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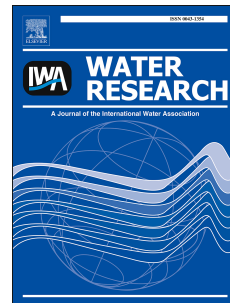
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# Accepted Manuscript

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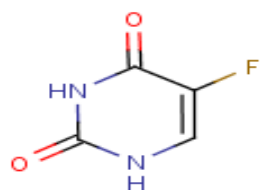
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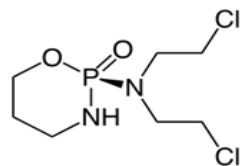


Oncological  
department

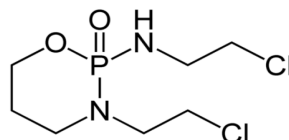
WASTEWATER WITH A HIGH CONCENTRATION OF MOLECULES



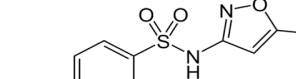
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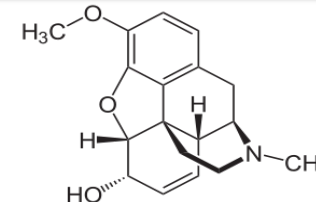
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(CP)



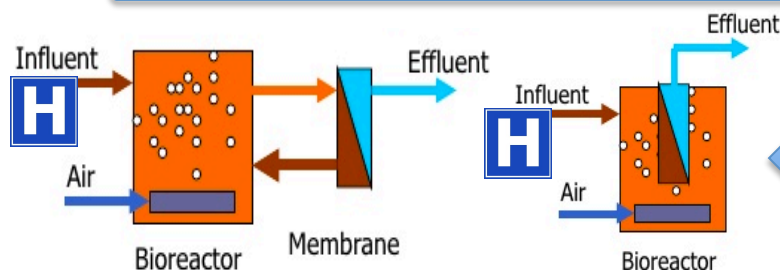
Ifosfamide (IF)



Sulfaméthoxazole  
(SM)



Codéine (CD)



MBR adapted to oncological wastewater

Biomass  
from



Urban waste water treatment plant

**Performance in terms of**

Biomass resistance

Processing capacity

Sorption or biotransformation

Removal of pharmaceutical  
molecules

**Performance of a biomass adapted to Oncological Ward Wastewater vs. biomass from municipal WWTP on the removal of pharmaceutical molecules**

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7  
8 **Abstract**

9 The performance of a biomass adapted to Oncological Ward Wastewater (OWW) in a  
10 membrane bioreactor (MBR) was compared with that of a municipal WWTP, on the  
11 removal of pharmaceutical molecules and more specifically on their overall resistance  
12 and purifying ability in the presence of pharmaceutical cocktails. Sorption and  
13 biotransformation mechanisms on two antineoplastics, one antibiotic and a painkiller  
14 were evaluated. Sludge acclimated to OWW allowed for a 34% increase in the removal  
15 rate and in the minimum inhibition concentration. The percentage of the amounts of  
16 specific pharmaceutical compounds removed by biotransformation or by sorption were  
17 measured. These results are positive, as they show that the observed removal of

18 pharmaceutical molecules by biomass acclimated to OWW can mostly be attributed to  
19 developed biotransformation, unlike the biomass from the municipal WWTP for which  
20 sorption is sometimes the only removal mechanism. The biotransformation kinetic and  
21 the solid-water distribution coefficients in this study show good agreement with  
22 literature data, even for much higher pharmaceutical concentrations in OWW.

23

24 **Keywords**

25 Pharmaceutical compounds; Acclimated sludge; pharmaceutical removal; sorption;

26 biotransformation

## 27 I. Introduction

28 The removal of pharmaceutical residues in wastewater treatment plants (WWTP) by  
29 activated sludge is carried out through two mechanisms: biotransformation (biological  
30 removal and metabolization of the parent molecule) and sorption; photo transformation  
31 and air-stripping are negligible (POSEIDON 2006). The biotransformation of  
32 pharmaceutical compounds follows a pseudo-first order model (Joss et al., 2006) in a  
33 concentration range which does not inhibit biomass. The Hydraulic Retention Time  
34 (HRT) may therefore be optimized according to concentrations at process input and to  
35 the value of the  $k_i,biol$  constant of the pharmaceutical molecule. So the  $k_i,biol$  constant  
36 depends on the degradability of the compound but also on the composition of the sludge,  
37 which influences the mechanism of biodegradation of pharmaceutical compounds in  
38 several ways. Joss et al. (2006) classified pharmaceutical compounds into 3 groups,  
39 according to their constants (i)  $k_i,biol < 0,1 \text{ L.gTSS}^{-1}.\text{d}^{-1}$ : no significant  
40 transformation/removal through biodegradation; (ii)  $0,1 < k_i,biol < 10 \text{ L.gTSS}^{-1}.\text{d}^{-1}$ :  
41 partial removal (20 % to 90 %) and (iii)  $k_i,biol > 10 \text{ L.gTSS}^{-1}.\text{d}^{-1}$ : more than 90%  
42 transformation/removal via biodegradation. Their results show that only 4 out of the 35  
43 pharmaceuticals molecules studied (estrone, estradiol, ibuprofen and paracetamol)  
44 could be removed by 90% through biotransformation but that this mechanism could be  
45 overestimated for a third of the compounds studied. It does not seem possible to  
46 conclude on the bio transformability of a pharmaceutical molecule because of the few  
47 exceptions that were obtained for antibiotics and anti-inflammatory agents. So the  $k_i,biol$   
48 constant must be determined experimentally. pH, redox potential, stereochemical  
49 structure and the chemical structure of the sorbent and of the sorbed molecule may  
50 influence the effect of the sorption mechanism on the activated sludge (Kümmerer,  
51 2009), be it through adsorption or absorption. Thus the influence of pH on the removal

52 of ionizable micropollutants in a Membrane Bioreactor (MBR) was confirmed by  
53 applying an acid pH which modified the hydrophobicity of some compounds which are  
54 not inclined to sorption on the bacterial flocs at a neutral pH (Urase et al., 2005;  
55 Tadkaew et al., 2010). The solid-water partition coefficient  $K_D$ , also called Nernst  
56 coefficient, was then introduced as the most appropriated parameter representing the  
57 sorbed fraction of a molecule on suspended matter (Schwarzenbach et al., 2003; Ternes  
58 et al., 2004). The sorption of a compound is considered negligible for municipal WWTP if  
59  $K_D$  is smaller than  $500 \text{ L.kg}_{\text{TSS}}^{-1}$  as it would represent less than 10% removal (Ternes et  
60 al., 2004). Joss et al. (2005) give a lower threshold value at  $300 \text{ L.kg}_{\text{TSS}}^{-1}$ , before taking  
61 the sorption mechanism into account. Sipma et al. (2010) conclude that the sorption of  
62 pharmaceutical compounds on activated sludge is generally a minor removal  
63 mechanism, due to the low values of  $K_D$  in pharmaceuticals. Numerous pharmaceutical  
64 molecules are hydrophilic, which a priori limits sorption phenomena. Nevertheless, very  
65 hydrophilic molecules, such as antibiotics from the fluoroquinolone class, are removed  
66 very efficiently through sorption due to electrostatic interactions (Göbel et al., 2007;  
67 Vieno et al., 2007). Out of 40 micropollutants that were studied in an MBR, the 14 very  
68 hydrophobic molecules were all removed at more than 85% (Tadkaew et al., 2011). It is  
69 necessary to distinguish between the 2 mechanisms of pharmaceutical micropollutants  
70 removal in order to estimate the proportion transferred to the sludge, which would  
71 allow for an assessment of the environmental relevance of the removal procedure /  
72 disposal of excess WWTP sludge.

73 Moreover, the treatment process may influence the ability of the biomass to resist toxic  
74 charges, as Henriques et al. (2005-2007) state that some processes are more sensitive to  
75 inhibition: it is the case of activated sludge flocs, which boost the formation of small  
76 aggregates (such as MBR) and processes involving a high shear. In their study,

77 respirometric tests on the biomasses of 2 MBR revealed an inhibition 1.25 and two times  
78 greater than that of a conventional activated sludge process while in contact with  
79 chemical toxins, with MBR bacterial flocs smaller by 41%.

80 The choice of treatment process configuration is very important in its ability to resist the  
81 presence of toxic material, as shear stress rate is different according to configuration. It  
82 is therefore expected that the MBR configuration may influence the ability of activated  
83 sludge to resist and to acclimate to a highly concentrated pharmaceuticals effluent. For  
84 this study, we decided to compare the performance of a biomass adapted to Oncological  
85 Ward Wastewater (OWW) with that of a municipal WWTP, on the removal of  
86 pharmaceutical molecules and more specifically on their overall resistance to the  
87 presence of pharmaceutical cocktails and the preservation of their purifying ability.  
88 Removal of one of the oncological ward's most consumed antineoplastics (5-FU) was  
89 quantified for both biomasses. Then removal of easily biodegradable substrate in the  
90 presence of pharmaceutical cocktails (antineoplastics and antibiotics) was measured for  
91 both biomasses in order to determine whether (i) adaptation to OWW permitted to  
92 increase resistance of the biomass to pharmaceuticals and whether (ii) one class of  
93 pharmaceuticals is more harmful than another to the performance of both biomasses.  
94 This objective arose from the different uses of antineoplastics and antibiotics in  
95 hospitals. While antineoplastics and antibiotics are administered continuously in  
96 oncologic wards, the antibiotics are given to prevent possible post-surgical infections  
97 and their concentrations in effluent can be strongly modified as a function of time and  
98 the number of patients. Hence it is assumed that the adaptation of the biomass to  
99 antibiotics is made all the more delicate by the occasional presence of concentration  
100 peaks in hospital effluents (OWW). Finally sorption and biotransformation mechanisms



101 on two antineoplastics, one antibiotic and a painkiller were studied for both activated  
102 sludge.

