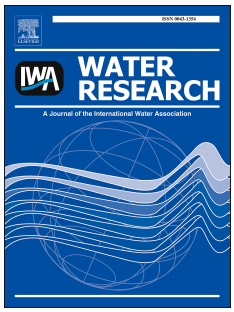
Performance of a biomass adapted to oncological ward wastewater vs. biomass from municipal WWTP on the removal of pharmaceutical molecules

Pierre Hamon, Philippe Moulin, Lionel Ercolei, Benoit Marrot

Accepted Manuscript



Performance of a biomass adapted to oncological ward wastewater vs. biomass from municipal WWTP on the removal of pharmaceutical molecules

P. Hamon, P. Moulin, L. Ercolei, B. Marrot

PII: S0043-1354(17)30874-6

DOI: 10.1016/j.watres.2017.10.037

Reference: WR 13294

To appear in: *Water Research*

Received Date: 3 April 2017

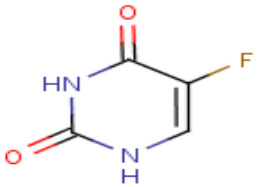
Revised Date: 6 October 2017

Accepted Date: 17 October 2017

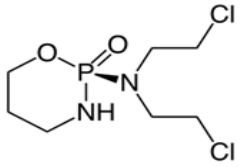
Please cite this article as: Hamon, P., Moulin, P., Ercolei, L., Marrot, B., Performance of a biomass adapted to oncological ward wastewater vs. biomass from municipal WWTP on the removal of pharmaceutical molecules, *Water Research* (2017), doi: 10.1016/j.watres.2017.10.037.

Oncological department

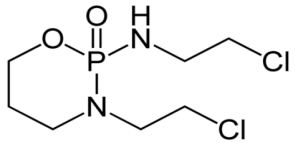
WASTEWATER WITH A HIGH CONCENTRATION OF MOLECULES



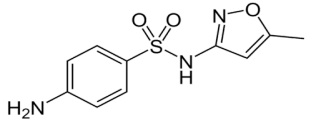
Fluorouracile (5-FU)



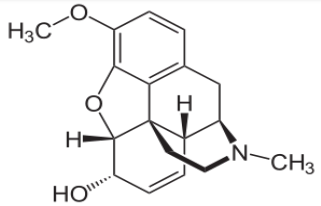
Cyclophosphamide (CP)



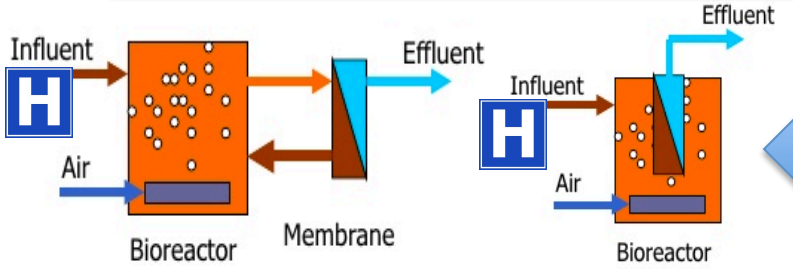
Ifosfamide (IF)



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

P. HAMON[(1-2)], P. MOULIN [(1*)], L. ERCOLEI[(2)], B. MARROT [(1)]

[(1)] *Aix Marseille Université, CNRS, Centrale Marseille, M2P2 UMR 7340, Equipe Procédés Membranaires (EPM), Europôle de l'Arbois, BP80, Pavillon Laennec, Hall C, 13545 Aix en Provence Cedex, France*

[(2)] *Société des Eaux de Marseille, 25 Rue Edouard Delanglade, 13006 Marseille, France*

**corresponding author: philippe.moulin@univ-amu.fr*

Tel.: +33 4 42 90 85 01, fax: +33 4 42 90 85 15.

Abstract

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

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

Keywords

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

I. Introduction

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

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

Moreover, the treatment process may influence the ability of the biomass to resist toxic charges, as Henriques et al. (2005-2007) state that some processes are more sensitive to inhibition: it is the case of activated sludge flocs, which boost the formation of small 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

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

II. Equipment and methods

II.1. MBR and hospital effluents

A pilot-scale membrane bioreactor was designed, built and set up underneath the oncological ward of the Timone hospital (Marseille, France). The MBR pilot was designed for treating 1 to 2 $L.h^{-1}$ of hospital effluent from the oncological ward. The pilot has a maximum capacity of 60 L, with an operating volume set at 32 L. A 3.1 kW refrigeration unit allowed for regulation of activated sludge temperature at 25 ± 2°C. OWW were kept in a storage tank with a maximum capacity of 200 L and were renewed every other day. First, OWW were sent into the denitrification tank (10.5 L) which was stirred through sludge recirculation carried out by a peristaltic pump. The dissolved oxygen concentration is continuously monitored in the denitrification reactor in order to check its zero value. The bacterial flocs were maintained in suspension without aerating the anoxic zone, which allowed the denitrification reaction to take place. A fraction of the recirculated sludge was transferred to the aerobic tank, which has a capacity of 21.5 L, and in which the nitrification reaction took place. The hydraulic retention time (HRT) in both tanks was set through the adjustment of valves, which established 1h/2h cycles in the anoxic and anaerobic tanks respectively. Aeration was performed by fine air bubbles delivered through four porous tubes connected to a compressor. This maintained oxygen concentration above 2 $mg.L^{-1}$ and ensured the stirring of the aerobic tank. A centrifugal pump (B3, Motovario) located at the foot of the nitrification tank performed 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 $N\text{-}NH_4^+$ concentrations were measured. COD and
135 $N\text{-}NH_4^+$ 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 $mg.L^{-1}$ as
139 sugar $C_6H_{12}O_6$) and $N\text{-}NH_4^+$ (31 $mg.L^{-1}$ as $(NH_4)_2SO_4$) 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 $\mu g.L^{-1}$. The detection limits were obtained
160 with several injections of compounds from 1 to 10 $\mu g.L^{-1}$. An accurate detection and
161 repeatability were obtained from the concentration at 5 $\mu g.L^{-1}$. In detail, the analytical
162 system was divided into three parts: (A) a mobile phase composed of 0.05 M
163 monopotassium phosphate (KH_2PO_4) was adjusted to pH 3 with orthophosphoric acid
164 and filtration at 0.2µm. (B) 500µL of the sample to be analyzed was acidified with 20µL
165 of 5% orthophosphoric acid; ibromouracil (50mL, 10$\mu g.mL^{-1}$) was added. The sample
166 was extracted with 6 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 g at 4°C.
168 The organic phase was sampled and evaporated in a water bath under nitrogen. The dry
169 residue was recovered in 100 µL 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

