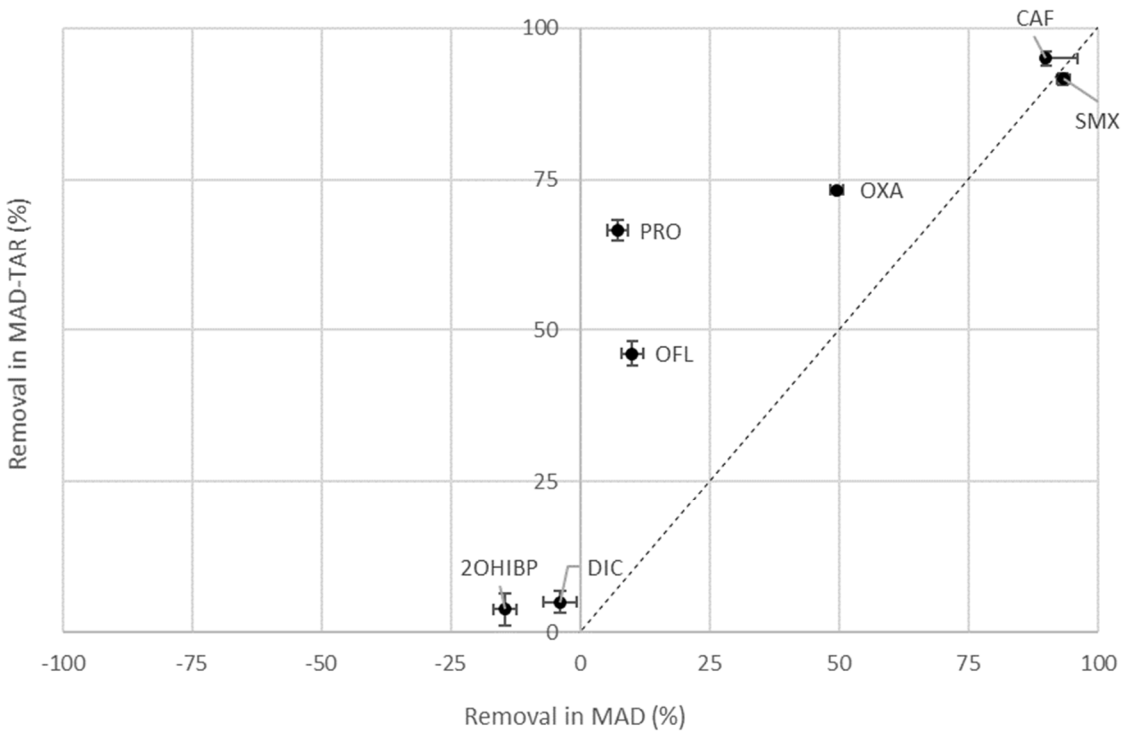


Figure 2. Comparison of compounds removal in conventional vs innovative process for biodegradable molecule. The error bars represent the 95% confidence interval.

a) b)