103

## 104 **II. Equipment and methods**

### 105 **II.1. MBR and hospital effluents**

106 A pilot-scale membrane bioreactor was designed, built and set up underneath the  
107 oncological ward of the Timone hospital (Marseille, France). The MBR pilot was designed  
108 for treating 1 to 2 L.h<sup>-1</sup> of hospital effluent from the oncological ward. The pilot has a  
109 maximum capacity of 60 L, with an operating volume set at 32 L. A 3.1 kW refrigeration  
110 unit allowed for regulation of activated sludge temperature at 25 ± 2°C. OWW were kept  
111 in a storage tank with a maximum capacity of 200 L and were renewed every other day.  
112 First, OWW were sent into the denitrification tank (10.5 L) which was stirred through  
113 sludge recirculation carried out by a peristaltic pump. The dissolved oxygen  
114 concentration is continuously monitored in the denitrification reactor in order to check  
115 its zero value. The bacterial flocs were maintained in suspension without aerating the  
116 anoxic zone, which allowed the denitrification reaction to take place. A fraction of the  
117 recirculated sludge was transferred to the aerobic tank, which has a capacity of 21.5 L,  
118 and in which the nitrification reaction took place. The hydraulic retention time (HRT) in  
119 both tanks was set through the adjustment of valves, which established 1h/2h cycles in  
120 the anoxic and anaerobic tanks respectively. Aeration was performed by fine air bubbles  
121 delivered through four porous tubes connected to a compressor. This maintained  
122 oxygen concentration above 2 mg.L<sup>-1</sup> and ensured the stirring of the aerobic tank. A  
123 centrifugal pump (B3, Motovario) located at the foot of the nitrification tank performed  
124 suction of the activated sludge towards the membrane module. Two acclimation

125 campaigns to OWW were carried out: one using an external membrane bioreactor  
126 (eMBR) and another using an external submerged membrane bioreactor (sMBRe). The  
127 biomasses from the eMBR and sMBRe were acclimated to effluents from the Timone  
128 oncological ward (Marseille) for more than 150 days each (Hamon, 2014). The pipe  
129 collected wastewater from 6 rooms without dilution by the ward's other activities.  
130 Pretreatment consisted of maceration with a Saniflo (Plus Silence, SFA) and 0.5 mm cut  
131 off filtration. After a few days of operation sampling of the OWW was carried out at night  
132 in order to avoid dilution by shower drain water, thus an effluent with a higher  
133 ammonium content was obtained. In spite of the standardization of the sampling  
134 method, large fluctuations in COD and  $\text{N-NH}_4^+$  concentrations were measured. COD and  
135  $\text{N-NH}_4^+$  concentrations were stabilized by feeding the MBR pilot with half OWW and half  
136 synthetic substrate during the second half of the first acclimation campaign in an eMBR  
137 configuration. Composition of the synthetic substrate which allowed for dilution of the  
138 polluting charge specific to OWW was determined using the average COD ( $800 \text{ mg.L}^{-1}$  as  
139 sugar  $\text{C}_6\text{H}_{12}\text{O}_6$ ) and  $\text{N-NH}_4^+$  ( $31 \text{ mg.L}^{-1}$  as  $(\text{NH}_4)_2\text{SO}_4$ ) concentrations, which were  
140 measured over a two-month period. Concentrations in mineral salts were set according  
141 to literature (Han et al., 2005 ; Barrioz-Martinez, 2006): C/N/P ratio of the synthetic  
142 effluent was 100/4/2. In both MBR configurations the retentate was returned to the  
143 nitrification tank while the permeate was sent back to the oncological ward waste water  
144 pipe. The features of both MBR, of the acclimation parameters and of the activated  
145 sludge are listed in table 1.

146

## 147 **II.2 Compounds and analysis**

148 The three most consumed antineoplastics in the oncological ward of the Timone hospital  
149 are among the seven antineoplastics on the French national agency of sanitary safety of  
150 food, environment and work (Anses) list: ifosfamide (IF), fluorouracil (5-FU) and  
151 cyclophosphamide (CP). Fluorouracil was analyzed by the pharmacology and  
152 toxicokinetics laboratory of the Timone hospital (Marseille, France). Ifosfamide,  
153 cyclophosphamide, codeine and sulfamethoxazole were analyzed by the Ianesco  
154 laboratory (Institut d'Analyses et d'Essais en Chimie de l'Ouest, Poitiers, France). This  
155 laboratory is COFRAC-certified to analyze the specific molecules studied in our paper.  
156 The COFRAC accreditation certifies the technical competence of testing and calibration  
157 laboratories to perform specific tasks. The procedure for dosing 5-FU in blood plasma  
158 was successfully applied to OWW and treated water. 5-FU was analyzed with HPLC-UV  
159 (254 nm). The limit of quantification was  $5 \mu\text{g}\cdot\text{L}^{-1}$ . The detection limits were obtained  
160 with several injections of compounds from 1 to  $10 \mu\text{g}\cdot\text{L}^{-1}$ . An accurate detection and  
161 repeatability were obtained from the concentration at  $5 \mu\text{g}\cdot\text{L}^{-1}$ . In detail, the analytical  
162 system was divided into three parts: (A) a mobile phase composed of 0.05 M  
163 monopotassium phosphate ( $\text{KH}_2\text{PO}_4$ ) was adjusted to pH 3 with orthophosphoric acid  
164 and filtration at  $0.2 \mu\text{m}$ . (B)  $500 \mu\text{L}$  of the sample to be analyzed was acidified with  $20 \mu\text{L}$   
165 of 5% orthophosphoric acid; ibromouracil ( $50 \text{mL}$ ,  $10 \mu\text{g}\cdot\text{mL}^{-1}$ ) was added. The sample  
166 was extracted with  $6 \text{mL}$  of n-propanol/diethylether 10:90 (v:v), mixed with an  
167 automatic vortex for 10 minutes before centrifugation for 15 minutes at  $3000 \text{g}$  at  $4^\circ\text{C}$ .  
168 The organic phase was sampled and evaporated in a water bath under nitrogen. The dry  
169 residue was recovered in  $100 \mu\text{L}$  of mobile phase and was centrifuged for 4 minutes. (C)  
170 The samples were analyzed by HPLC-UV: 7 solvent samples were injected for calibration  
171 (the 7 samples covered the whole concentration range), then the samples to be analyzed  
172 were injected, and finally 3 samples were injected for quality control. The four

173 remaining molecules (cyclophosphamide, ifosfamide, sulfamethoxazole and codeine)  
174 were analyzed simultaneously by liquid chromatography combined with tandem mass  
175 spectrometry (LC/MS-MS). Detection limits were first estimated by calculation by  
176 injecting a low-concentration standard solution: the limit of detection is at least equal to  
177 3 times the background noise and the limit of quantification is, to a minimum, equal to  
178 10 times the signal of the background noise. The quantification limits were controlled by  
179 injecting a standard solution at this given concentration. Then real samples were doped  
180 at this given concentration in order to assess the accuracy and reliability of the  
181 analytical method. The limit of quantification of the method was  $2.5 \mu\text{g.L}^{-1}$ . The LC/MS-  
182 MS was calibrated with a  $200\text{mg.L}^{-1}$  solution of our molecules of interest in methanol. A  
183 calibration range of 0, 1, 2.5, 10, 30, 50 and  $100 \mu\text{g.L}^{-1}$  in ultrapure water / methanol  
184 (80/20) was obtained by diluting the deuterated internal standards to  $30 \mu\text{g.L}^{-1}$   
185 (sulfamethoxazole-D4, diclofenac-D4, ketoprofenD4 and caffeine). In detail, the  
186 analytical system used was composed of an AGILENT 1100 HPLC equipped with a high  
187 pressure pump, an automatic injector (thermostated by Peltier effect) and a SCIEX,  
188 API400 tandem mass spectrometer. Quantification was carried out with a calibration in  
189 solutions containing internal standards. The analytical conditions were: (i) an analytical  
190 column: ZORBAX Eclipse Plus C18 (100mm x 2.1mm x  $3.5\mu\text{m}$ ) with guard pre-column  
191 ZORBAX Eclipse Plus C18 ( $5 \mu\text{m}$  x 12.5 mm). (ii) The solvent gradient parameters were  
192 set through two channels: channel A: ultra-pure water with 0.1% formic acid and  
193 channel B: methanol, with a flow rate of  $0.35 \text{ mL.min}^{-1}$ . The solvent gradient was  
194 modified at 0-4/12/16/20/24/25/40 min with the respective ratios (A-B) 95-5/70-  
195 30/30-70/2-98/1-99/0-100/95-5/95-5%. The injected volume was  $20 \mu\text{L}$  and the oven  
196 temperature was  $25^\circ\text{C}$ . The electrospray ionization mode (positive-mode Turbo-V) was  
197 positive mode (ion formation  $[\text{M}+\text{H}]^+$  mostly but also potentially  $\text{Na}^+$  or  $\text{K}^+$  adducts). The

198 de-solvation temperature, the acquisition mode, the duration of the MRM windows and  
199 the duration of analysis were respectively 550°C, MRM, 200s and 44 minutes. The  
200 retention times for codeine, sulfamethoxazole, ifosfamide and cyclophosphamide were  
201 respectively 5.4/8.5/10.5/11 min. Prior to analysis, wastewater was decanted then  
202 filtered on a 0.45 µm porosity filter. Removal of the coarsest solid materials should not  
203 lead to under-estimating the pharmaceutical concentration in OWW, as the selected  
204 pharmaceuticals are excreted solely through the urinary tract and are hydrophilic. Thus  
205 sorption on TSS of OWW is negligible. The analytical LOQ might seem high regarding  
206 pharmaceutical concentration in municipal wastewater but is satisfying regarding the  
207 oncological ward wastewater which was investigated.

208

### 209 **II.3 Pharmaceutical cocktails**

210 The influence of various pharmaceutical cocktails on the performance of both biomasses  
211 (acclimated to OWW and municipal WWTP) was assessed by using the kinetics of  
212 degradation of easily degradable substrates (COD, NH<sub>4</sub><sup>+</sup>) in a batch reactor. The  
213 pharmaceutical cocktails were prepared using hospital pharmaceuticals. The  
214 composition of the antineoplastics cocktail was based on the maximum concentration of  
215 5-FU found in OWW during the acclimation period of the biomass, i.e. 1287 µg.L<sup>-1</sup>, on the  
216 metabolization rate of each pharmaceutical and on the maximum quantity consumed in  
217 the oncological ward unit to which the MBR was connected. The concentrations thus  
218 calculated are shown in Table 2.

$$219 \quad [pharmaceutical] = [5 - FU]_{max} \cdot \frac{n_{pharmaceutical}}{n_{5-FU}} \cdot \frac{1 - \tau_{pharmaceutical}}{1 - \tau_{5-FU}} \quad \text{Eq. 1}$$

220

221 with:

222  $[5 - FU]_{max}$ : Maximum concentration in 5-FU detected in OWW during the first  
223 experimental campaign ( $\mu\text{g.L}^{-1}$ )

224  $n_{\text{pharmaceutical}}$ : annual consumption of the pharmaceutical in the unit ( $\text{mg.year}^{-1}$ )

225  $n_{5-FU}$ : annual 5-FU consumption in the unit ( $\text{mg.year}^{-1}$ )

226  $1 - \tau_{\text{pharmaceutical}}$ : non-metabolized pharmaceutical rate (-)

227  $1 - \tau_{5-FU}$ : non-metabolized 5-FU rate (-)

228

229 The concentrations of the antibiotics cocktail were defined arbitrarily in order to obtain  
230 a total concentration in the same order of magnitude as that of the antineoplastics. Thus  
231 the concentration of each of the ward's four most consumed antibiotics was set at 1  
232  $\text{mg.L}^{-1}$  (Table 2).

233 It should be noted that these cocktails do not in any way represent the average or the  
234 maximum concentrations that could be detected in OWW. Five original COD  
235 concentrations plotted to the quantity of TSS were tested for each "type of biomass –  
236 pharmaceutical cocktail" pair: 0.1 – 0.2 – 0.5 – 1 – 3  $\text{gCOD.gTSS}^{-1}$ . Total duration of the  
237 tests was 4h. However, the duration that was used to calculate the maximum  
238 degradation velocity varied according to original concentrations and corresponded to  
239 the linear degradation velocity of the COD.