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

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

II.3 Pharmaceutical cocktails

The influence of various pharmaceutical cocktails on the performance of both biomasses (acclimated to OWW and municipal WWTP) was assessed by using the kinetics of degradation of easily degradable substrates (COD, NH_4^+) in a batch reactor. The pharmaceutical cocktails were prepared using hospital pharmaceuticals. The composition of the antineoplastics cocktail was based on the maximum concentration of 5-FU found in OWW during the acclimation period of the biomass, i.e. 1287 µg.L^{-1}, on the metabolization rate of each pharmaceutical and on the maximum quantity consumed in the oncological ward unit to which the MBR was connected. The concentrations thus calculated are shown in Table 2.

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

with:

$[5-FU]_{max}$: Maximum concentration in 5-FU detected in OWW during the first experimental campaign (µg.L^{-1})

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

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

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

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

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

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

II.4 Sorption and biotransformation tests

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

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

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

III. Results

III.1. Removal Performance

III.1.1. 5-FU

During the acclimation phase, 5-FU was almost consistently detected in OWW at concentrations up to 1287 $\mu g.L^{-1}$ (minimum 49.6 $\mu g.L^{-1}$ / average 440 $\mu g.L^{-1}$ / 150 days). These concentrations are very high compared with those measured in previous research: between 11.5 and 122 $\mu g.L^{-1}$ for Mahnik et al. (2007) and between 35 and 92 $ng.L^{-1}$ for Kosjek et al. (2013). The permeate samples which were analyzed show good removal of 5-FU by acclimated activated sludge, as reductions are above 90% in spite of high initial concentrations, sometimes greater than 1 $mg.L^{-1}$. Specific degradation velocity seems relatively proportional to the 5-FU initial concentration (pseudo-first order) (Figure 1). If the $V_{specificMBR}$ > LOQ and $V_{specificMBR}$ < LOQ, the velocity was 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_2). Thus Kümmerer (1997) observed
307 contradictory results: he found no biotransformation of 5-FU for very high 5-FU
308 concentrations (9 and 850 $mg.L^{-1}$). 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 $\mu g.L^{-1}$. 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 $g.L^{-1}$. 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

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

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

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

III.1.2. Performance with pharmaceutical cocktails

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

On Figure 4, positive and negative velocities respectively show COD degradation and an inhibitory effect exerted by pharmaceuticals. Non-acclimated WWTP sludge (Rousset, France) proved to be totally impacted by the presence of pharmaceutical cocktails: degradation velocity of the COD was zero at low concentrations and even became negative for high charges. A negative degradation velocity means that the presence of pharmaceuticals triggered cell lysis of activated sludge. Conversely, positive COD degradation velocities were measured for sludge acclimated to OWW, which means that it retains a capacity for purification in the presence of pharmaceuticals. However, COD degradation velocities were slower than that of the control group without pharmaceuticals, indicating that pharmaceutical cocktails still partially inhibit the performance of the biomass, which would be logical given the high concentrations used in the cocktail. Optimal degradation velocity was around 0.2 $gCOD.gTSS^{-1}$ for the acclimated sludge. An inhibition of COD degradation by pharmaceutical materials was observed from 0.1 $gCOD.gTSS^{-1}$ for the sludge in sole presence of the antineoplastic cocktail and from 0.2 $gCOD.gTSS^{-1}$ for the antineoplastic cocktail with antibiotics. Thus inhibition seems stronger for the antineoplastic cocktail on its own than for the combination of antineoplastic and antibiotic cocktails. This surprising observation could 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 $L.kgTSS^{-1}$
413 for CP, IF and SM and smaller than 120 $L.kgTSS^{-1}$ for CD in municipal WWTP. Joss et al.
414 (2005) claimed that for a value of K_D smaller than 300 $L.kgTSS^{-1}$ sorption of a compound
415 is negligible and its removal may be assessed using input and output concentrations.

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

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

K_D coefficients of both antineoplastics (CP and IF) found in the literature are generally low (Seira, 2013 ; Ternes et al., 2004). High values of K_D for CP of 794.3 L.kgTSS^{-1} (Delgado, 2009) and of 111.4 L.kgTSS^{-1} (Zaviska, 2013) could be due to the thermic inhibition technique used, which completely breaks down the biomass (Hamon et al., 2014). For activated sludge from an MBR pilot, Seira (2013) obtained similar results to those of this study, with a K_D of 15 L.kgTSS^{-1} for CP and of 22 L.kgTSS^{-1} 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 $L.kgTSS^{-1}$. 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 $L.kgTSS^{-1}$ measured in this study for sludge acclimated to OWW and of 21.4
449 $L.kgTSS^{-1}$ 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

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

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

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

for SM and around 70% for CD in only 4 h (Figure 6). These results agree with those measured in the supernatant of the MBR during the acclimation period of the sludge. However, maximum removal of CP at 36% and of IF at 38% in that reduced time scale seems better than the removal obtained in the MBR. This could stem from the nature of the substrate, which was far less rich and complex than real OWW, and from the initial absence of these molecules in the supernatant, which was not the case in the MBR. It should also be noted that exclusively aerobic conditions (applied in batch reactors) are known to favor degradation of micropollutants as opposed to aerobic/anoxic processes (applied in the MBR) (Suarez et al., 2010), even if a 4 h test remains far shorter than the HRT of the MBR. Although these removals are incomplete, it should be noted that the differences in the associated concentrations are substantial since they are in the order of 700 $\mu g.L^{-1}$ for CD and 400 $\mu g.L^{-1}$ for SM, CP and IF.

