Environ Sci Pollut Res (2017) 24:6779–6793
DOI 10.1007/s11356-017-8413-y

RESEARCH ARTICLE

Biodegradability of fluoxetine, mefenamic acid, and metoprolol using different microbial consortiums

Yolanda Flores Velázquez[1] · **Petia Mijaylova Nacheva**[2]

Received: 5 August 2016 / Accepted: 5 January 2017 / Published online: 14 January 2017

Abstract The biodegradation of fluoxetine, mefenamic acid, and metoprolol using ammonium-nitrite-oxidizing consortium, nitrite-oxidizing consortium, and heterotrophic biomass was evaluated in batch tests applying different retention times. The ammonium-nitrite-oxidizing consortium presented the highest biodegradation percentages for mefenamic acid and metoprolol, of 85 and 64% respectively. This consortium was also capable to biodegrade 79% of fluoxetine. The heterotrophic consortium showed the highest ability to biodegrade fluoxetine reaching 85%, and it also had a high potential for biodegrading mefenamic acid and metoprolol, of 66 and 58% respectively. The nitrite-oxidizing consortium presented the lowest biodegradation of the three pharmaceuticals, of less than 48%. The determination of the selected pharmaceuticals in the dissolved phase and in the biomass indicated that biodegradation was the major removal mechanism of the three compounds. Based on the obtained results, the biodegradation kinetics was adjusted to pseudo-first-order for the three pharmaceuticals. The values of k_{biol} for fluoxetine, mefenamic acid, and metoprolol determined with the three consortiums indicated that ammonium-nitrite-oxidizing and heterotrophic biomass allow a partial biodegradation of the compounds, while no substantial biodegradation can be expected using nitrite-oxidizing consortium. Metoprolol was the less biodegradable compound. The sorption of fluoxetine and mefenamic acid onto biomass had a significant contribution for their removal (6–14%). The lowest sorption coefficients were obtained for metoprolol indicating that the sorption onto biomass is poor (3–4%), and the contribution of this process to the global removal can be neglected.

Keywords Biodegradation · Microbial consortiums · Pharmaceutical compounds · Sorption

Responsible editor: Diane Purchase

✉ Petia Mijaylova Nacheva
petiam@tlaloc.imta.mx

Yolanda Flores Velázquez
flveyola@hotmail.com

[1] National Autonomous University of Mexico, Campus IMTA, Paseo Cuauhnáhuac 8532, Progreso, 62550 Jiutepec, Morelos, Mexico

[2] Mexican Institute of Water Technology, Paseo Cuauhnáhuac 8532, Progreso, 62550 Jiutepec, Morelos, Mexico

Introduction

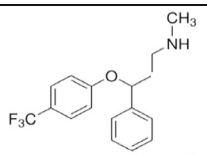
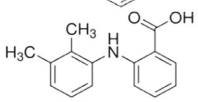
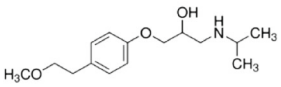
Pharmaceuticals are a class of emerging contaminants that may cause acute and chronic negative effects on aquatic organisms; these effects can take place under low concentrations of ng L^{-1} to μg L^{-1} (Escher et al. 2011; Vulliet and Cren-Olivé 2011). Pharmaceutical compounds have been found in municipal and hospital wastewater, surface water, groundwater, and even in drinking water (Stuart et al. 2012; Birkholz et al. 2014). The compounds identified in the environment belong to several classes such as analgesics/anti-inflammatories, β-blockers, psychiatric drugs, antibiotics, lipid regulators, contrast agents, anti-cancer agents, hormones, and products of contrasts (Deblonde et al. 2011; Martínez et al. 2012). These compounds with their synthetic precursors and transformation products are continually released into the aquatic environment, as a result of their manufacturer and use (via excretion, mainly in urine and feces). The aquatic organisms are captives of their environment and therefore suffer perpetual exposure. Although most pharmaceuticals are designed to target specific metabolic pathways in humans and domestic animals, they can have numerous often unknown effects on metabolic systems of nontarget organisms, especially invertebrates. Despite