240

#### 241 **II.4 Sorption and biotransformation tests**

242 The technique to inhibit activated sludge must be adapted to sorption tests: it must  
243 allow for complete inhibition of the biotransformation mechanism and for preservation

244 of the structural integrity of the activated sludge. Prior to sorption text, it was necessary  
245 to determine the most suitable activated sludge inhibition technique for carrying out  
246 these tests in order to ensure the consistency of batch tests with sorption phenomenon  
247 in the MBR: gas purging is the only technique that does not affect the structure of the  
248 biomass (Hamon et al. 2014). Moreover, this inhibition is very easily implemented and  
249 the inhibition state is reached immediately, as the concentration in dissolved oxygen  
250 drops down almost instantaneously to zero. The quantity of the selected  
251 pharmaceuticals, which were removed thanks to the sorption mechanism was evaluated  
252 for the activated sludge from the sMBRe pilot and from the municipal WWTP of Rousset  
253 (France). The sorption tests were carried out over 4h as this duration allows ifosfamide  
254 and cyclophosphamide to reach sorption equilibrium, whatever the origin of the  
255 activated sludge (Seira, 2013). Sorption equilibrium of sulfamethoxazole was reached in  
256 2h (Yang et al., 2011). There is no available data concerning codeine. Activated sludge  
257 filtered with coffee filters (average pore size 100  $\mu\text{m}$ ) was re-suspended with distilled  
258 water, so as not to use the supernatant from the sMBRe pilot, as it was likely to contain  
259 the selected pharmaceuticals at considerable concentrations, which could distort results.  
260 Activated sludge concentration in TSS was brought down to 4  $\text{g.L}^{-1}$  using coffee filters.  
261 Sorption tests were performed on pharmaceutical cocktails in 4 different original  
262 concentrations close to 100 – 250 – 500 and 1000  $\mu\text{g.L}^{-1}$ . The original measured  
263 concentrations were slightly different but in the correct order of magnitude. Thus, to  
264 allow for easier reading, results are presented according to that concentration factor (1  
265 – 2.5 – 5 and 10). Reductions and  $K_D$  values were calculated using actual original  
266 concentrations. The activated sludge was placed in anaerobic conditions. Water was  
267 deoxygenated with dinitrogen, the pharmaceuticals were introduced and the initial  
268 sample was taken. Sorption tests were carried out in closed 200 mL brown glass vials

269 filled completely and slightly stirred to ensure homogeneous mixing and avoid  
270 sedimentation of sludge particles. The null value of the dissolved oxygen was checked  
271 once every hour during tests. In biotransformation tests degradation kinetics of the  
272 selected pharmaceuticals were performed in brown glass vials, aerated over 4h, with  
273 sludge acclimated to OWW and WWTP sludge. Similarly to sorption tests, activated  
274 sludge concentration in TSS was brought down to 4 g.L<sup>-1</sup>. Filtered sludge was re-  
275 suspended with distilled water. The initial concentrations of the pharmaceutical  
276 cocktails were identical to that of the sorption tests, i.e. close to the targeted  
277 concentrations 100 – 250 – 500 and 1000 µg.L<sup>-1</sup>, and allowed us to respect the  
278 concentration factor 1 – 2.5 – 5 and 10.

279

### 280 **III. Results**

#### 281 **III.1. Removal Performance**

##### 282 **III.1.1. 5-FU**

283 During the acclimation phase, 5-FU was almost consistently detected in OWW at  
284 concentrations up to 1287 µg.L<sup>-1</sup> (minimum 49.6 µg.L<sup>-1</sup> / average 440 µg.L<sup>-1</sup> / 150 days).  
285 These concentrations are very high compared with those measured in previous  
286 research: between 11.5 and 122 µg.L<sup>-1</sup> for Mahnik et al. (2007) and between 35 and 92  
287 ng.L<sup>-1</sup> for Kosjek et al. (2013). The permeate samples which were analyzed show good  
288 removal of 5-FU by acclimated activated sludge, as reductions are above 90% in spite of  
289 high initial concentrations, sometimes greater than 1 mg.L<sup>-1</sup>. Specific degradation  
290 velocity seems relatively proportional to the 5-FU initial concentration (pseudo-first  
291 order) (Figure 1). If the  $V_{\text{specificMBR}} > \text{LOQ}$  and  $V_{\text{specificMBR}} < \text{LOQ}$ , the velocity was  
292 calculated from the value of the measured concentration and from the value of the LOQ



293 respectively. Removal of 5-FU was assessed during similar research on treatment of  
294 OWW in an Austrian hospital using MBR (Mahnik et al., 2007). Results proved similar,  
295 with total removal of 5-FU, as 5-FU could no longer be quantified at process output. In  
296 the present study and even if the LOQ can be considered to be high, the concentrations  
297 in the effluent are so high that removal is always higher than 95%. Obviously, each  
298 removal rate is calculated from specific measured data. By using batch degradation tests  
299 with radiolabeled compounds Mahnik et al. (2007) noticed total 5-FU removal from the  
300 liquid phase and negligible sorption onto the activated sludge, ranging from 2 to 5%.  
301 Thus 5-FU is almost totally removed by biotransformation. The capacity of 5-FU to be  
302 biotransformed at low and high concentrations had already been shown by some  
303 authors (Kiffmeyer et al., 1998 ; Yu et al., 2006). However, these results were obtained  
304 by conducting tests on high concentrations which do not reflect the actual situation, as  
305 there might be an inhibitory effect and the analytical methods used were sometimes  
306 unsuitable (measurement of COD or of produced CO<sub>2</sub>). Thus Kümmerer (1997) observed  
307 contradictory results: he found no biotransformation of 5-FU for very high 5-FU  
308 concentrations (9 and 850 mg.L<sup>-1</sup>). In this present study, degradation kinetics for 5-FU  
309 were performed on sludge from the municipal WWTP and on sludge acclimated to OWW  
310 in batch reactors for 5 initial concentrations: 50 – 1000 µg.L<sup>-1</sup>. The kinetics were  
311 performed over 21h so as to match the HRT of the eMBR pilot at the time of sampling.  
312 The sludge from the municipal WWTP was adjusted to the concentration of the  
313 acclimated sludge, i.e. 4.1 g.L<sup>-1</sup>. Whatever the concentration, 5-FU reduction was always  
314 slightly greater for acclimated sludge than for sludge sampled from municipal WWTP. In  
315 that concentration range the activated sludge from municipal WWTP also seemed very  
316 efficient for the removal of 5-FU, as the minimum reduction was always greater than

317 80%. 5-FU reductions by both types of sludge, as well as associated specific degradation  
318 velocities are presented in Figure 2.

319 Just as with sludge acclimated to OWW, the higher the initial concentration, the greater  
320 the reduction was, which shows that the concentration range, which was tested (50 –  
321 1000  $\mu\text{g.L}^{-1}$ ) was lower than a possible inhibition threshold; the kinetics remained  
322 pseudo-first order. Acclimation to OWW allowed the biomass to be slightly more  
323 efficient at initial low concentrations (50 – 200  $\mu\text{g.L}^{-1}$ ). This improvement is minor as 5-  
324 FU proved to be a very easily removable molecule. Thus the specific removal velocities  
325 of 5-FU were almost the same: 0.0115  $\text{g}_{\text{TSS}}^{-1}.\text{h}^{-1}$  for the acclimated sludge and 0.0114  $\text{g}_{\text{TSS}}^{-1}.\text{h}^{-1}$   
326 for the municipal WWTP. However it is very important to note that in spite of the  
327 numerous pharmaceuticals, metabolites and cleaning products contained in the OWW  
328 which was used for acclimation, the acclimated biomass proved to be at least as efficient  
329 as the WWTP sludge, which only removed 5-FU during those tests. Nevertheless the  
330 kinetics study allowed for identification of a few behavioral differences in both types of  
331 sludge. The degradation kinetic constants  $k_{\text{biol}}$  were calculated between  $t=15$  min and  
332  $t=90$  min as the term  $\ln(C/C_0)$  is linear in that range, thus confirming that the  
333 degradation kinetics is pseudo-first order for the first 90 minutes (Figure 3).

334 The evolution of the degradation constant  $k_{\text{biol}}$  shows that acclimation at the source  
335 allows for faster removal of 5-FU. Thus an average 34% increase was reached for the  
336 acclimated sludge, compared with WWTP sludge. The variation of  $k_{\text{biol}}$  constants shows  
337 that WWTP reached a threshold, while the  $k_{\text{biol}}$  constants continue to evolve beyond  
338 1000  $\mu\text{g.L}^{-1}$  for the acclimated sludge. Hence, it seems that the minimum inhibition  
339 concentration is lower for WWTP sludge than for sludge acclimated to OWW in the MBR.

340

### 341 **III.1.2. Performance with pharmaceutical cocktails**

342 The influence of antineoplastics and antibiotics cocktails was quantified in the biomass  
343 acclimated to OWW and in a biomass from a municipal WWTP, by monitoring the  
344 degradation of an easily biodegradable substrate. Specific degradation velocities of the  
345 COD were calculated for each “type of biomass – pharmaceutical cocktail” configuration  
346 and were represented according to the COD concentration plotted to the amount of TSS  
347 (Figure 4).

348 On Figure 4, positive and negative velocities respectively show COD degradation and an  
349 inhibitory effect exerted by pharmaceuticals. Non-acclimated WWTP sludge (Rousset,  
350 France) proved to be totally impacted by the presence of pharmaceutical cocktails:  
351 degradation velocity of the COD was zero at low concentrations and even became  
352 negative for high charges. A negative degradation velocity means that the presence of  
353 pharmaceuticals triggered cell lysis of activated sludge. Conversely, positive COD  
354 degradation velocities were measured for sludge acclimated to OWW, which means that  
355 it retains a capacity for purification in the presence of pharmaceuticals. However, COD  
356 degradation velocities were slower than that of the control group without  
357 pharmaceuticals, indicating that pharmaceutical cocktails still partially inhibit the  
358 performance of the biomass, which would be logical given the high concentrations used  
359 in the cocktail. Optimal degradation velocity was around  $0.2 \text{ gCOD.gTSS}^{-1}$  for the  
360 acclimated sludge. An inhibition of COD degradation by pharmaceutical materials was  
361 observed from  $0.1 \text{ gCOD.gTSS}^{-1}$  for the sludge in sole presence of the antineoplastic  
362 cocktail and from  $0.2 \text{ gCOD.gTSS}^{-1}$  for the antineoplastic cocktail with antibiotics. Thus  
363 inhibition seems stronger for the antineoplastic cocktail on its own than for the  
364 combination of antineoplastic and antibiotic cocktails. This surprising observation could  
365 be due to (i) interactions between antineoplastics and antibiotics, which brought about a

366 decrease in total pharmaceutical toxicity, or (ii) to the absence of toxicity of the  
367 antibiotics cocktails on acclimated sludge, hence the differences observed in specific  
368 removal velocities of the COD would be due only to experimental uncertainties (COD  
369 measurement precision). A contrasting behavior was observed for the municipal WWTP  
370 sludge. The antineoplastic cocktail in the presence of antibiotics triggered a more  
371 pronounced cell lysis than the sole antineoplastic cocktail, showing that antibiotics have  
372 a bactericidal effect on non-acclimated activated sludge. Thus the acclimated biomass  
373 acquired resistance to the tested antibiotics and may have been able to metabolize them  
374 partially. These results clearly demonstrate that biomass acclimation allowed for the  
375 development of capacities of high resistance to antineoplastics and antibiotics, since, at  
376 low charge, the sludge developed in the hospital MBR was only slightly affected by their  
377 presence.