These removals were obtained without adding a cosubstrate in the batch reactors. Thus actual biotransformation of the selected pharmaceuticals could be achieved through a direct metabolic pathway. Seira (2013) did not observe any biotransformation of CP and IF without adding a cosubstrate, but the concentrations tested in his study, respectively 6 and 2 $\mu g.L^{-1}$, were much lower than those of the present study (100 to 1,000 $\mu g.L^{-1}$). The higher concentrations used here are representative of the concentrations measured in OWW. Such concentrations could allow pharmaceuticals to be used as primary substrate for the biomass. The data obtained in this study permits to calculate kinetic parameters for removal by both types of tested activated sludge (Eq.2) for each pharmaceutical.

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

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

518

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

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 $[Pharmaceutical]_{eq}$ and
533 final $[Pharmaceutical]_f$ during the total removal test (Eq.4 and Table 6):

534

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

536

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

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

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

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

From 0 to 4 h:

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

Then with t > 4 h:

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

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

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

These profiles show the extent of the capacity for biotransformation developed by activated sludge thanks to on-site treatment of OWW. It seems that SM is the pharmaceutical which presents the smallest number of differences between the two types of sludge. The clearest gain from treatment with activated sludge is for the two 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_{global} constant are above the associated profile calculated
589 with the k_{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_{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 ± 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

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

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

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

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

Acknowledgements

The authors acknowledge the Ianesco laboratory (Institut d'Analyses et d'Essais en Chimie de l'Ouest, Poitiers, France) for their help.

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 ($g.L^{-1)}$
COD	chemical oxygen demand ($mgO_2.L^{-1}$)
CP	cyclophosphamide
IF	ifosfamide
SM	sulfamethoxazole
CD	codeine
CF	concentration factor
SRT	sludge retention time
HRT	hydraulic retention time (h)

671

672

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

673

674

675 **Reference**

676

677 Abegglen C., Joss A., McArdell C., Fink G., Schlüsener M., Ternes T., Siegrist H., 2009. The fate of
678 selected micropollutants in a single-house MBR. Water Research 43, 2036-2046.

679 Barrios-Martinez, A., E. Barbot, B. Marrot, P. Moulin and N. Roche, Degradation of phenol-containing
680 effluents by MBR. J. Membrane Sci., 288 (2006) 288-296.

681 Buerge I., Buser H., Poiger T., Müller M., 2006. 1st Network Conference on Persistent Organic
682 Pollutants: Human Exposure and Impacts, University of Birmingham, March 29-30, 2006.

683 Delgado L., 2009. Bioréacteur à membrane externe pour le traitement d'effluents contenant des
684 médicaments anticancéreux : élimination et influence du cyclophosphamide et de ses principaux
685 métabolites sur le procédé. Thèse de doctorat, INP Toulouse.

686 Göbel A., Thomsen A., McArdell C., Joss A., Giger W., 2005. Occurrence and Sorption Behavior of
687 Sulfonamides, Macrolides, and Trimethoprim in Activated Sludge Treatment. Environ. Sci. Technol.
688 39, 3981-3989.

689 Hann, S., Æ Zs. Stefanka Æ K. Lenz Æ G. Stingeder. Novel separation method for highly sensitive
690 speciation of cancerostatic platinum compounds by HPLC–ICP–MS. Anal Bioanal Chem (2005) 381:
691 405–412.

692 Hamon, P., 2013. Traitement des effluents d'un service d'oncologie par bioréacteur à membranes :
693 faisabilité d'acclimatation et gain apporté sur l'élimination de molécules médicamenteuses, Thèse
694 d'Aix Marseille Université

695 Hamon P., Villain M., Marrot B., 2014. Determination of sorption properties of micropollutants: What
696 is the most suitable activated sludge inhibition technique to preserve the biomass structure?
697 Chemical Engineering Journal 242, 260-268.

698 Henriques I., Holbrook R., Kelly R., Love N., 2005. The impact of floc size on respiration inhibition by
699 soluble toxicants—a comparative investigation. Water Research 39, 2559-2568.

700 Henriques I., Love N., 2007. The role of extracellular polymeric substances in the toxicity response of
701 activated sludge bacteria to chemical toxins. Water Research 41, 4177-4185.

702 Hörsing M., Ledin A., Grabic R., Fick J., Tysklind M., Jansen J., Andersen H., 2011. Determination of
703 sorption of seventy-five pharmaceuticals in sewage sludge. Water Research 45, 4470-4482.

704 Joss A., Keller E., Alder A., Göbel A., McArdell C., Ternes T., Siegrist H., 2005. Removal of
705 pharmaceuticals and fragrances in biological wastewater treatment. Water Research 39, 3139-3152.

706 Joss A., Zabczynski S., Göbel A., Hoffmann B., Löffler D., McArdell C., Ternes T., Thomsen A., Siegrist
707 H., 2006. Biological degradation of pharmaceuticals in municipal wastewater treatment: Proposing a
708 classification scheme. Water Research 40, 1686-1696.

709 Kiffmeryer T., Götze H., Jursch M., Lüders U., 1998. Trace enrichment, chromatographic separation
710 and biodegradation of cytostatic compounds in surface water. Fresenius J Anal Chem 361, 185–191

711 Kosjek T., Perko S., Zigon D., Heath E., 2013. Fluorouracil in the environment: Analysis, occurrence,
712 degradation and transformation. Journal of Chromatography A 1290, 66-72.

713 Kümmerer K., Steger-Hartmann T., Meyer M., 1997. Biodegradability of the anti-tumour agent
714 ifosfamide and its occurrence in hospital effluents and communal sewage. Water Research 31 (11),
715 2705-2710.

716 Kummerer K., Al-Ahmad A., 1997. Biodegradability of the anti-tumor agents 5-fluorouracil,
717 cytarabine, and gemcitabine: impact of the chemical structure and synergistic toxicity with hospital
718 effluent. Acta Hydrochim. Hydrobiol. 25 (4), 166–172.

719 Kummerer K., Helmers E., 1997. Hospital effluents as a source of platinum for the environment.
720 Science of Total Environment 193, 179-184.

721 Kümmerer K., 2009. The presence of pharmaceuticals in the environment due to human use –
722 present knowledge and future challenges. Journal of Environmental Management 90 (8), 2354 –
723 2366.