some pharmaceuticals often show poor solubility in water, leading to preferential sorption to suspended particles, they can sorb to colloids, and therefore, they can be discharged in the aqueous effluents (Daughton and Ternes 1999). Municipal wastewater treatment plant effluents represent one of the main sources of pharmaceuticals, since conventional wastewater treatment systems are not designed to remove them (Luo et al. 2014). For these reasons, it is necessary to achieve deeper knowledge about the removal of the compounds with high environmental risk within each class. Three pharmaceuticals from different classes of action and with different physicochemical properties were selected for this study: fluoxetine (psychiatric), mefenamic acid (analgesic/anti-inflammatory), and metoprolol (β-blocker). The model compounds were selected on the basis of their widespread use (Tauxe-Wuersch et al. 2005; Deblonde et al. 2011), their toxicological effects on aquatic organisms (Escher et al. 2011; Roos et al. 2012; Verlicchi et al. 2012; Mansour et al. 2016), and their concentrations in the effluents from wastewater treatment plants or in the aquatic environment (Ternes 1998; Miège et al. 2009; Rosal et al. 2010).

Biodegradation and sorption are the most important removal mechanisms of pharmaceuticals in biological treatment processes. Biodegradation has been identified as the major elimination pathway in particular for hydrophilic nonpersistent pharmaceuticals (Ternes and Joss 2006; Majewsky et al. 2011). The compound structure plays an important role in determining the resistance to biodegradation. The persistent micropollutants contain halogen or electron withdrawing functional groups. In contrast, high removal efficiency has been observed with most compounds bearing electron donating functional groups. Nevertheless, the analyses have revealed several exceptions which remained unexplainable given the lack of data about the compounds (Tadkaew et al. 2011). The studies have shown that biodegradation of pharmaceuticals in wastewater is due to cometabolic activity of autotrophic microorganisms, while heterotrophs degrade pharmaceuticals via cometabolism and/or metabolism mechanisms. If the compounds are present only in trace levels, the removal does not result in any significant biomass growth, and the compounds are usually transformed by cometabolism under the supplement of growth substrates (Tran et al. 2013). Moreover, low food to microorganism ratios at a long solid retention time (oligotrophic conditions) may enable microorganisms to use pharmaceuticals as carbon sources (Maeng et al. 2013). High removal rates are achieved at solid retention times (SRT) higher than 10 days; the long SRT allow the enrichment of slow-growing bacteria such as nitrifying bacteria (Clara et al. 2005). The nitrifying activity significantly contributes to the biotransformation of a number of pharmaceuticals; ammonia-oxidizing activity appears as a good indicator for estimation of the biodegradation potential of pharmaceuticals, especially at low concentrations (Dawas et al. 2014; Rattier et al. 2014). Good-nitrifying activities increased the biodegradation of pharmaceuticals like ibuprofen, naproxen, trimethoprim, erythromycin, roxithromycin, and fluoxetine; cometabolic biodegradation seemed to be responsible for the initial biotransformation due to the action of ammonium monooxygenase enzyme, which catalyzes the first step of nitrification and of other enzymes present in nitrifying bacteria, whose activity is also enhanced by the high nitrification rates, can be involved in the biotransformation (Fernandez-Fontaina et al. 2012). However, in other studies, no clear correlation was detected between the SRT applied in the wastewater treatment plants and the elimination of several pharmaceuticals (Joss et al. 2005; Vieno et al. 2007). Some experiments have indicated that high degradation of pharmaceuticals like ibuprofen, ketoprofen, naproxen, fenoprofen, indomethacin, diclofenac, clofibric acid, mefenamic acid, gemfibrozil, carbamazepine, sulfamethoxazole, and paracetamol was observed due to the heterotrophic activity in the bioreactors (Tran et al. 2009; Majewsky et al. 2011; Maeng et al. 2013). Therefore, more studies are still required to explore the role of the different microbial consortiums in the biodegradation process of the pharmaceutical micropollutants. The contribution of autotrophs and heterotrophs in the biotransformation has been evaluated by the addition of inhibitors. Nitrification has been suppressed by the addition of allylthiourea, allylthiourea targets ammonium monooxygenase enzyme, but inhibition of other bacterial activity could be observed which might explain the reduced removal of pharmaceuticals in the presence of this inhibitor (Falås et al. 2012). This is the reason why inhibitors were not used in this study. The sorption of pharmaceutical compounds during the biological wastewater treatment is dependent on their hydrophobicity. The removal by sorption onto suspended solids is an important mechanism for hydrophobic compounds (Poseidon 2004); the sorption increases with hydrophobicity as expected considering the higher sorption of nonpolar compounds on sludge (Rosal et al. 2010). The octanol-water partition coefficient K_{ow} is used as hydrophobicity descriptor, log $K_{ow} < 2.5$ indicates low sorption potential, $2.5 < \log K_{ow} < 4$ indicates medium sorption potential, and log $K_{ow} > 4$ indicates high sorption potential (Rogers 1996). The physical-chemical properties of the selected pharmaceuticals are presented in Table 1.