378

## 379 **III.2. Removal mechanisms of the selected pharmaceuticals**

### 380 **III.2.1 Sorption**

381 Degradation tests in a batch reactor were performed so as to determine the influence of  
382 each of the two removal mechanisms coupled with the purifying biomass: sorption and  
383 biotransformation. These tests must show whether the apparent removal with sludge  
384 acclimated to OWW is mainly linked to a pollutant transfer from the liquid to the solid  
385 phase, or whether there is a biological metabolization by bacteria from the purifying  
386 biomass. Lastly, these tests were also carried out using municipal WWTP activated  
387 sludge from Rousset, so as to quantify the improvement brought by acclimation of  
388 sludge to OWW.

389 Sorption of the selected pharmaceuticals seemed relatively low for the antineoplastics  
390 molecules on the activated sludge of the SMBRe hospital pilot and for the municipal

391 WWTP, as it turned out to be lower than 10% (Figure 5a). Although its sorption  
392 remained very low, ifosfamide seems to have more affinities with sorption than  
393 cyclophosphamide, which is coherent with Seira's results (2013). Sulfamethazole was  
394 removed a few percent more than antineoplastics for both types of sludge, but its  
395 sorption remained limited as its removal reached a maximum 13% for sludge acclimated  
396 to OWW. Codeine seems to have much more pronounced sorption affinities, since its  
397 removal through sorption reached up to 30% for WWTP sludge. Whichever sludge was  
398 used, the proportion of sorption of pharmaceuticals tended to decrease as its initial  
399 concentration increased, because of a constant number of sorption sites on bacterial  
400 flocs for a larger amount of pollutant.

401 Thus apparent removal of cyclophosphamide, ifosfamide and sulfamethazole by  
402 acclimated sludge may be attributed to biotransformation.

403 Although transfer of pollutant from the liquid phase to sludge appears limited, the very  
404 high concentrations in pharmaceuticals in OWW may include significant amounts of  
405 pharmaceuticals sorbed onto the sludge of an MBR treating these OWW. These amounts,  
406 calculated from average concentrations measured in OWW and from corresponding  
407 concentration factors, show that ifosfamide and sulfamethazole might be present in high  
408 concentrations in MBR sludge and should be taken into account when choosing the  
409 appropriate treatment method for excess sludge (Table 3).

410 The evolution of distribution coefficients  $K_D$  according to the concentration factor is  
411 logically identical to that of reduction through sorption (Figure 5b). The selected  
412 pharmaceuticals have low distribution coefficients  $K_D$ .  $K_D$  was smaller than 40 L.kgTSS<sup>-1</sup>  
413 for CP, IF and SM and smaller than 120 L.kgTSS<sup>-1</sup> for CD in municipal WWTP. Joss et al.  
414 (2005) claimed that for a value of  $K_D$  smaller than 300 L.kgTSS<sup>-1</sup> sorption of a compound  
415 is negligible and its removal may be assessed using input and output concentrations.

416 According to Ternes et al. (2004) sorption may be considered as a significant removal  
417 mechanism at a threshold value of 500 L.kgTSS<sup>-1</sup>. Even though this seems justified for  
418 both antineoplastics, SM sorption represents more than 10% of removal for acclimated  
419 sludge, and more importantly, sorption of CD allows for a removal above 30%. These  
420 observations match Seira's (2013), who noted that low values of  $K_D$  could not be  
421 systematically neglected since sometimes significant removal could occur even for  
422 molecules presenting low  $K_D$ . He proposed to highlight the particle concentrations  
423 associated to any suggestion of  $K_D$  limit value from which sorption may be considered  
424 negligible.

425 Sorption of CP, IF and SM proved to be in the same order of magnitude for both tested  
426 activated sludge. The nature of the sludge could have significantly influenced the  
427 sorption affinities of a compound, but comparison between the sludge acclimated to  
428 OWW and that of the Rousset WWTP provided no evidence of this. This was probably  
429 due to the fact that the MBR of the WWTP and that of the MBR which was used for  
430 acclimation had the same configuration (submerged external membrane bioreactor).  
431 Another factor could be the origin of the sludge which was used as a base for  
432 acclimation, which came from the Rousset WWTP. Comparing several studies would be  
433 difficult because of differences in experimental procedures and in the nature of the  
434 sludge (Table 4).

435  $K_D$  coefficients of both antineoplastics (CP and IF) found in the literature are generally  
436 low (Seira, 2013 ; Ternes et al., 2004). High values of  $K_D$  for CP of 794.3 L.kgTSS<sup>-1</sup>  
437 (Delgado, 2009) and of 111.4 L.kgTSS<sup>-1</sup> (Zaviska, 2013) could be due to the thermic  
438 inhibition technique used, which completely breaks down the biomass (Hamon et al.,  
439 2014). For activated sludge from an MBR pilot, Seira (2013) obtained similar results to  
440 those of this study, with a  $K_D$  of 15 L.kgTSS<sup>-1</sup> for CP and of 22 L.kgTSS<sup>-1</sup> for IF. It should

441 be noted that Seira's study measured the most pronounced sorption of CP and IF for  
442 eMBR sludge. This was attributed to the smaller-sized flocs, which provided more  
443 sorption sites to compounds.  $K_D$  coefficients of SM found in the literature seem very  
444 heterogeneous as they range from 3.2 to 370 L.kgTSS<sup>-1</sup>. These differences could be  
445 explained by the nature of the activated sludge and by the inhibition techniques that  
446 were used, but also by SM photosensitivity (Hörsing et al., 2011), which is negligible in  
447 this study since sorption tests were carried out in brown glass vials. Average values of  
448 34.1 L.kgTSS<sup>-1</sup> measured in this study for sludge acclimated to OWW and of 21.4  
449 L.kgTSS<sup>-1</sup> for Rousset WWTP sludge were in the same order of magnitude as  $K_D$   
450 measured in the studies of Abbeglen (2009) for MBR sludge and Yang et al. (2011) for  
451 conventional WWTP sludge. Significant differences were observed for values of  $K_D$   
452 obtained for CD. Again, these differences could be due to the inhibition techniques that  
453 were used and/or to the nature of the activated sludge. It should also be noted that the  
454 sorption assessed in this study is competitive as pharmaceuticals were added as a  
455 cocktail, while the results of some studies in the literature are sorption values of isolated  
456 compounds.

457 Although sorption of the selected pharmaceuticals may not be totally ignored, it proved  
458 to be a minor removal mechanism for acclimated sludge, except for codeine, which could  
459 be removed through sorption in proportions ranging around 30%.

460

### 461 **III.2.2. Biotransformation**

462 Concerning biotransformation tests, a definite improvement in the total removal of the 4  
463 pharmaceuticals was observed with sludge acclimated to OWW (Figure 6). The initial  
464 concentration was analysed. Except for codeine, which was always removed very

465 efficiently whatever the concentration factor, total removal of pharmaceuticals with  
466 acclimated sludge seemed to increase with their initial concentration. Conversely and  
467 except for ifosfamide, removal with sludge from the municipal WWTP seemed to  
468 stagnate when initial concentration increased. Capacities for biotransformation were  
469 developed by sludge acclimated to OWW while removal of selected pharmaceuticals  
470 from the Rousset municipal WWTP sludge was mainly due to a sorption mechanism, as  
471 the following ratio shows:

472

473  $\frac{\text{Biotransformation}}{\text{Sorption}}$  smaller than 1 (Table 5).

474

475 In accordance with literature, the biotransformation measured for the 2 antineoplastics  
476 CP and IF by municipal WWTP sludge proved to be low, even zero (Kümmerer et al.,  
477 1997; Buerge et al., 2006). Removal of CD and SM through biotransformation in the  
478 Rousset WWTP sludge was low, as it was in the order of 8% in 4 h. Total removal after 4  
479 h seems to confirm partial removal of these two pharmaceuticals, as mentioned in the  
480 literature. The stagnation of removal which was observed for the municipal WWTP  
481 sludge could show the inhibition effect exerted by the most concentrated  
482 pharmaceutical cocktails. Besides, the 1000  $\mu\text{g.L}^{-1}$  cocktail of each of these  
483 pharmaceuticals seemed to trigger a very strong inhibition, as a significant decrease in  
484 the reduction of CD was noted. Thus increase in the removal of CP and IF for the most  
485 concentrated cocktail could be due to the sorption of these molecules onto soluble  
486 microbial products which were released during a possible cell lysis brought about by the  
487 pharmaceutical cocktails in the non-acclimated municipal WWTP sludge. The IANESCO  
488 Laboratory is certified and these conclusions are validated by the results of analyses.  
489 These batch reactor tests confirm the correct reductions with 43% maximum removal



490 for SM and around 70% for CD in only 4 h (Figure 6). These results agree with those  
491 measured in the supernatant of the MBR during the acclimation period of the sludge.  
492 However, maximum removal of CP at 36% and of IF at 38% in that reduced time scale  
493 seems better than the removal obtained in the MBR. This could stem from the nature of  
494 the substrate, which was far less rich and complex than real OWW, and from the initial  
495 absence of these molecules in the supernatant, which was not the case in the MBR. It  
496 should also be noted that exclusively aerobic conditions (applied in batch reactors) are  
497 known to favor degradation of micropollutants as opposed to aerobic/anoxic processes  
498 (applied in the MBR) (Suarez et al., 2010), even if a 4 h test remains far shorter than the  
499 HRT of the MBR. Although these removals are incomplete, it should be noted that the  
500 differences in the associated concentrations are substantial since they are in the order of  
501  $700 \mu\text{g.L}^{-1}$  for CD and  $400 \mu\text{g.L}^{-1}$  for SM, CP and IF.  
502 These removals were obtained without adding a cosubstrate in the batch reactors. Thus  
503 actual biotransformation of the selected pharmaceuticals could be achieved through a  
504 direct metabolic pathway. Seira (2013) did not observe any biotransformation of CP and  
505 IF without adding a cosubstrate, but the concentrations tested in his study, respectively  
506 6 and  $2 \mu\text{g.L}^{-1}$ , were much lower than those of the present study ( $100$  to  $1,000 \mu\text{g.L}^{-1}$ ).  
507 The higher concentrations used here are representative of the concentrations measured  
508 in OWW. Such concentrations could allow pharmaceuticals to be used as primary  
509 substrate for the biomass. The data obtained in this study permits to calculate kinetic  
510 parameters for removal by both types of tested activated sludge (Eq.2) for each  
511 pharmaceutical.