724 Mahnik S., Lenz K., Weissenbacher N., Mader R., Fuerhacker M., 2007. Fate of 5-fluorouracil,
725 doxorubicin, epirubicin, and daunorubicin in hospital wastewater and their elimination by activated
726 sludge and treatment in a membrane-bio-reactor system. Chemosphere 66, 30-37.

727 Okuda T., Yamashita N., Tanaka H., Matsukawa H., Tanabe K., 2009. Development of extraction
728 method of pharmaceuticals and their occurrences found in Japanese wastewater treatment plants.
729 Environment International 35, 815-820.

730 POSEIDON, 2006. Assessment of Technologies for the Removal of Pharmaceuticals and Personal Care
731 Products in Sewage and Drinking Water Facilities to Improve the Indirect Potable Water Reuse.

732 Radjenovic J., Petrovic M., Barcelo D., 2009. Fate and distribution of pharmaceuticals in wastewater
733 and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor
734 (MBR) treatment. Water Research 43, 831-841.

735 Schwarzenbach R., Gschwend P., Imboden D., 2003. Environmental Organic Chemistry. Wiley-
736 Interscience.

737 Seira J., 2013. Rôle de la sorption et de la biodégradation dans l'élimination de micropolluants par
738 des procédés d'épuration biologique : application aux molécules anticancéreuses traitées par
739 bioréacteur à membrane. Thèse de doctorat, Université de Toulouse.

740 Sipma J., Osuna B., Collado N., Monclus H., Ferrero G., Comas J., Rodriguez-Roda I., 2010. Comparison
741 of removal of pharmaceuticals in MBR and activated sludge systems. Desalination 250 (2), 653-659.

742 Stevens-Garmon J., Drewes J., Khan S., McDonald J., Dickenson E., 2011. Sorption of emerging trace
743 organic compounds onto wastewater sludge solids. Water Research 45, 3417-3426.

744 Suarez S., Lema J., Omil F., 2010. Removal of pharmaceuticals and personal care products (PPCPs)
745 under nitrifying and denitrifying conditions. Water Research 44, 3214-3224.

746 Tadkaew N., Sivakumar M., Khan S., McDonald J., Nghiem L., 2010. Effect of mixed liquor pH on the
747 removal of trace organic contaminants in a membrane bioreactor. Bioresource Technology 101,
748 1494-1500.

749 Tadkaew N., Hai F., McDonald J., Khan S., Nghiem L., 2011. Removal of trace organics by

750 Ternes T., Herrmann N., Bonerz M., Knacker T., Siegrist H., Joss A., 2004. A rapid method to measure
751 the solid–water distribution coefficient (Kd) for pharmaceuticals and musk fragrances in sewage
752 sludge. Water Research 38, 4075-4084.

753 Urase T., Kagawa C., Kikuta T., 2005. Factors affecting removal of pharmaceutical substances and
754 estrogens in membrane separation bioreactors. Desalination 178, 107-113.

755 Vieno N., Tuhkanen T., Kronberg L., 2007. Elimination of pharmaceuticals in sewage treatment plants
756 in Finland. Water Research 41, 1001 – 1012.

757 Wick A., Fink G., Joss A., Siegrist H., Ternes T., 2009. Fate of beta blockers and psycho-active drugs in
758 conventional wastewater treatment. Water Research 43, 1060-1074.

759 Yang S., Lin C., Lin A., Hong P., 2011. Sorption and biodegradation of sulfonamide antibiotics by
760 activated sludge: Experimental assessment using batch data obtained under aerobic conditions.
761 Water Research 45, 3389-3397.

762 Yu J., Bouwer E, Coelhan M., 2006. Occurrence and biodegradability studies of selected
763 pharmaceuticals and personal care products in sewage effluent. Agricultural Water Management 86,
764 72-80.

765 Zaviska F., Drogui P., Grasmick A., Azais A., Héran M., 2013. Nanofiltration membrane bioreactor for
766 removing pharmaceutical compounds. Journal of Membrane Science 429, 121-129.

767

768

769

770

771

772 **List of Tables and Figures**

773

774

788

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

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

795 Table 7. Required duration to achieve 95 % removal of the selected pharmaceuticals for both
796 activated sludge.

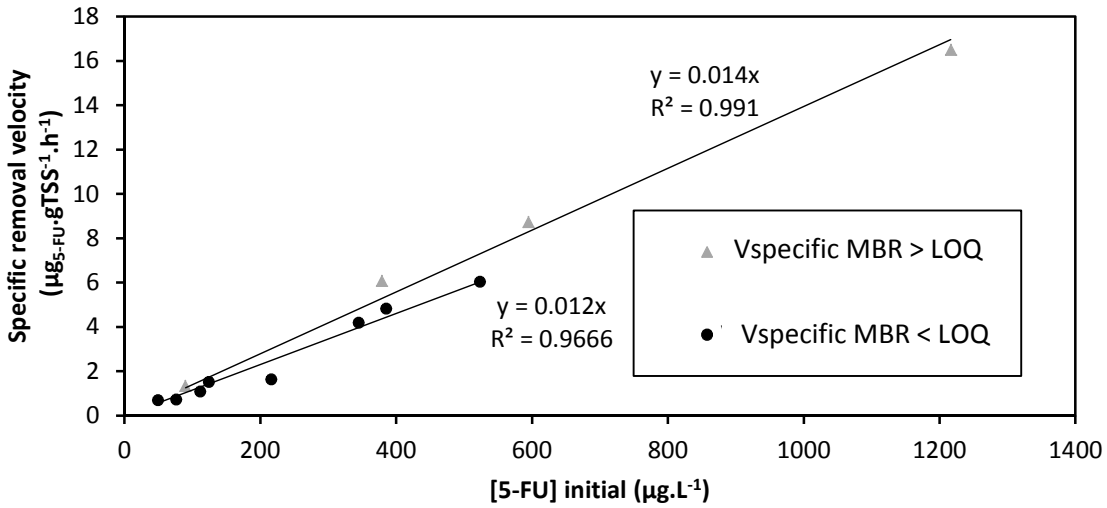
797 Table 8. Removals of the selected pharmaceuticals for both activated sludge at the average
798 HRT of 29h of the sMBRe pilot treating OWW.