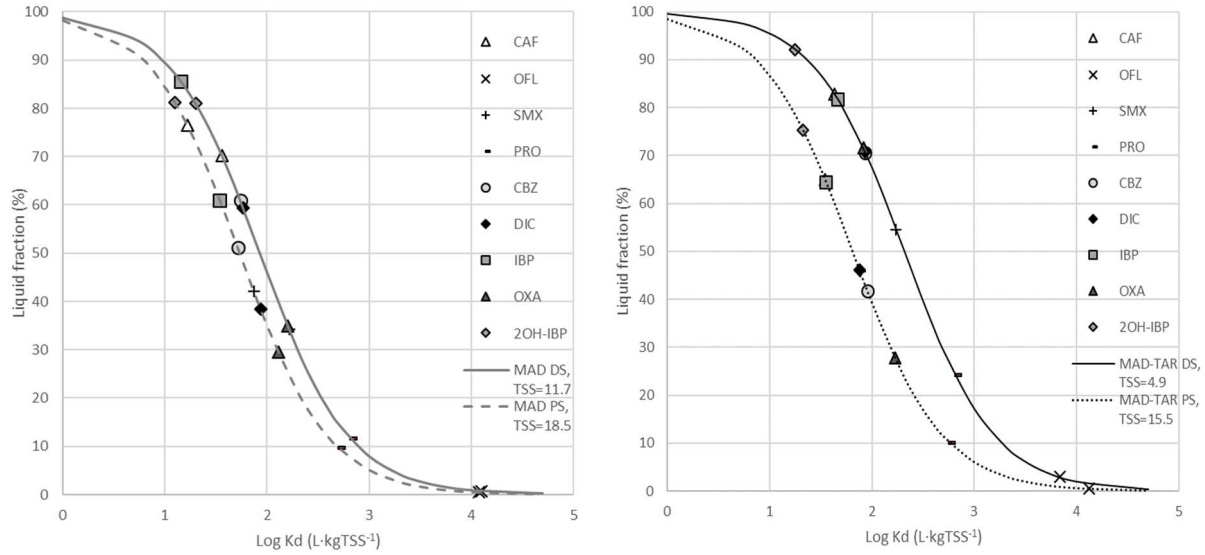
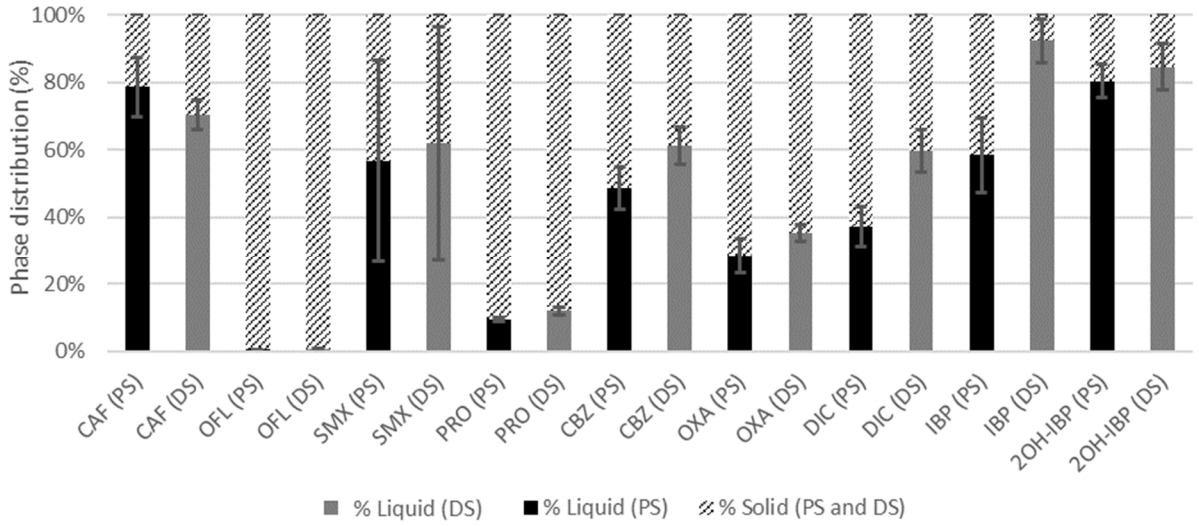


Figure 3. Logarithm of solid-liquid partition coefficient Kd (L·kgTSS^{-1}) of target molecules in primary (PS) and digested (DS) sludge of MAD (a) and MAD-TAR (b) campaigns. Lines are calculated from eq. 1.

a)



b)