512

$$513 \quad \frac{d[\text{Pharmaceutical}]}{dt} = k_{global} \cdot [\text{Pharmaceutical}]_{initial}^n \quad \text{Eq.2}$$

514

515  $k_{global}$ , which is the kinetic constant of total removal measured during the 4h test ( $\mu\text{g}^{-1}$ -  
 516  $^n \cdot \text{L}^{n-1} \cdot \text{h}^{-1}$ ), and  $n$ , which is the order of the reaction, were then determined through  
 517 linearization (Eq.3) :

518

$$519 \quad \log_{10} \frac{d[\text{Pharmaceutical}]}{dt} = \log_{10} k_{global} + n \cdot \log_{10} [\text{Pharmaceutical}]_{initial} \quad \text{Eq.3}$$

520

521 Representing this equation allows for the determination of the kinetics constant  $k_{global}$   
 522 (Table 6).

523 It appears that some degradation kinetics deviate from pseudo-first order, such as that  
 524 of cyclophosphamide by acclimated sludge. This deviation may be attributed to  
 525 experimental uncertainties or to inhibition by the pharmaceutical cocktail at the highest  
 526 concentrations. However, it seems obvious that the order greater than 2 for IF obtained  
 527 with the Rousset WWTP sludge is due to the WWTP sludge's inability to biotransformate  
 528 that compound. It is not possible to use a mathematical model for IF. The traditional  
 529 model of pseudo-first order was not used and the values of the kinetic constants were  
 530 not compared to literature data because their units depend on the order of the reaction.  
 531 The biotransformation kinetic constant  $k_{biol}$  and the associated order of the reaction  $n$   
 532 may be determined from concentrations at sorption equilibrium  $[\text{Pharmaceutical}]_{eq}$  and  
 533 final  $[\text{Pharmaceutical}]_f$  during the total removal test (Eq.4 and Table 6):

534

$$535 \quad \log_{10} \frac{[\text{Pharmaceutical}]_{eq} - [\text{Pharmaceutical}]_f}{t_f - t_i} = \log_{10} k_{biol} + n \cdot \log_{10} [\text{Pharmaceutical}]_{initial} \quad \text{Eq.4}$$

536

537 After integrating equation 2, previously determined kinetic constants and orders of  
 538 reaction allow for the calculation of the residual concentration of pharmaceuticals  
 539 according to time (Eq.5):

540

$$541 \quad [Pharmaceutical](t) = \left[ k \cdot t \cdot (n - 1) + [Pharmaceutical]_{initial}^{(1-n)} \right]^{\frac{1}{1-n}} \quad \text{Eq.5}$$

542

543 Thus it is possible to represent a removal profile for each pharmaceutical for both types  
 544 of activated sludge. A profile is proposed with the  $k_{global}$  constant representing maximum  
 545 removal of a pharmaceutical, when biotransformation does not limit sorption kinetics  
 546 and allows for the release of sorption sites onto bacterial flocs. Another profile is  
 547 represented with the  $k_{global}$  constant for the first 4 hours, then with only  $k_{biol}$ , which  
 548 would be the most unfavorable case, i.e. sorption which does not repeat because of very  
 549 low biotransformation, which does not allow for the rapid release of sites onto bacterial  
 550 flocs.

551 Final concentration in pharmaceuticals was calculated using Eq.6 up to 4 h then Eq.7  
 552 from 4 h respectively, for the profile, which only takes into account the  
 553 biotransformation mechanism after 4 h.

554 From 0 to 4 h:

$$555 \quad [Pharmaceutical](t) = \left[ k_{global} \cdot t \cdot (n - 1) + [Pharmaceutical]_{initial}^{(1-n)} \right]^{\frac{1}{1-n}} \quad \text{Eq.6}$$

556

557 Then with  $t > 4$  h:

$$558 \quad [Pharmaceutical](t) = \left[ k_{biol} \cdot (t - 4) \cdot (n - 1) + [Pharmaceutical]_{4h}^{(1-n)} \right]^{\frac{1}{1-n}} \quad \text{Eq.7}$$

559

560 Removal profiles were calculated using the average concentrations of the selected  
561 pharmaceuticals measured in the OWW: 26 – 290 – 1664 - 422  $\mu\text{g}\cdot\text{L}^{-1}$  for codeine (CD) –  
562 Cyclophosphamide (CP) – Isofofammide (IF) – Sulfamethoxazole (SM respectively). The  
563 temporary absence of a molecule in OWW was not taken into account in the calculation  
564 of the average concentration. The evolution of the removal of the 4 pharmaceuticals  
565 from the liquid phase was represented for both types of sludge (Figure 7). It should be  
566 specified that most of the profiles using  $k_{\text{global}}$  overestimated the removal kinetics of the  
567 liquid phase, particularly for the sludge from the Rousset WWTP, as the renewal of  
568 sorption sites depends on the biotransformation mechanism. Hence a low  
569 biotransformation will limit the sorption kinetics once sorption equilibrium has been  
570 attained.

571 Removal of a pharmaceutical from the liquid phase in a batch reactor is situated  
572 between the two curves which were calculated from  $k_{\text{global}}$  and  $k_{\text{biol}}$ . So the actual  
573 evolution of removal of CD, CP and IF for the Rousset WWTP sludge should follow the  
574 curve for removal through biotransformation, as this mechanism limits sorption  
575 kinetics. This kinetics is probably close to the  $k_{\text{global}}$  curve for sludge acclimated to OWW,  
576 because of the developed biotransformation which is greater than sorption, and close to  
577  $k_{\text{biol}}$  for the sludge from the Rousset municipal WWTP. They are the solid lines in Figure  
578 7.

579 These profiles show the extent of the capacity for biotransformation developed by  
580 activated sludge thanks to on-site treatment of OWW. It seems that SM is the  
581 pharmaceutical which presents the smallest number of differences between the two  
582 types of sludge. The clearest gain from treatment with activated sludge is for the two  
583 antineoplastics (CO and IF), as developed biotransformation enabled us to obtain

584 significant removals. Moreover, it is logical to suppose that the profile that best  
585 represents removal of antineoplastics for the Rousset WWTP is the profile which only  
586 takes into account biotransformation after 4 h, as sorption does not limit the  
587 biotransformation mechanism, which proved to be quasi null. Obviously all the removal  
588 profiles calculated with the  $k_{\text{global}}$  constant are above the associated profile calculated  
589 with the  $k_{\text{biol}}$  constant, except ifosfamide for acclimated sludge, whose two profiles are  
590 practically superposed. This superposing clearly shows that sorption is renewed as the  
591 biotransformation process of IF takes place. Consequently it is possible to determine the  
592 time needed to reach a given reduction. The time needed to obtain a 95% reduction was  
593 determined for both types of sludge (Table 7).

594 These results clearly demonstrate the gain from the acclimation in an MBR of sludge  
595 acclimated to OWW. However, these durations remain far longer than the average HRT  
596 of the sMBRe pilot used for the 29 h acclimation during the experiment. The removals  
597 that correspond to this average HRT are presented in Table 8.

598 This calculated data should be interpreted with caution. Comparing removal kinetics of a  
599 batch reactor and of a continuous process may prove delicate, especially as retention of  
600 pharmaceuticals by the membrane, as was observed in this study (Hamon, 2013),  
601 strongly limits that comparison. This data, calculated from kinetic parameters, would  
602 show an excellent removal of IF for acclimated sludge. Still, the performance of the  
603 hospital MBR pilot which was used for acclimation proved to be consistently lower.  
604 These differences may be attributed to the pharmaceutical cocktail created for the tests  
605 in a batch reactor. This cocktail only contained 4 pharmaceuticals, which is far from the  
606 great complexity of OWW as to quantity and quality, without even mentioning  
607 metabolites. Thus the profile of IF removal by sludge acclimated to OWW may be

608 questioned for the reasons mentioned above, because of experimental mistakes, or  
609 because of the low experimental concentrations used in IF, compared to OWW  
610 concentrations, which would only trigger an inhibitory effect restricted to the biomass.  
611 Removal of CP at average HRT seems more reliable as the 46% removal calculated from  
612  $k_{\text{biol}}$  is relatively close to reduction in the MBR during acclimation. It should be noted  
613 that subtracting the average sorption part of 3.7% (previously observed for the  
614 activated sludge of the hospital sMBRe pilot) from the 46% removal of CP would give a  
615 biotransformation part of 42.3%. This biotransformed fraction of 42.3% is in the same  
616 order of magnitude as the biotransformation measured by Seira (2013) of  $39 \pm 5$  % in an  
617 eMBR pilot treating urban wastewater with a CP dopant. The developed model also  
618 seems reliable for SM, as its average removal by the MBR during acclimation (75%) was  
619 between the calculated maximum removal and removal through biotransformation.  
620 Lack of data about the sorption of codeine on sludge acclimated to OWW makes it  
621 impossible to conclude on the validity of the model for that pharmaceutical.

622

#### 623 **IV. Conclusion**

624 Removal of the selected pharmaceutical molecules by activated sludge acclimated to  
625 OWW and non-acclimated sludge from the municipal WWTP confirms literature  
626 observations on the heterogeneity of the removal of pharmaceuticals. 5-FU was almost  
627 systematically removed beyond 90%. This molecule is easily removable, as the  
628 performance of WWTP sludge confirmed. It is important to specify that (i) even if  
629 removal of 5-FU by sludge acclimated to OWW seems identical to that by WWTP sludge,  
630 sludge acclimation was obtained in the presence of other inhibitory compounds  
631 contained in the effluents of the oncological ward (pharmaceuticals, surface-active

632 agents), which makes the results all the more remarkable, and (ii) using sludge  
633 acclimated to OWW allowed for a 34% increase in the degradation kinetic constant and  
634 in the minimum inhibition concentration.

635 Acclimation of activated sludge to OWW in an MBR brought about the creation of  
636 extensive capacity for biotransformation and the acquisition of a very pronounced  
637 resistance to the most widely consumed antibiotics in the oncological ward. While  
638 sorption is the main, or even the only, removal mechanism by non-acclimated WWTP  
639 sludge, treatment by acclimated sludge provides a significant improvement in the  
640 removal of the selected pharmaceuticals. Hence 20% of the amounts of CP, IF and SM  
641 can be removed by biotransformation in a mere 4 h. With the exception of codeine for  
642 which sorption reaches 30%, sorption of the selected pharmaceuticals onto sludge  
643 proved minor, as it was lower than or in the order of 10% for both types of tested  
644 sludge. If removal by sorption is low, adsorbed quantities still remain significant,  
645 because of the high concentrations in pharmaceuticals of hospital effluents, and more  
646 specifically effluents from a care unit. Thus pollutant transfer from the liquid to the solid  
647 phase must be taken into account when determining the suitable process for the  
648 treatment of sludge.

649 These results are positive, as they show that the observed removal of pharmaceutical  
650 molecules by an acclimated biomass can mostly be attributed to developed  
651 biotransformation, in comparison with the sorption phenomenon. The acclimated  
652 activated sludge showed a great capacity for adaptation to the pharmaceuticals  
653 contained in the OWW. That observation is supported by the conservation of the  
654 purifying capacities of the biomass in the presence of a pharmaceutical cocktail, the  
655 acquisition of a pronounced resistance to antibiotics and, most of all, by the creation of

656 biotransformation capacities on the selected pharmaceuticals. A systematic  
657 improvement of the performance of the acclimated activated sludge, compared to that of  
658 activated WWTP sludge, was obtained in spite of the presence of numerous compounds  
659 (pharmaceuticals, metabolites, and cleaning products) in the OWW. These compounds  
660 sometimes inhibited the development of the biomass and its purifying performance on  
661 the COD, ammonium and nitrates during acclimation. Furthermore, it has to be noted  
662 that the development of a pronounced resistance to antibiotics must be seriously  
663 studied with regard to human health and the environment in order to validate the  
664 biological treatment at the source of highly concentrated antibiotics effluent.