799

800

List of Tables and Figures

30



31

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

33

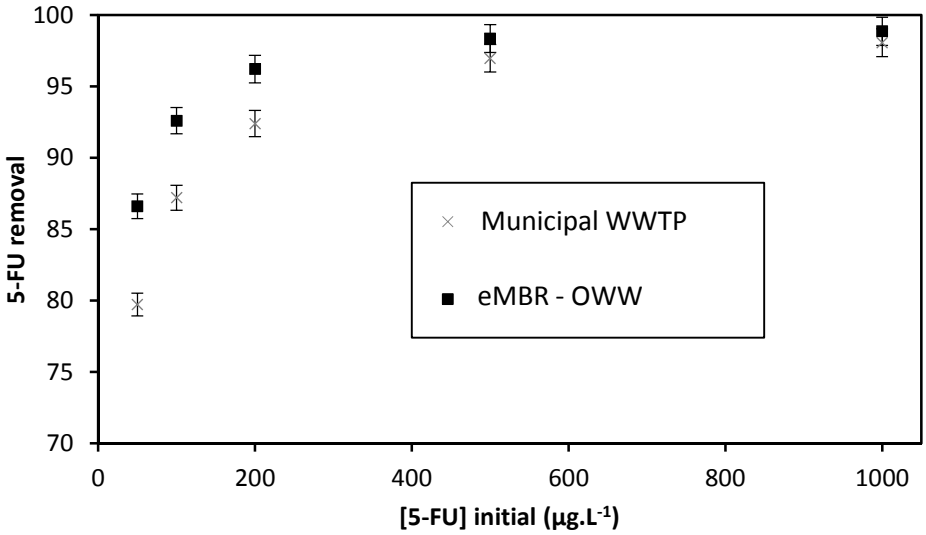
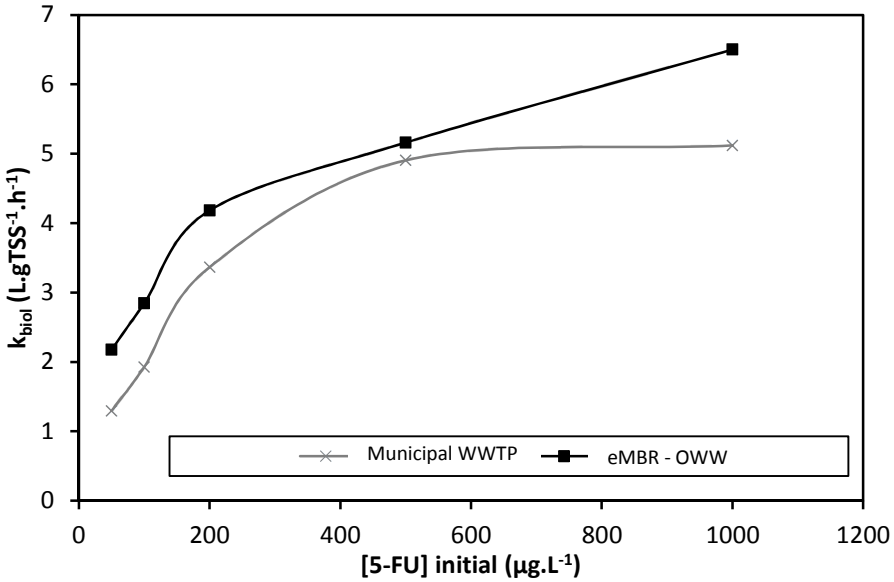


Figure 2. 5-FU removal in batch reactor for activated sludge from municipal WWTP and activated sludge from the eMBR treating OWW.

38



39

40 **Figure 3. Evolution of k_{biol} for activated sludge from municipal WWTP and eMBR treating OWW.**