In general, compounds such as fluoxetine that tend to be sorbed onto solids are expected to be better eliminated by activated sludge processes, while the polar compounds such as metoprolol are more likely to be found in wastewater treatment plants effluents (Luo et al. 2014). While many pharmaceuticals are acids or bases, there are compounds (such as mefenamic acid) that have high K_{ow} values and a dissociation constant pK_a lower than the typical pH of the wastewater; these compounds can be found dissociated in the aqueous phase and not bound to the particles (Sui et al. 2010). That is why the liposome-water distribution coefficient (D_{lipw}) at a

Table 1 Physical-chemical properties of the pharmaceuticals

Compound	Chemical structure	Chemical formula	Molecular weight (g mol^{-1})	Water solubility, 25 °C (mg mL^{-1})	pK$_a$	log K$_{ow}$	log D$_{lipw}$, pH=7 (L kg^{-1})
Fluoxetine		$C_{17}H_{18}F_3NO$	309.13	0.06 [c]	9.8 [f] 10.3 [b]	4.05 [b] 4.17 [f]	3.28 [e]
Mefenamic Acid		$C_{15}H_{15}NO_2$	241.29	0.02 [d]	3.89 [f] 4.48 [e]	5.12 [a] 5.4 [f]	4.16 [e]
Metoprolol		$C_{15}H_{25}NO_3$	267.18	14 [g]	9.67 [f]	1.76 [f] 1.88 [g]	1.25 [e]

References: [a] Jones et al. 2002; [b] Ternes and Joss 2006; [c] Trenholm et al. 2006; [d] Chen et al. 2011; [e] Escher et al. 2011; [f] Kovalova et al. 2012; [g] Grossberger et al. 2014

[a] Jones et al. 2002

[b] Ternes and Joss 2006

[c] Trenholm et al. 2006

[d] Chen et al. 2011

[e] Escher et al. 2011

[f] Kovalova et al. 2012

[g] Grossberger et al. 2014

defined pH value has replaced the K_{ow} as a descriptor for the compound uptake in biological wastewater treatment processes (Escher et al. 2011). The analysis of sorption coefficients data set performed by Sathyamoorthy and Ramsburg (2013) suggested that models employing log K_{ow} or the apparent partition coefficient (K_{ow} corrected to the experimental pH) generally offer insufficient predictive capability. The predictive capability of log D or log K_{ow} based models only becomes meaningful when the percentage of uncharged pharmaceuticals is >99%, and the lack of predictive power of models based upon log D and log K_{ow} highlights the importance of considering the type of solutes and sorbents. The pharmaceutical compounds display a wide range of sorption behaviors which are highly dependent on the chemical characteristics of each compound and on the physicochemical properties of sludge, sediment, or soil particles. The complexity of the sorbate-sorbent interactions means that the approaches developed for predicting the sorption can be inappropriate for use on pharmaceuticals in sludge, because the sorption behavior is influenced by the properties of the system; therefore, the sorption coefficient should be measured for each type of sludge. The water-solid distribution coefficient (K_d) has been used to determine the sorption of pharmaceuticals onto sludge, and it is the key for understanding the mobility of a pharmaceutical through the systems and its availability for degradation (Ternes and Joss 2006). In activated sludge processes, the solid-water distribution coefficient (K_d) is defined as the partition of a compound between the sludge and the water phase at equilibrium. For compounds having a K_d of below 300 L kg^{-1} (log K_d < 2.48), the sorption onto sludge can be considered to be insignificant (Joss et al. 2005). The sorption behaviors vary from compound to compound and are difficult to predict because the sorption is often controlled by interactions with specific functional groups or complicated pH-dependent speciation (Kibbey et al. 2007). A number of hydrophobicity-independent mechanisms such as cation exchange, cation bridging at clay surfaces, surface complexation, and hydrogen bonding appear to be involved. Further, organic matter, pH, cation exchange capacity, clay, and various ions may affect sorption processes (Monteiro and Boxall 2010). The aim of this study was to elucidate the biodegradability of fluoxetine, mefenamic acid, and metoprolol using ammonium-nitrite-oxidizing consortium, nitrite-oxidizing consortium, and heterotrophic bacteria. Biodegradation coefficients were determined for each microbial consortium. Additionally, the removal by sorption onto suspended solids was evaluated, and the solid-water distribution coefficients were determined.