665

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669

670



List of symbols

5-FU	Fluorouracile
OWW	oncological ward wastewater
WWTP	wastewater treatment plant
eMBR	external membrane bioreactor
sMBRe	external submerged membrane bioreactor
TSS	Total Suspended Solids ( $\text{g.L}^{-1}$ )
COD	chemical oxygen demand ( $\text{mgO}_2.\text{L}^{-1}$ )
CP	cyclophosphamide
IF	ifosfamide
SM	sulfamethoxazole
CD	codeine
CF	concentration factor
SRT	sludge retention time
HRT	hydraulic retention time (h)

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$k_{\text{biol}}$	Biotransformation kinetic constant	$\text{L.gTSS}^{-1}.\text{d}^{-1}$
$K_D$	Solid-water distribution coefficient	$\text{L.kgTSS}^{-1}$
$k_{\text{global}}$	Maximum removal constant	$\mu\text{g}^{1-n}.\text{L}^{n-1}.\text{d}^{-1}$
$[5\text{-FU}]_{\text{max}}$	5-FU maximum concentration detected in OWW	$\mu\text{g.L}^{-1}$
$n_{\text{med}}$	Yearly amount of pharmaceutical molecule consumed in the oncological ward	$\text{mg.year}^{-1}$
$n_{5\text{-FU}}$	Yearly amount of 5-FU consumed in the oncological ward	$\text{mg.year}^{-1}$
$1-\tau_{\text{med}}$	Rate of unmetabolized pharmaceutical	-
$1-\tau_{5\text{-FU}}$	Rate of unmetabolized 5-FU	-

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674

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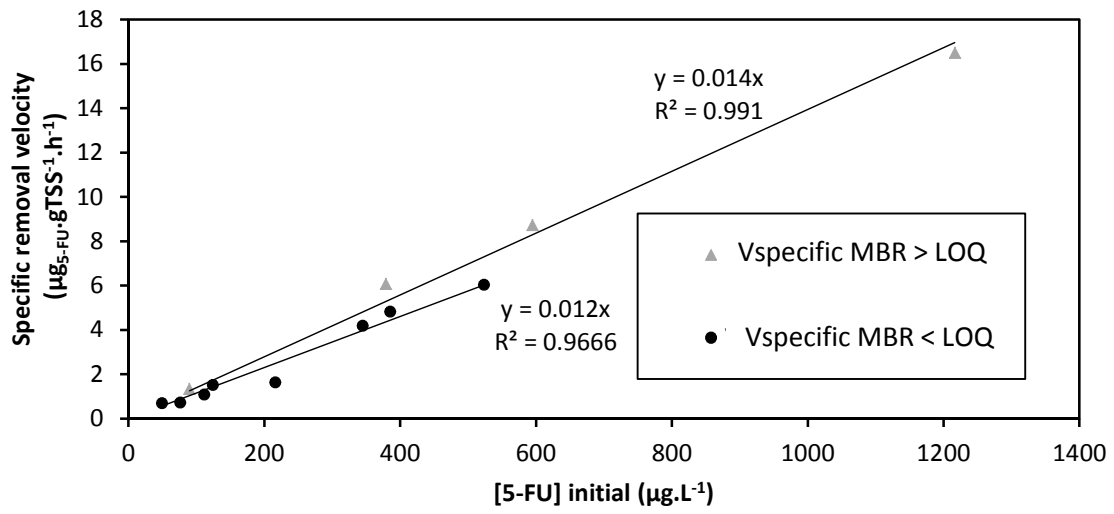
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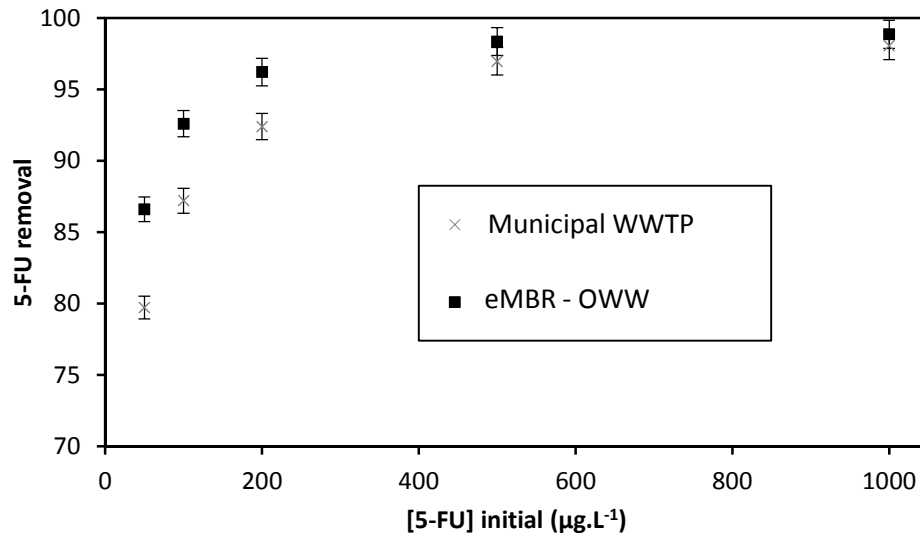


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Figure 1. Specific removal velocity of 5-FU in eMBR treating OWW.



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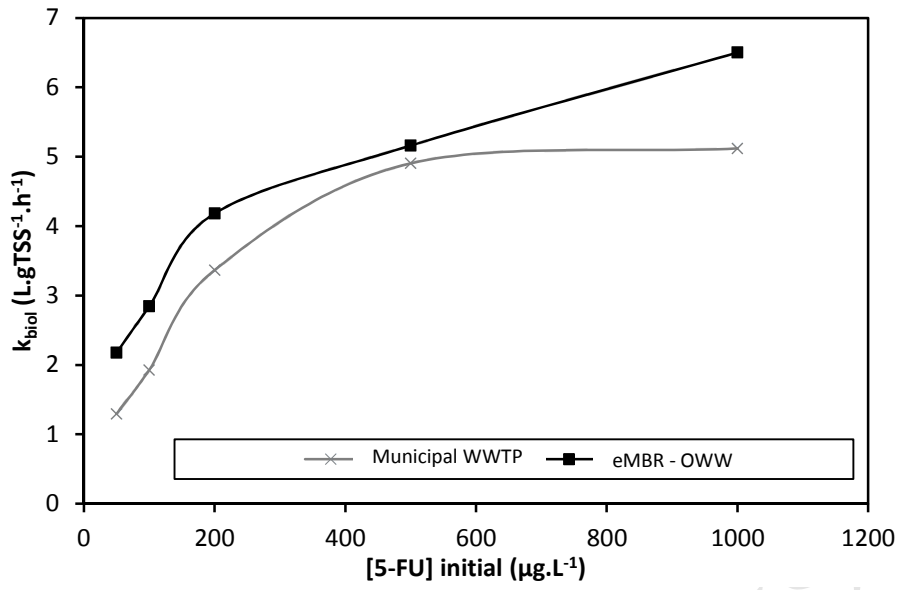
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Figure 2. 5-FU removal in batch reactor for activated sludge from municipal WWTP and activated sludge from the eMBR treating OWW.

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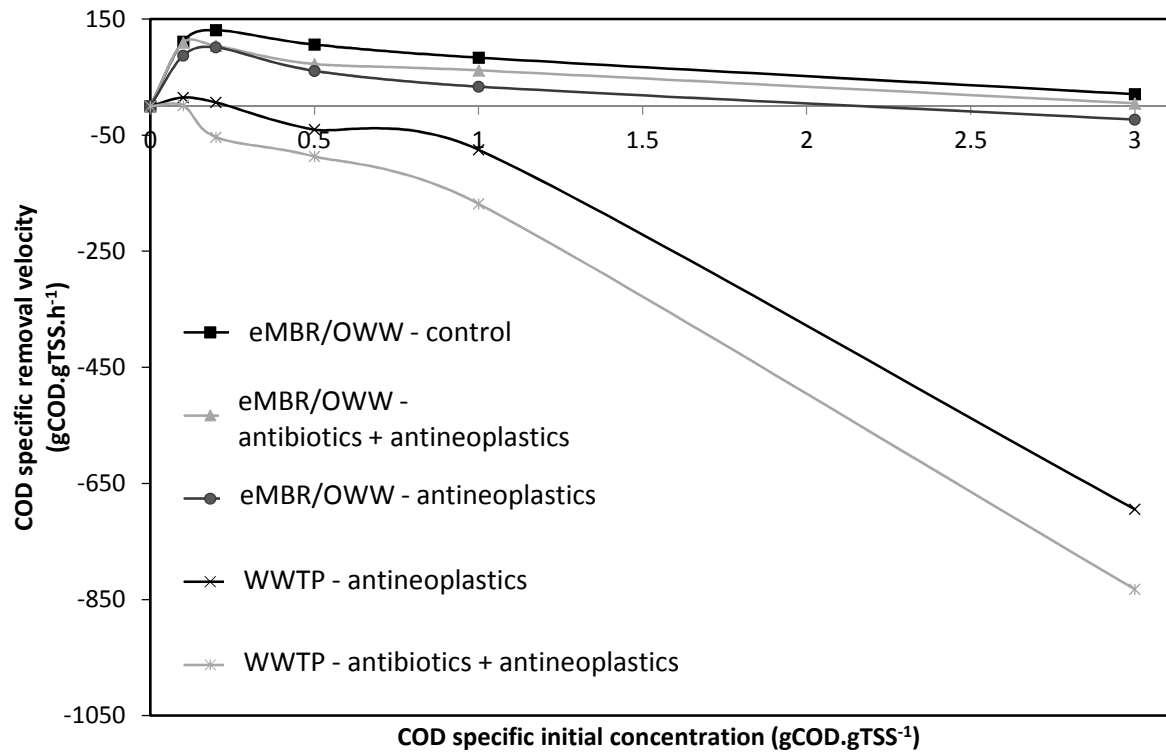


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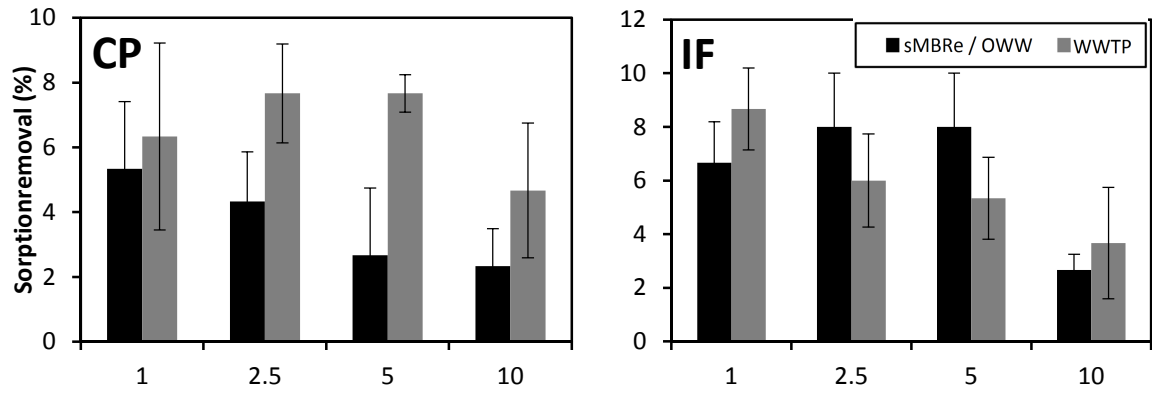
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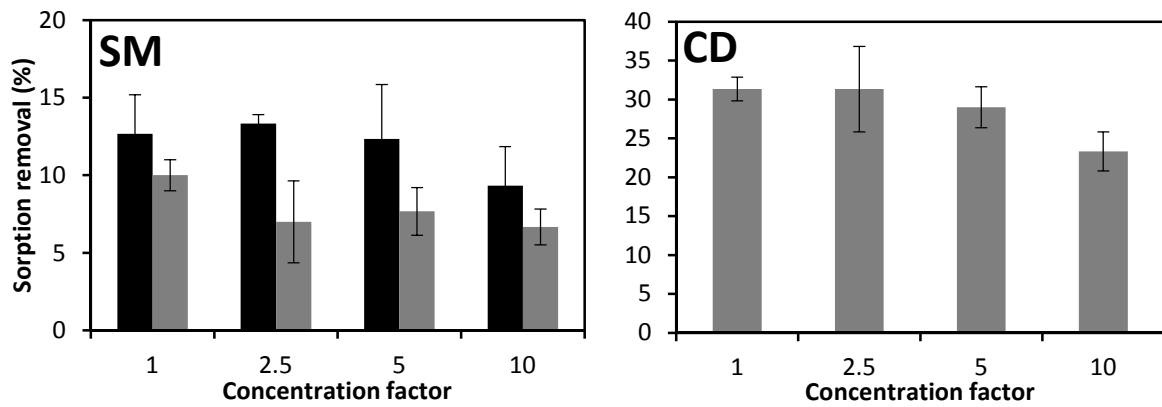


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Figure 4. COD specific removal velocity in presence of pharmaceuticals for activated sludge from municipal WWTP and the eMBR treating OWW.