41

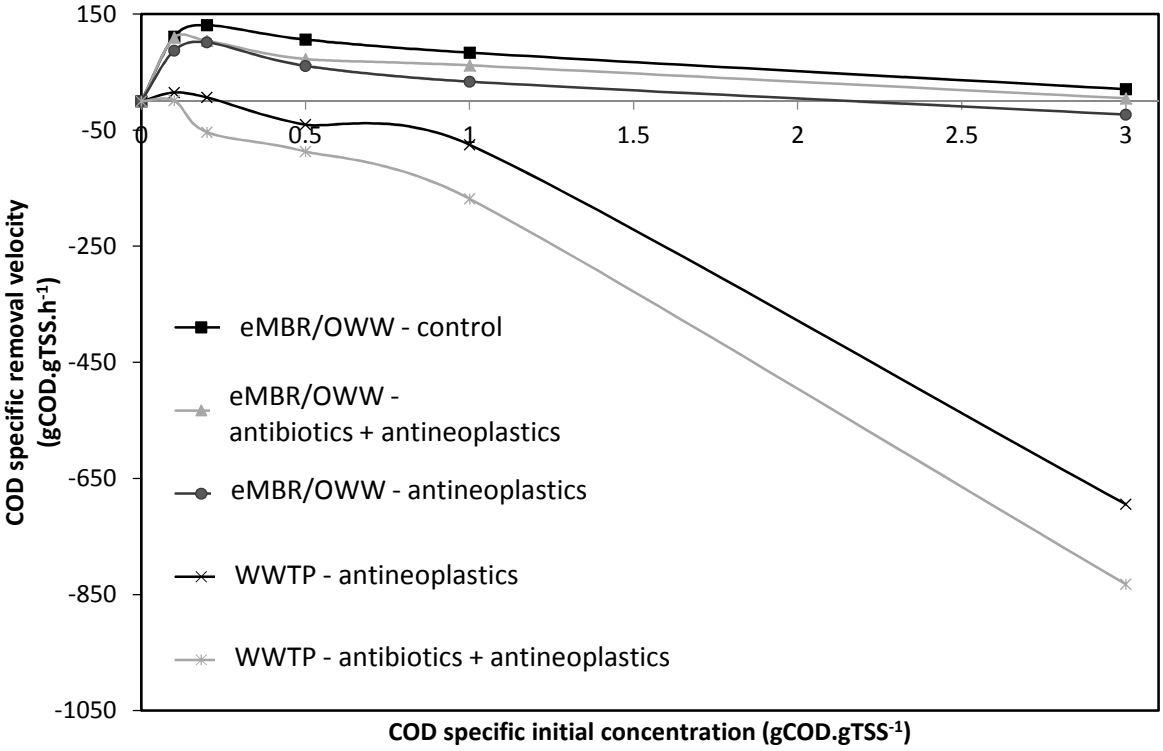
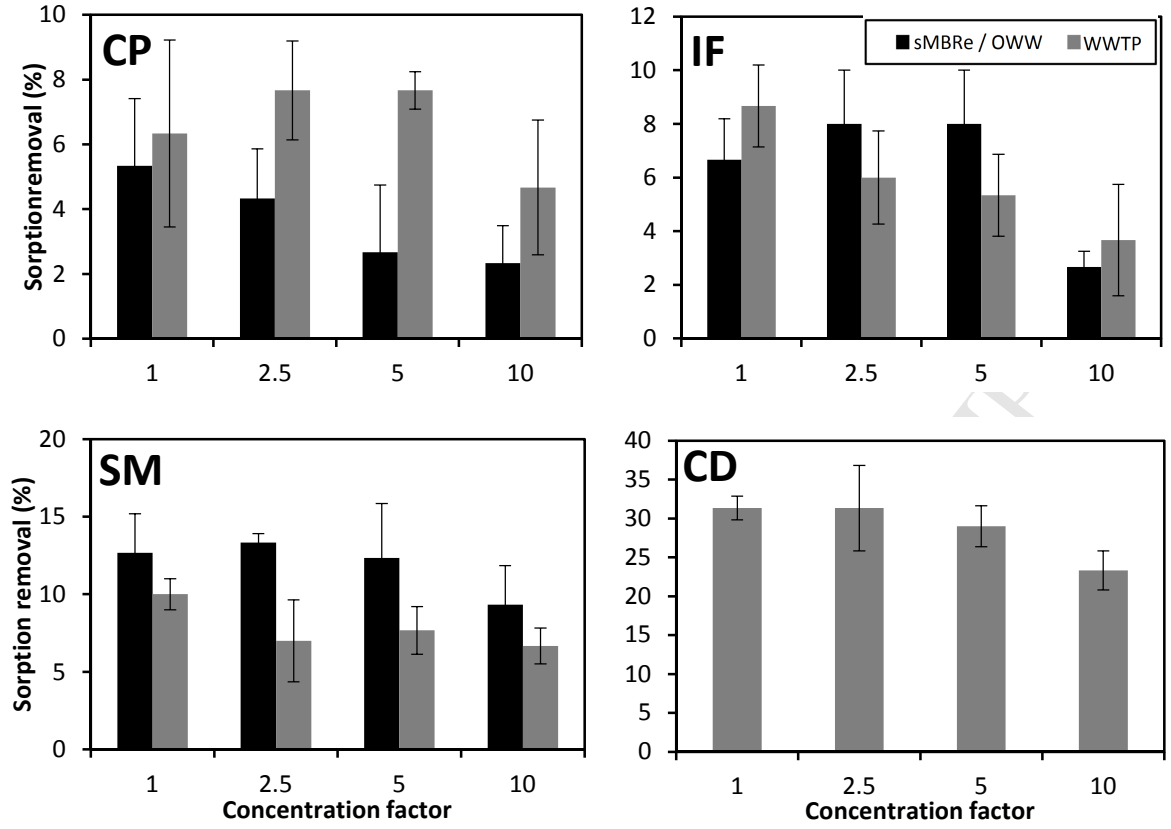
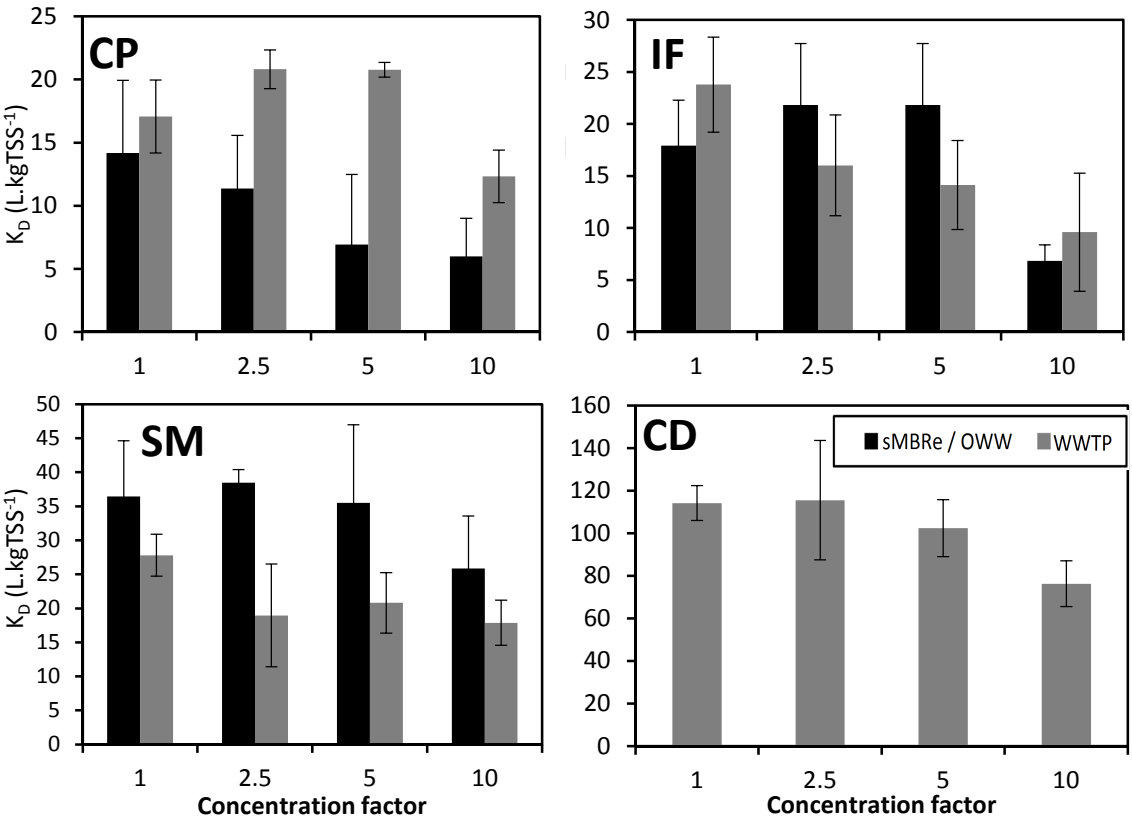


Figure 4. COD specific removal velocity in presence of pharmaceuticals for activated sludge from municipal WWTP and the eMBR treating OWW.