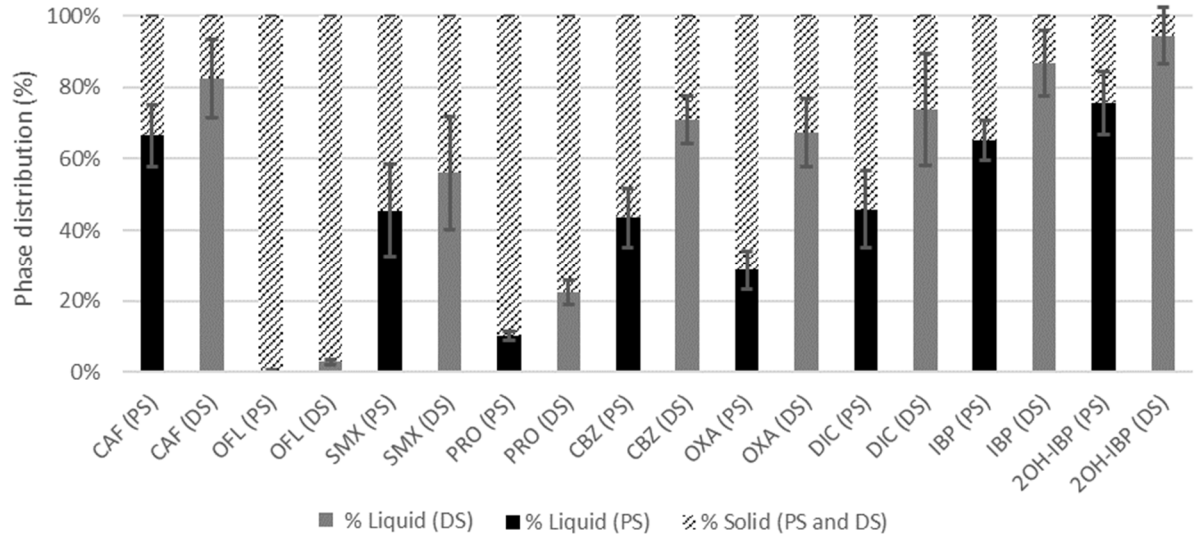


Figure 4. Distribution of the target compounds in particulate and liquid phases for primary (PS) and digested (DS) sludge: a) MAD b) MAD-TAR.

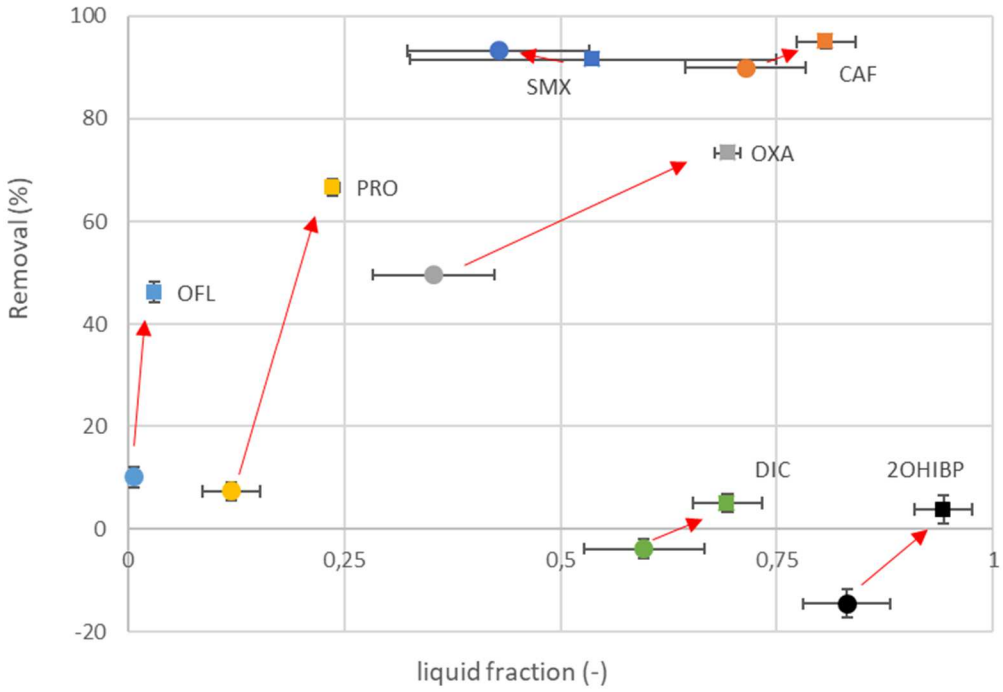


Figure 5. Removal efficiencies versus fraction in liquid phase for MAD (●) and MAD-TAR (■) for biodegradable compounds. The error bars represent the 95% confidence interval.