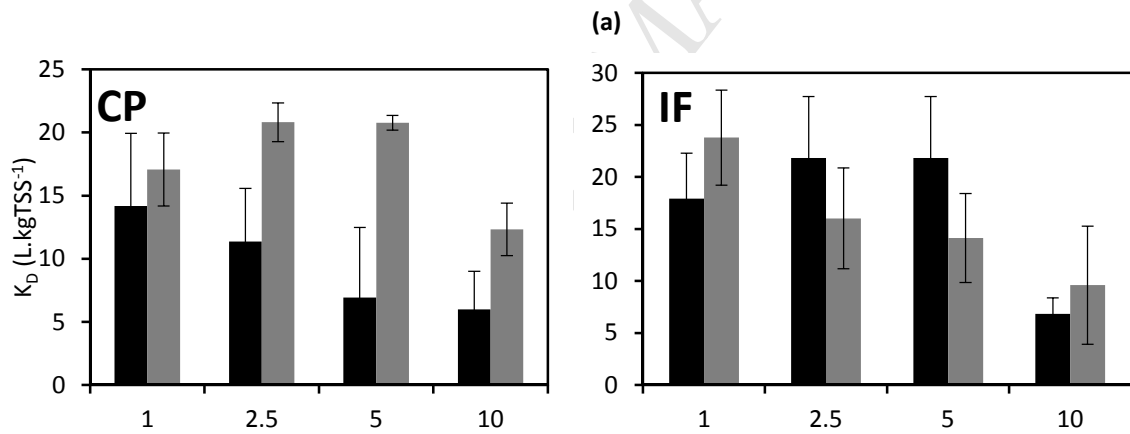


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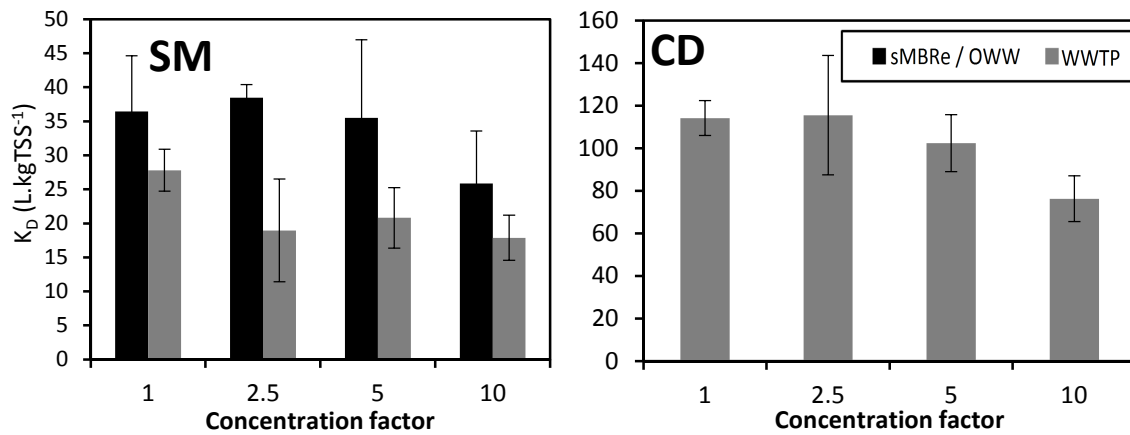


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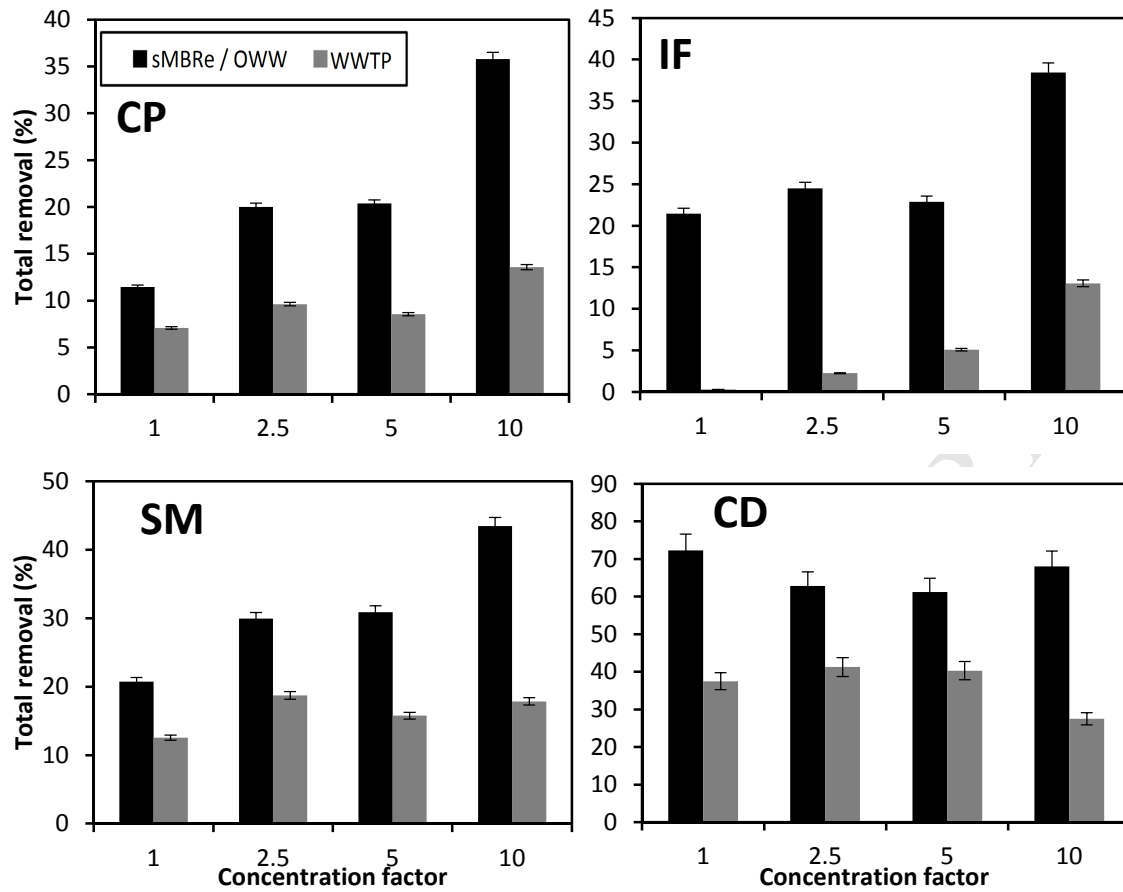
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(b)

52 **Figure 5. Removal of the selected pharmaceuticals due to sorption (a) and values of  $K_p$  for the selected**  
53 **pharmaceuticals (b) for both activated sludge from municipal WWTP and the sMBRe treating OWW.**  
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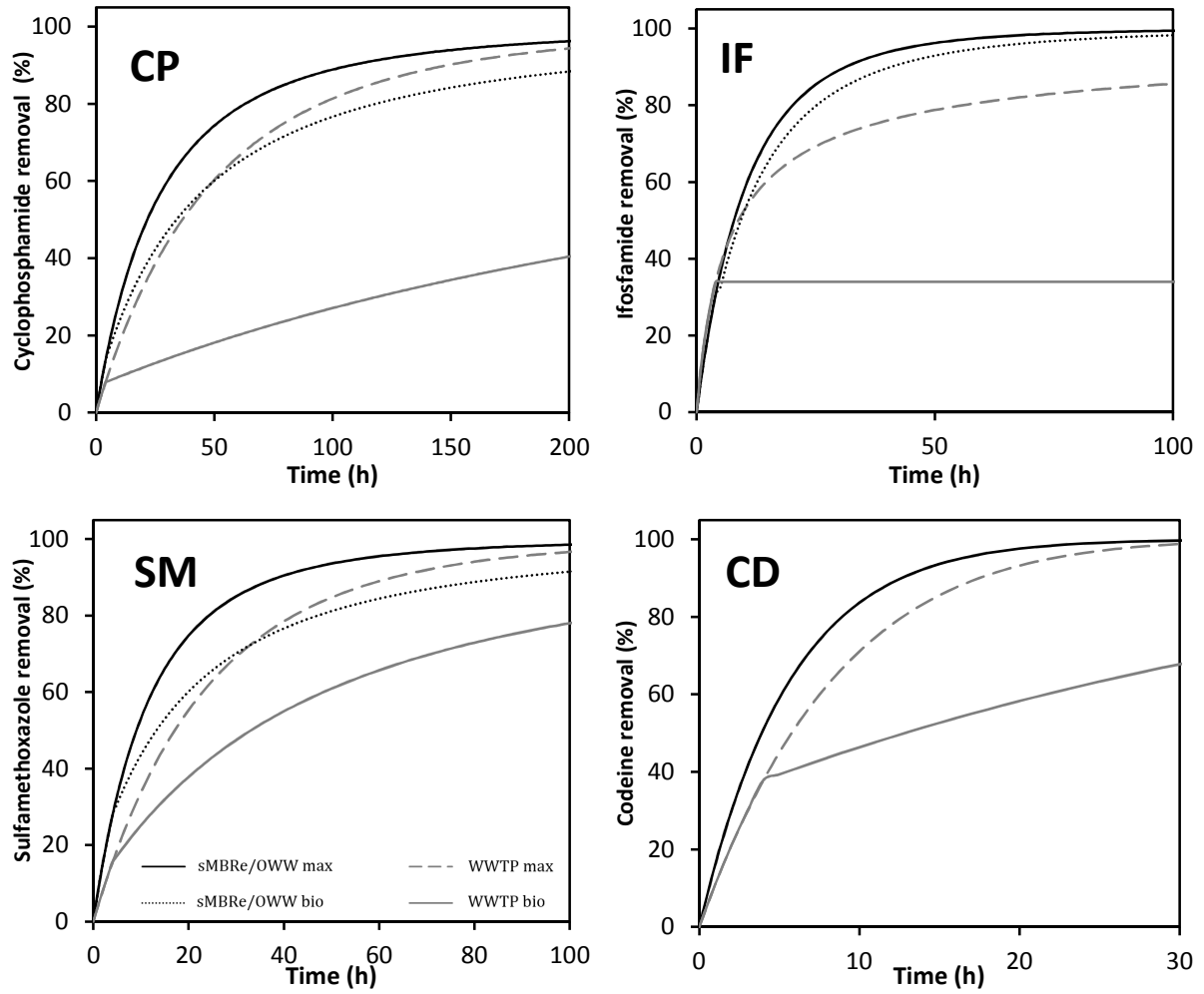
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57 **Figure 6. 4hours removal of the selected pharmaceuticals for both activated sludge from municipal WWTP**  
 58 **and the SMBRe treating OWW.**

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Figure 7. Removal evolution of the 4 selected pharmaceuticals by both activated sludge.