(a)



(b)

52 **Figure 5. Removal of the selected pharmaceuticals due to sorption (a) and values of K_D for the selected**
53 **pharmaceuticals (b) for both activated sludge from municipal WWTP and the sMBRe treating OWW.**

54

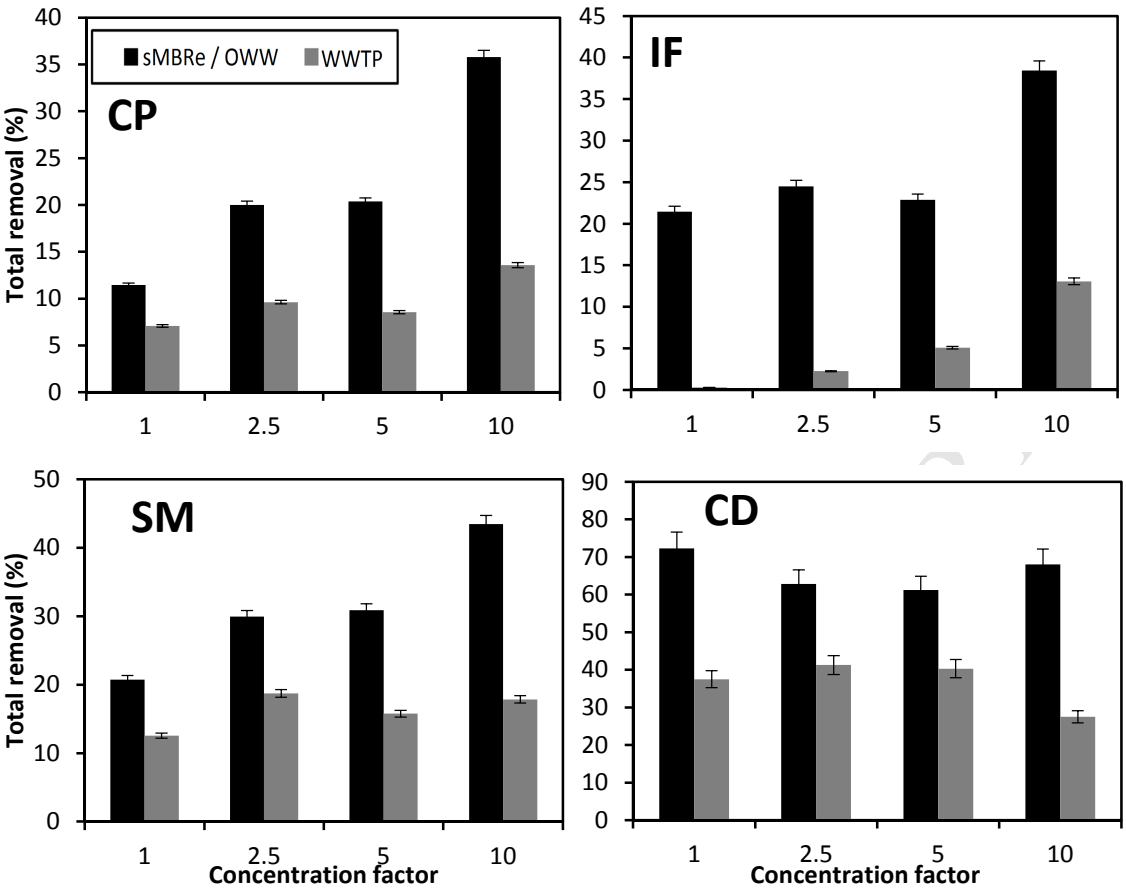


Figure 6. 4hours removal of the selected pharmaceuticals for both activated sludge from municipal WWTP and the sMBRe treating OWW.