Materials and methods

Microbial consortiums

Three different kinds of microbial consortiums were used to assess the biodegradability of the selected pharmaceutical

compounds: ammonium-nitrite-oxidizing consortium, nitrite-oxidizing consortium, and heterotrophic bacteria. The consortiums were enriched from a nitrifying-denitrifying reactor activated sludge using different feed strategies. Three sequencing batch reactors (SBR) with effective volume of 10 L each one were used during the enrichment. The ammonium-nitrite-oxidizing biomass was obtained feeding one of the reactors with ammonium sulfate as energy source, the nitrite-oxidizing biomass was obtained feeding the second reactor with sodium nitrite, and the heterotrophic biomass was obtained feeding the third reactor with methanol as energy and carbon source. The autotrophic bacteria use an inorganic carbon source; thus, sodium bicarbonate was supplied to the first two reactors. Each reactor was inoculated with 10 L of activated sludge (MLVSS of 2000 mg L^{-1}). The operation cycles lasted 24 h (23 h of aeration and 1 h for sedimentation, water discharging and feeding). The reactors were operated without sludge extraction in order to avoid biomass losses. The compositions of the synthetic wastewater and the quantity of each compound used for the enrichment of the three consortiums are shown in Table 2 (no pharmaceuticals were added). The ammonium concentration was gradually increased from 100 to 350 mg NH_4-N L^{-1} in the ammonium-nitrite-oxidizing reactor during the enrichment. The nitrite concentration was increased from 250 to 495 mg NO_2-N L^{-1} in the nitrite-oxidizing reactor. In the case of ammonium-oxidizing consortium, the alkalinity decreases during the nitrification process; 7.14 mg of alkalinity (as $CaCO_3$) is used per milligram of ammonium ions oxidized to nitrite. Thus, the required amount of sodium bicarbonate was added in order to avoid pH decreases in the reactor. To avoid nitrification in the reactor with heterotrophic bacteria, the dissolved oxygen (DO) concentration was kept below 2 mg L^{-1}, while the OD was kept above 3 mg L^{-1} in the reactors with ammonium-nitrite-oxidizing and nitrite-oxidizing consortiums, as maximum nitrification occurs at DO of almost 3 mg L^{-1} (EPA 2002; Gerardi 2002).

The enrichment of the three consortiums was inferred through ammonium-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), and heterotrophic bacteria activity measurements. In order to allow the enrichment of the consortiums, the reactors were operated until the process stabilization was achieved, as indicated by a constant percentage removal (greater than 90%) of NH_4-N, NO_2-N, and chemical oxygen demand (COD). Once stabilization was obtained, the biomass was used for the biodegradability tests. To determine the biomass activity, the ammonium nitrogen (NH_4-N) and nitrite nitrogen (NO_2-N) uptake, as well as NO_2-N and nitrate nitrogen (NO_3-N) production were measured in both ammonium- and nitrite-oxidizing reactors. COD removal was determined in the heterotrophic reactor. Total suspended solids (TSS) and volatile suspended solids (VSS) were determined once a week in all the reactors; pH and temperature were measured daily.