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65

66 **Table 1: State and performance of activated sludge at the sampling date**

MBR characteristics	<b>Supplier</b>	CTI			Polymem		
	<b>Type</b>	Carbosep® M1			M2		
	<b>Configuration</b>	Tubular-monocanal			Frontal extern-intern / 260 hollow fibers		
	<b>Material</b>	ZrO <sub>2</sub> -TiO <sub>2</sub>			Polysulfone		
	<b>Initial permeability (L.h<sup>-1</sup>.m<sup>-2</sup>.bar<sup>-1</sup>)</b>	110			130		
	<b>Molecular weight cut-off (kDa)</b>	150			100		
	<b>Total filtration surface (m<sup>2</sup>)</b>	0.0222			0.4		
	<b>Length (m)</b>	1.20			0.6		
	<b>Internal diameter/external diameter (mm)</b>	06 / 10			0.85 / 1.44		
Operating conditions	<b>Permeate flow rate (L.h<sup>-1</sup>)</b>	min	max	average	min	max	average
		1	2	1.42 ± 0.22	0.8	2	1.13 ± 0.27
	<b>Hydraulic retention time (h)</b>	16	32	23.0 ± 3.6	16	40	29 ± 8
		<b>Transmembrane pressure (bar)</b>	0.8	2.2	1.30 ± 0.23	0.06	0.75
	<b>Sludge retention time</b>	Infinite			Infinite		
	<b>Cycle aerated / not aerated (h)</b>	2 / 1			2 / 1		
Operating conditions at the sampling time	<b>Acclimation to OWW duration (d)</b>	160			180		
	<b>TSS (g.L<sup>-1</sup>)</b>	4.0			4.1		
	<b>Biomass evolution</b>	growth			stabilisation		
	<b>CODS removal (%)</b>	94			35		
	<b>CODP removal (%)</b>	98			89		
	<b>N-NH<sub>4</sub><sup>+</sup> removal (%)</b>	99			100		

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**Table 2: Concentrations of the antineoplastic and antibiotic cocktail**

<b>Antineoplastic</b>	<b>Concentration (<math>\mu\text{g}\cdot\text{L}^{-1}</math>)</b>
5-FU	1300
Carboplatin	600
Etoposide	600
Doxorubicin	400
Cisplatin	400
Cytarabine	200
Methotrexate	150
Gemcitabine	150
<b>Total</b>	<b>3800</b>

<b>Antibiotic</b>	<b>Concentration (<math>\mu\text{g}\cdot\text{L}^{-1}</math>)</b>
Ticarcillin	1000
Amoxicillin	1000
Ciprofloxacin	1000
Ceftriaxone	1000
<b>Total</b>	<b>4000</b>

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**Table 3: Quantity of sorbed pharmaceutical onto activated sludge.**

Molecule	OWW <sub>average</sub> ( $\mu\text{g}\cdot\text{L}^{-1}$ )	Corresponding CF	Corresponding removal efficiency (%)	Specific quantity of sorbed pharmaceutical ( $\mu\text{g}\cdot\text{gTSS}^{-1}$ )
CD	26	1	31.3 (WWTP)	2
CP	290	2.5	4.3	3
IF	1664	10	2.7	11
SM	422	5	12.3	13

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**Table 4.  $K_D$  values of selected pharmaceuticals reported in previous studies.**

Molecule	$K_D$ (L.kgTSS <sup>-1</sup> )	Activated sludge origin	Inhibition technique	Reference
	-	<b>sMBRe – infinite SRT</b>	<b>Anaerobic</b>	<b>Present study</b>
	<b>102.1 ± 8.9</b>	<b>sMBRe WWTP</b>	<b>Anaerobic</b>	<b>Present study</b>
CD	Sorption too low to be quantified	primary - secondary at weak SRT – secondary at long SRT	AS are slightly frozen then sterilized at 103°C during 3h	Hörsing et al., 2011
	14 ± 1	Conventional WWTP	Sodium azide 0.2 % (v/v)	Wick et al., 2009
	<b>9.6 ± 3.8</b>	<b>sMBRe – infinite SRT</b>	<b>Anaerobic</b>	<b>Present study</b>
	<b>17.7 ± 4.0</b>	<b>sMBRe WWTP</b>	<b>Anaerobic</b>	<b>Present study</b>
CP	15 - 12 - 0 – 47 - 20 - 32	eMBR - Conventional WWTP – Sludge thickener - Conventional WWTP – sMBRe - Conventional WWTP	Gas purging	Seira (2013)
	794.28	eMBR	Thermal	Delgado (2009)
	2.4 ± 0.5	WWTP	Gas purging	Ternes et al., 2004
	log $K_D$ about 3.2 --> $K_D = 1600$	Primary sludge	-	Okuda et al., 2009
	<b>17.1 ± 2.1</b>	<b>sMBRe – infinite SRT</b>	<b>Anaerobic</b>	<b>Present study</b>
	<b>15.9 ± 0.6</b>	<b>sMBRe WWTP</b>	<b>Anaerobic</b>	<b>Present study</b>
IF	22-71-7-87-55-63	eMBR - Conventional WWTP – Sludge thickener - Conventional WWTP – sMBRe - Conventional WWTP	Gas purging	Seira (2013)
	1.4 ± 0.4	WWTP	Gas purging	Ternes et al., 2004
	<b>34.1 ± 7.3</b>	<b>sMBRe – infinite SRT</b>	<b>Anaerobic</b>	<b>Present study</b>
	<b>21.4 ± 2.0</b>	<b>sMBRe WWTP</b>	<b>Anaerobic</b>	<b>Present study</b>
	256 ± 169	Conventional WWTP	Freeze-drying	Göbel et al., 2005
	3.2 ± 4.5 - 77 ± 60 - 60 ± 49 - 63 ± 42	Primary sludge – Secondary sludge – MBR flat sheets – MBR hollow fibers	Freeze-drying	Radjenovic et al., 2009
SM	40 ± 13 - 50 ± 13	MBR	-	Abbeglen et al., 2009
	320 - 370 - 280	primary - secondary at weak SRT – secondary at long SRT	AS are slightly frozen then sterilized at 103°C during 3h	Hörsing et al., 2011
	<30	Primary and two different secondary activated sludge	Lyophilization and dry-heat	Stevens-Garmon et al., 2011
	28.6 ± 1.9	Conventional WWTP	Sodium azide 1 g.L <sup>-1</sup>	Yang et al., 2011

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**Table 5. 4 hours removal of the selected pharmaceuticals by both activated sludge.\***

Molecule	sMBRe – acclimated to OWW				sMBRe municipal WWTP			
	Total (%)	Biotransformation (%)	Sorption (%)	$\frac{\text{Biotransformation}}{\text{Sorption}}$	Total (%)	Biotransformation (%)	Sorption (%)	$\frac{\text{Biotransformation}}{\text{Sorption}}$
CD	66.1 ± 5.1	-	-	-	36.6 ± 6.3	7.9	28.7 ± 3.8	0.3
CP	21.9 ± 10.1	18.2	3.7 ± 1.4	4.9	9.7 ± 2.8	3.1	6.6 ± 1.4	0.5
IF	26.8 ± 7.9	20.5	6.3 ± 2.5	3.2	5.2 ± 5.6	-0.7	5.9 ± 2.1	Solely sorption
SM	31.3 ± 9.4	19.4	11.9 ± 1.8	1.6	16.2 ± 2.7	8.4	7.8 ± 1.5	1.1

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\*Values presented relate the average removals calculated from the 4 concentration factors.

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83 **Table 6. k and n values for the total and biotransformation removal by both activated sludge.**

<b>Total removal</b>				
TSS = 4 g.L <sup>-1</sup> – t = 4h				
Molecule	sMBRe/OWW		sMBRe/municipal WWTP	
	k <sub>global</sub> (μg <sup>1-n</sup> .L <sup>n-1</sup> .d <sup>-1</sup> )	n	k <sub>global</sub> (μg <sup>1-n</sup> .L <sup>n-1</sup> .d <sup>-1</sup> )	n
Codeine (CD)	4.680	1	4.056	0.9
Cyclophosphan	0.072	1.5	0.120	1.2
Ifosfamide (IF)	0.456	1.2	1.30E-05	2.7
Sulfamethoxaz	0.336	1.3	0.480	1.1
<b>Biotransformation removal</b>				
Molecule	sMBRe/OWW		sMBRe/municipal WWTP	
	k <sub>biol</sub> (μg <sup>1-n</sup> .L <sup>n-1</sup> .d <sup>-1</sup> )	n	k <sub>biol</sub> (μg <sup>1-n</sup> .L <sup>n-1</sup> .d <sup>-1</sup> )	n
Codeine (CD)	-	-	0.792	0.9
Cyclophosphan	0.012	1.7	5.28E-04	1.9
Ifosfamide (IF)	0.192	1.3	no biotransformation	
Sulfamethoxaz	0.048	1.6	0.024	1.6

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85 **Table 7. Required duration to achieve 95 % removal of the selected pharmaceuticals for both activated**  
 86 **sludge.**

Molecule	sMBRe/municipal		sMBRe/municipal	
	sMBRe/OWW	WWTP	sMBRe/OWW	WWTP
	t95% max (h)	t95% bio (h)	t95% max (h)	t95% bio (h)
Codeine (CD)	16	22	-	94
Cyclophosphamide (CP)	170	212	408	4849
Ifosfamide (IF)	44	619	61	Never
Sulfamethoxazole (SM)	57	86	150	336

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89 **Table 8. Removals of the selected pharmaceuticals for both activated sludge at the average HRT of 29h of the**  
90 **sMBRe pilot treating OWW.**

Molecule	sMBRe/OWW		sMBRe/municipal WWTP	
	Max removal (%)	Bio removal (%)	Max removal (%)	Bio removal (%)
Codeine (CD)	99.6	-	98.6	67.0
Cyclophosphamide (CP)	58.8	46.0	42.9	13.7
Ifosfamide (IF)	88.6	91.8	71.5	34.0
Sulfamethoxazole (SM)	84.3	69.3	68.1	46.6

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- (i) Acclimated sludge allowed for a 34% increase in the degradation kinetic constant
- (ii) Acclimated sludge allowed an increase in the minimum inhibition concentration.
- (iii) Sorption of pharmaceuticals onto sludge proved minor in comparison of biosorption
- (iv) Removal of pharmaceuticals is attributed to developed biotransformation
- (v) High removal factor for 5-FU whatever the presence of other inhibitory compounds