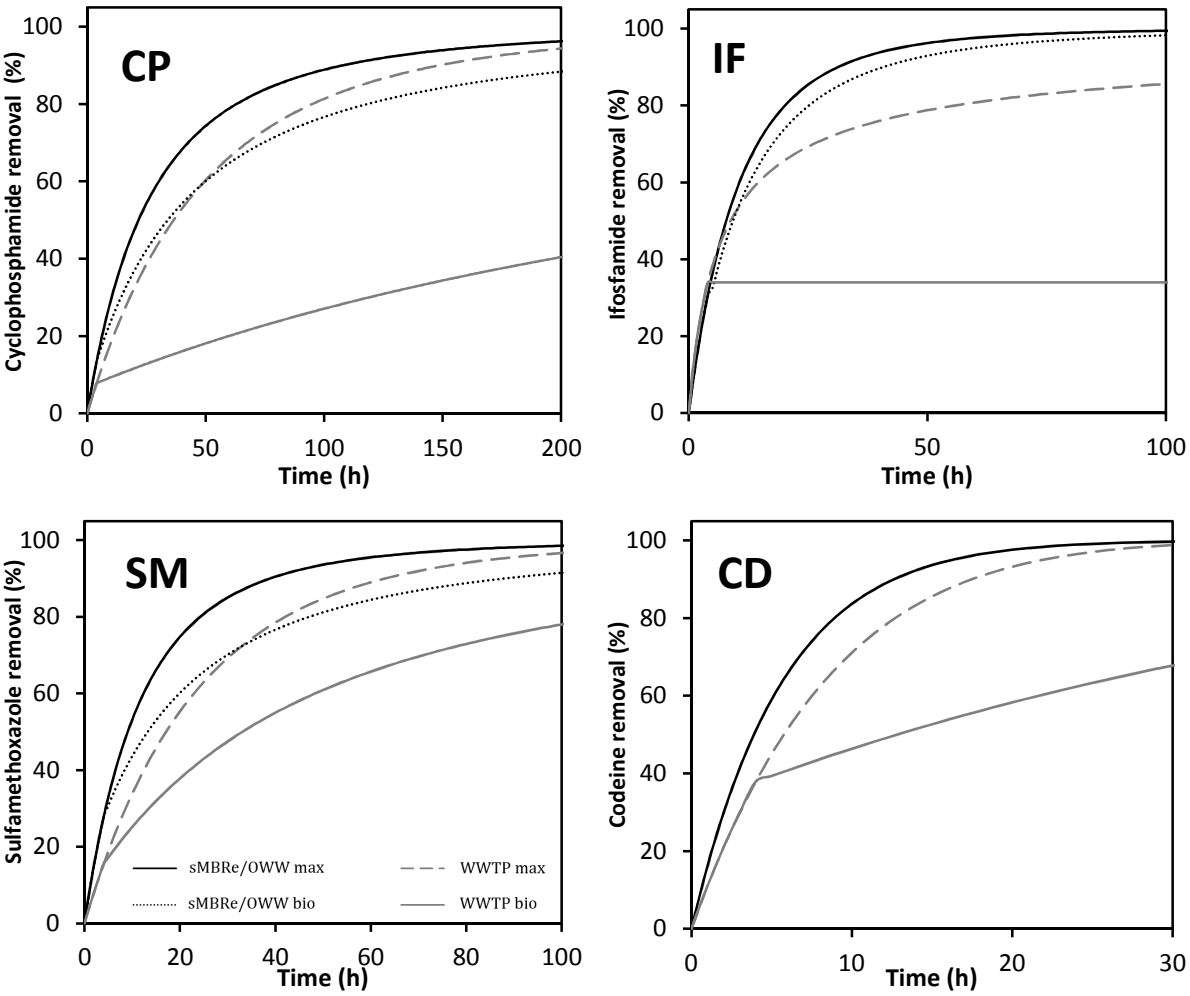


Figure 7. Removal evolution of the 4 selected pharmaceuticals by both activated sludge.

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

MBR characteristics	**Supplier**	CTI			Polymem		
	Type	Carbosep® M1			M2		
	Configuration	Tubular-monocanal			Frontal extern-intern / 260 hollow fibers		
	Material	ZrO_2-TiO_2			Polysulfone		
	Initial permeability ($L.h^{-1}.m^{-2}.bar^{-1}$)	110			130		
	Molecular weight cut-off (kDa)	150			100		
	Total filtration surface (m^2)	0.0222			0.4		
	Length (m)	1.20			0.6		
	Internal diameter/external diameter (mm)	06 / 10			0.85 / 1.44		
Operating conditions		min	max	average	min	max	average
	Permeate flow rate ($L.h^{-1}$)	1	2	1.42 ± 0.22	0.8	2	1.13 ± 0.27
	Hydraulic retention time (h)	16	32	23.0 ± 3.6	16	40	29 ± 8
	Transmembrane pressure (bar)	0.8	2.2	1.30 ± 0.23	0.06	0.75	0.39 ± 0.18
	Sludge retention time	Infinite			Infinite		
	Cycle aerated / not aerated (h)	2 / 1			2 / 1		
Operating conditions at the sampling time	**Acclimation to OWW duration (d)**	160			180		
	TSS ($g.L^{-1}$)	4.0			4.1		
	Biomass evolution	growth			stabilisation		
	CODS removal (%)	94			35		
	CODP removal (%)	98			89		
	$N\text{-}NH_4^+$ removal (%)	99			100		

68

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

Antineoplastic	Concentration ($\mu g.L^{-1}$)
5-FU	1300
Carboplatin	600
Etoposide	600
Doxorubicin	400
Cisplatin	400
Cytarabine	200
Methotrexate	150
Gemcitabine	150
Total	**3800**
Antibiotic	**Concentration ($\mu g.L^{-1}$)**
Ticarcillin	1000
Amoxicillin	1000
Ciprofloxacin	1000
Ceftriaxone	1000
Total	**4000**

70

71

72

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

Molecule	$OWW_{average}$ ($\mu g.L^{-1}$)	Corresponding CF	Corresponding removal efficiency (%)	Specific quantity of sorbed pharmaceutical ($\mu g.gTSS^{-1}$)
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

74

75

76

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

Molecule	K_D (L.kgTSS^{-1})	Activated sludge origin	Inhibition technique	Reference
CD	-	**sMBRe – infinite SRT**	**Anaerobic**	**Present study**
	102.1 ± 8.9	**sMBRe WWTP**	**Anaerobic**	**Present study**
	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
CP	**9.6 ± 3.8**	**sMBRe – infinite SRT**	**Anaerobic**	**Present study**
	17.7 ± 4.0	**sMBRe WWTP**	**Anaerobic**	**Present study**
	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
IF	**17.1 ± 2.1**	**sMBRe – infinite SRT**	**Anaerobic**	**Present study**
	15.9 ± 0.6	**sMBRe WWTP**	**Anaerobic**	**Present study**
	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
SM	**34.1 ± 7.3**	**sMBRe – infinite SRT**	**Anaerobic**	**Present study**
	21.4 ± 2.0	**sMBRe WWTP**	**Anaerobic**	**Present study**
	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
	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^{-1}	Yang et al., 2011

78

79

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

Molecule	sMBRe – acclimated to OWW				sMBRe municipal WWTP			
	Total (%)	Biotrans formation (%)	Sorption (%)	$\frac{Biotransformation}{Sorption}$	Total (%)	Biotrans formation (%)	Sorption (%)	$\frac{Biotransformation}{Sorption}$
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

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

82

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

Total removal TSS = 4 $g.L^{-1}$ – t = 4h

Molecule	sMBRe/OWW k_{global} ($\mu g^{1-n}.L^{n-1}.d^{-1}$)	n	sMBRe/municipal WWTP k_{global} ($\mu g^{1-n}.L^{n-1}.d^{-1}$)	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

Biotransformation removal

Molecule	sMBRe/OWW k_{biol} ($\mu g^{1-n}.L^{n-1}.d^{-1}$)	n	sMBRe/municipal WWTP k_{biol} ($\mu g^{1-n}.L^{n-1}.d^{-1}$)	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

84

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

Molecule	sMBRe/OWW t95% max (h)	sMBRe/municipal WWTP t95% bio (h)	sMBRe/OWW t95% max (h)	sMBRe/municipal WWTP 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

87

88

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

91

92

93

(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