Biodegradability tests and kinetics

The enriched ammonium-nitrite-oxidizing, nitrite-oxidizing, and heterotrophic biomasses were used to perform the fluoxetine, mefenamic acid, and metoprolol biodegradability tests. Pharmaceuticals were added to the synthetic wastewater to

Table 2 Composition of the synthetic wastewater

Compound	Ammonium-nitrite oxidizing[e] Concentration (mg L^{-1})	Nitrite oxidizing[b, c, d]	Heterotrophic[a]
CH_3OH	–	–	850
$(NH_4)_2SO_4$	500–1600	–	200
$NaNO_2$	–	350–750	–
$NaHCO_3$	1000–3500	250	–
KH_2PO_4	200	300	100
K_2HPO_4	200	300	100
$MgSO_4$ $7H_2O$	90	125	50
$FeSO_4$ $7H_2O$	3	10	10
$CaCl_2$ $2H_2O$	40	80	50
EDTA $2H_2O$	5	10	5
$ZnSO_4$	0.5	0.5	0.5
$CuSO_4$	3	2.5	–

[a] Li and Bishop 2004

[b] Spieck and Bock 2005

[c] Nogueira and Melo 2006

[d] Spieck and Lipski 2011

[e] Torres and Buitrón 2012

obtain concentrations of 10 μg L^{-1} for fluoxetine, mefenamic acid, and metoprolol. The compounds mefenamic acid and metoprolol were dissolved in methanol and the fluoxetine in acetone, stirring the solutions during 5 min at 25 °C to create a stock solution of 1000 μg mL^{-1}, which was subsequently frozen and used to spike into the synthetic wastewater. The biodegradability tests were conducted in batch mode using 500-mL amber bottles to prevent photolysis. Each bottle was filled with 450 mL of sterilized synthetic wastewater (Table 2) and 50 mL of biomass (inoculum), obtaining a biomass concentration of almost 400 mg VSS L^{-1}. The bottles were incubated at 30 °C in an orbital shaker agitated at 80 rpm to facilitate oxygen transfer into the liquid. Ten degradation times were evaluated (ten tests in duplicate) collecting the samples at intervals of 48 h during the first 12 days and every 96 h during the next 12 days. Two bottles were used for sampling and analysis for each degradation time, and the averages were reported. Finally, mass balance was performed considering the concentration of pharmaceuticals in the liquid and solid (biomass) phases. Three controls were prepared (in duplicate) for each biomass: (1) synthetic wastewater with pharmaceutical compounds, (2) synthetic wastewater (without test substance) and biomass to ensure microbial activity, and (3) inactive biomass and pharmaceutical compounds (sorption controls). To assess the activity of the biomass, NH_4-N, NO_2-N, NO_3-N, and COD were determined in addition of the pharmaceuticals analysis. Ammonium nitrogen and nitrite consumption, as well as nitrites and nitrates production were calculated in the tests with ammonium-nitrite-oxidizing and nitrite-oxidizing biomasses. Biodegradation rate based on COD was obtained for the heterotrophic biomass.

A pseudo-first-order kinetic model was used to describe the removal of the pharmaceuticals in this study. The term "pseudo" refers to its proportionality to the sludge concentration (Joss et al. 2006; Tran et al. 2009; Suarez et al. 2010; Dawas et al. 2014), and the compound concentration decrease can be expressed:

$$\frac{dC}{dt} = -k_{\text{biol}} X_{VSS} C,$$

where C is the compound concentration (μg L^{-1}), t is the time (d), k_{biol} is the reaction rate constant (L $gVSS^{-1}$ d^{-1}), and X_{VSS} is the volatile suspended solids concentration (gVSS L^{-1}). The results obtained from the biodegradability tests were adjusted to pseudo-first-order graphically, by plotting -ln (C/Co) versus t, the data were linearly regressed and R^2 values were calculated. In order to determine k_{biol}, each slope was divided by the VSS concentration. The amounts of the pharmaceuticals were measured in the liquid and solid phase (biomass) at the different times, after which the mass balance was performed and the graphics were done considering the biodegradation of fluoxetine, mefenamic acid, and metoprolol. The pseudo-first-order approach is frequently used to model the biodegradation of micropollutants despite the fact that this model dismisses the effect of biomass activity and does not link micropollutants with primary substrate degradation (Urase and Kikuta 2005; Joss et al. 2006; Helbling et al. 2012; Dawas et al. 2014; Fernandez-Fontaina et al. 2012, 2014, 2016). Fernandez-Fontaina et al. (2014) applied cometabolic Monod-type kinetic model for the biotransformation of organic micropollutants by nitrifying biomass, comparing its accuracy with a pseudo-first-order kinetic model. The cometabolic model was able to fit for ibuprofen and naproxen better, regardless of fitting the experimental results to different k_{biol} in each of the kinetic assays. On the contrary, erythromycin, roxithromycin, galaxolide, tonalide, fluoxetine, and sulfamethoxazole were best fitted by the pseudo-first-order kinetics.