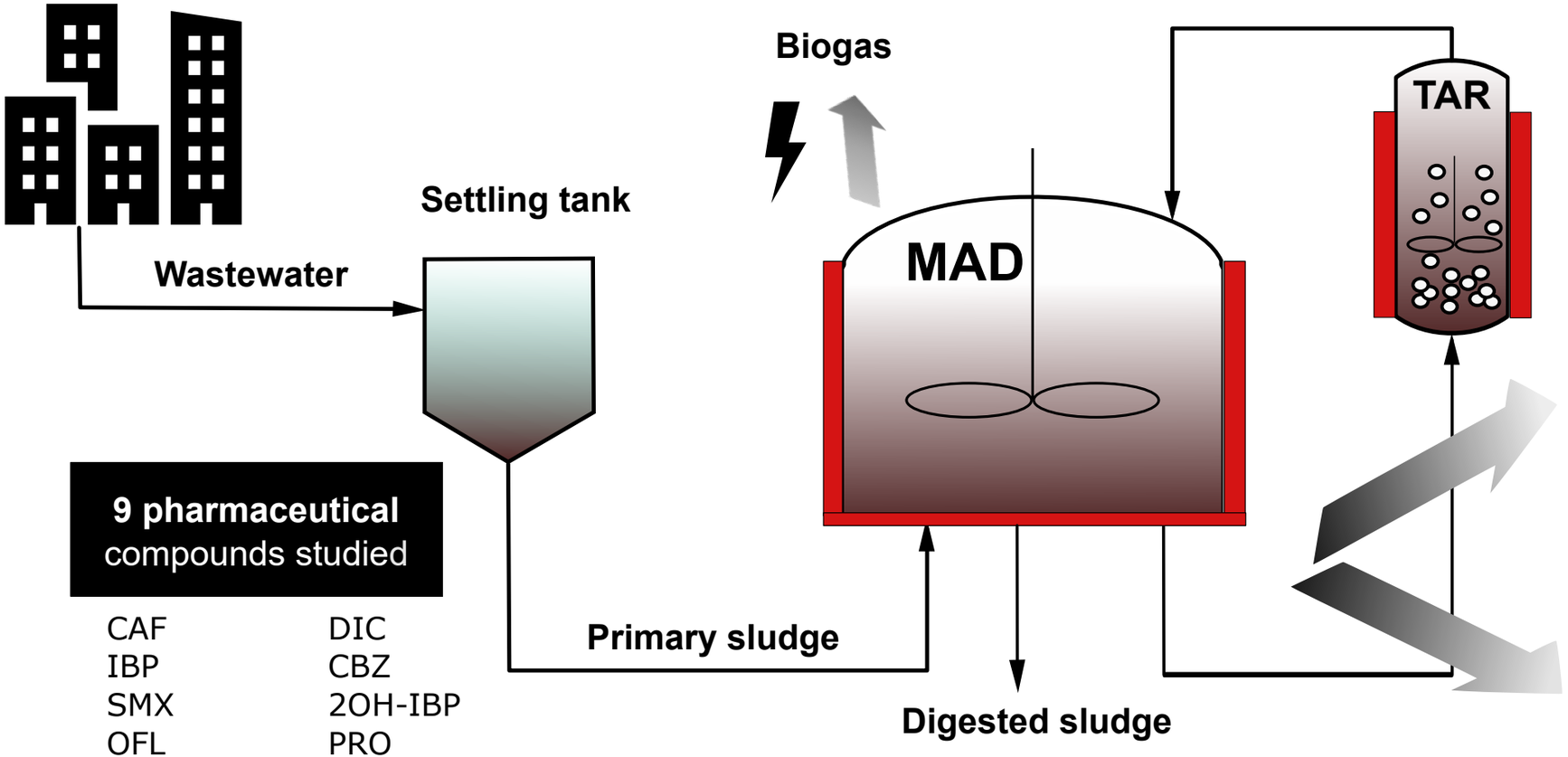
Table 1: Logarithm of solid-liquid partition coefficient K_d ($L.kgTSS^{-1}$) of target molecules in primary sludge.