Determination of the sorption coefficient (K_d)

Sorption was assessed for each type of biomass. The sorption equilibrium time was determined as the amount of solute sorbed per unit mass of sorbent at equilibrium state. The equilibrium is reached when the sorption and desorption rates are equal. The biomass was inactivated by adding 200 mg L^{-1} of Hg_2SO_4, and the exposure time was of 24 h. After this time, the biomass was washed several times with water to eliminate the toxic compound. The tests were carried out in duplicate using 500 mL amber bottles, the media contained 0.01 M of $CaCl_2$, 1 g L^{-1} TSS, and 10 μg L^{-1} of the pharmaceuticals, the bottles were agitated at 120 rpm by an orbital shaker, and the concentrations were measured in liquid and solid phase after defined exposure times (1, 3, 6, 12, 24 and 48 h). The equilibrium was indicated by the constant values of the compound over time. Once the equilibrium time was determined, the sorption isotherms were obtained at 30 °C using the inactivated biomass. Amber bottles were used and the media contained 0.01 M of $CaCl_2$, 1 g L^{-1} TSS, and six different concentration of the pharmaceuticals (0.5, 1, 2.5, 5, 7.5 and 10 μg L^{-1}). The tests were carried out in duplicate, the bottles were mixed at 120 rpm with an orbital shaker for 24 h, and the liquid samples were analyzed. The amount of the compound sorbed was calculated by the difference between the initial amount of compound in solution and the amount at the end of the experiment. The amount of pharmaceutical compound sorbed per gram of TSS at the equilibrium state can be calculated by the equation:

$$q_e = \frac{x}{m} = \frac{(C_0 - C_e)V}{m},$$

where x is the mass sorbed (μg), m is the sludge weight (g), V is the volume of the solution (L), C_0 is the initial concentration (μg L^{-1}), and C_e is the equilibrium concentration of contaminant in solution (μg L^{-1}).

The sorption isotherms of pharmaceuticals were determined with the Freundlich isotherm model:

$$q_e = \frac{x}{m} = K_f \cdot C_e{}^{1/n},$$

where K_f is the Freundlich sorption capacity parameter ($\mu g_{compound}\ g^{-1}{}_{sludge}$) ($L_{water}\ \mu g^{-1}{}_{compound}$)$^{1/n}$, and $1/n$ is the Freundlich sorption intensity parameter (unitless). When the value of n is 1, Freundlich model turns into a simple linear sorption model which describes the sorption as partitioning between the liquid and solid phases (Zhao et al. 2008). The linear isotherm is valid for the dissolved species that are present at a concentration of less than one half of its solubility; it is applicable only at low concentrations like the pharmaceutical compounds. The linear sorption isotherm is expressed as

$$q_e = K_d \cdot C_e$$

The slope of the linear isotherm is the distribution coefficient K_d.