	CAF	OFL	SMX	PRO	CBZ	DIC	IBP
Log K_d	1.4 – 2.0 [a)]	3.0 - 3.5 [a)]	2.6 [b)] 1.8 - 2.6 [a)] 0.50 - 0.89 [c)]	2.21-3.05 [c)]	2.4 [c)] 1.7 - 2.0 [a)] 2.04 - 2.71 [c)]	2.7 [b)] 2.3 - 3.1 [a)] 1.77 - 2.51 [c)] 2.7 [d)]	0.8 - 1.1 [c)]

a) Narumiya et al. (2013) b) Carballa et al. (2008) c) Radjenović et al. (2009) d) Ternes et al. (2004)

Table 1 Main characteristics of MAD and MADTAR digestion.

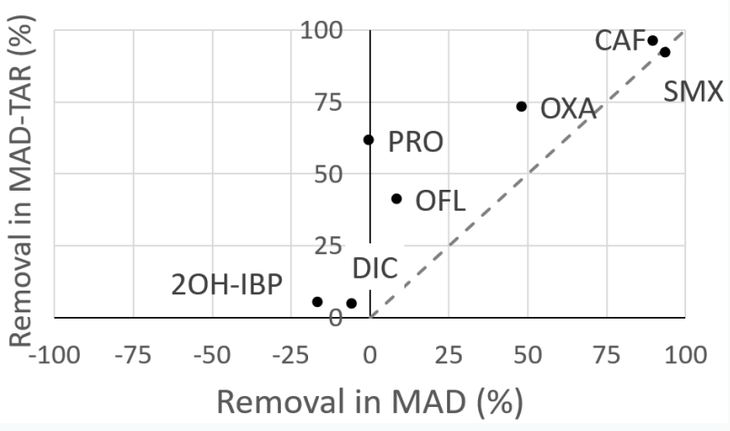
	MAD	MAD-TAR
Operational conditions		
OLR (kgCOD m^{-3} day^{-1})	1.2	0.9
OLR (kgVSS m^{-3} day^{-1})	0.8	0.6
SRT (days)	19.6	22.4
Temperature (°C)	35	35 (MAD), 60 (TAR)
Primary sludge		
TSS (g L^{-1})	18.5 ± 3.5	15.5± 2.1
VSS (g L^{-1})	15.1 ± 2.9	12.9± 1.7
COD_t (g L^{-1})	24.2 ± 4.6	20.2 ± 4.6
COD_s (g L^{-1})	0.63 ± 0.12	0.45 ± 0.15
pH	6.1 ± 0.1	6.2 ± 0.1
Reactor		
TSS (g L^{-1})	11.7 ± 0.5	4.9 ± 0.6
VSS (g L^{-1})	8.3 ± 0.4	3.4 ± 0.4
COD_t (g L^{-1})	14.1 ± 1.4	5.7 ± 0.8
COD_s (g L^{-1})	0.37 ± 0.06	0.36 ± 0.08
pH	7.5 ± 0.1	7.4 ± 0.1
Removal efficiencies (%)		
COD	46.7	72.9
VSS	47.0	75.7
Biogas production	151	186
(NL CH_4 Kg^{-1} COD_{fed})		
Average methane concentration (%)	57.4 %	56.3 %



9 pharmaceutical compounds studied

CAF	DIC
IBP	CBZ
SMX	2OH-IBP
OFL	PRO
OXA	

Removal of pharmaceutical compounds: MAD vs MAD-TAR



COD removal: 73%