Analytical methods

The concentrations of the pharmaceutical compounds were determined by gas chromatography using Varian gas chromatography CP-3800, fitted with a 30-m HP5-MS (5% phenyl, 95% dimethylpolysiloxane) fused silica capillary column (30 m × 0.25 mm, 0.25-μm film thickness), and connected to a Varian Saturn 2200 ion trap tandem mass spectrometer. The carrier gas was helium. The gas chromatography–mass spectrometry method was developed and validated for the simultaneous detection of the three pharmaceutical compounds (fluoxetine, mefenamic acid, and metoprolol) in liquid and solid phase. Solid phase extraction was used to concentrate the pharmaceutical compounds and remove interfering substances, and the compounds were extracted on Oasis HLB cartridges with hydrophilic-lipophilic balance (lipophilic divinylbenzene + hydrophilic N-vinyl pyrrolidone), 200 mg sorbent per cartridge, and 30-μm particle size. Cartridges were conditioned with 10 mL of methanol and 10 mL of water (HPLC grade), and the sample was passed through the cartridge by a vacuum manifold. Then, the remaining interfering components were washed from the adsorbent with 4 mL of methanol-water solution (5:95, *v*/*v*). To eliminate wetness, the cartridges were dried by negative pressure air flow during 3 h. The analytes were eluted with 4 mL of methanol. Finally, the eluted extract was concentrated under a gentle nitrogen stream for a subsequent derivatization. The analytes were derivatized by silylation using *N*,*O*-Bis(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane, 100 μL of the derivatizing agent was used, and the sample was heated at 80 °C during 60 min. After this drying, the sample was reconstituted with 1 mL of toluene to be analyzed. To quantify pharmaceuticals sorbed onto biomass, biomass was dried by lyophilization, after which methanol was added, the sample was sonicated during 20 min, and the obtained supernatant was filtered and collected in a round bottom flask. The same procedure was done three times, and the supernatants were concentrated in a rotating evaporator until there was 1 mL remaining. Finally, the sample was reconstituted to 350 mL with HPLC grade water to follow the same method for aqueous samples. The samples of pharmaceuticals in liquid and solid phases were taken in duplicate, and the average was reported. Standards and sample concentrates were injected using a Varian CP-8400 (Switzerland) automatic sample injector. During the qualitative analysis, the characteristic ions were fluoxetine (44), mefenamic acid (223), and metoprolol (72). In terms of operating conditions selected, the injected volume was 1 μL in splitless mode (which means that the whole 1 μL was used for the analysis) at injection temperature of 260 °C and a flow rate of 1 mL min^{-1}. The temperature ramping allows to separate the different analytes without the risk of breaking them down. The temperature ramping was initiated at 150 °C for 2 min, then it was increased at a rate of 10 °C min^{-1} up to 250 °C, and finally the temperature was elevated at 15 °C min^{-1} up to 290 °C, and as a result, a runtime of 14.67 min was obtained. The retention time for mefenamic acid was of 12.291 min, while fluoxetine retention time was 9.270 min and 11.135 min for metoprolol. The following conditions for the mass spectrometer operation were chosen: electron impact (EI)- ionization at 70 eV, ion trap temperature of 250 °C, multiplier voltage of 180 V, and selective ion monitoring (223 + 44 + 72).

The analytical methods for fluoxetine, mefenamic acid, and metoprolol determination were validated using standard solutions in liquid and solid phases. Calibration curves, response linearity, sensitivity, limit of detection and quantification, recovery, and precision of the analytical procedure were calculated. Validation was done using seven replicates at a concentration of 0.01 μg L^{-1} in liquid phase. High recoveries of pharmaceuticals were observed after solid phase extraction, mean recoveries of the three compounds were greater than 98%, the acceptance criteria must be 70–130% of the true value for each analyte, and thus, the acceptance criteria were fulfilled. This means that the method is accurate, and OASIS HLB cartridges are suitable for the retention of these compounds. On the other hand, the validation in solid phase was done using seven replicates at a concentration around 0.1 μg g^{-1}. Mean recoveries in biomass were also within the acceptance criteria (greater than 90%). The recoveries and quantification limits in water and biomass are presented in Table 3. The relative standard deviations (variation coeffi-cients) of the results of the seven replicates were less than 7% in liquid and solid phase, it must be less than 20%, and thus, the acceptance criteria were fulfilled and the values indicated that the method is precise. The quantification limits were lower than 0.17 μg L^{-1} in the liquid phase and lower

Table 3 Detection limits, accuracy, and precision of the analytical method

Compound	Ion	Recovery (%) Water	VC Water	DL (μg L^{-1}) Water	QL (μg L^{-1}) Water	Recovery (%) Biomass	VC Biomass	DL (μg g^{-1}) Biomass	QL (μg g^{-1}) Biomass
Fluoxetine	44	101 ± 6	6.36	0.002	0.017	99 ± 6	6.89	0.028	0.181
Mefenamic acid	223	109 ± 1	1.38	0.0006	0.012	90 ± 4	4.63	0.017	0.143
Metoprolol	72	99 ± 6	6.44	0.002	0.016	104 ± 5	5.12	0.021	0.164

DL detection limit, *QL* quantification limit, *VC* variation coefficient

than 0.181 μg g^{-1} in the solid phase (suspended biomass), while the detection limits were lower than 0.002 μg L^{-1} and 0.028 μg g^{-1}, respectively. The results summarized in Table 3 indicated that the methods allow accurate, precise, and reliable determination of the three compounds.

To assess the process performance in the reactors, in addition of the fluoxetine, mefenamic acid, and metoprolol analysis, the parameters NH_4-N, NO_2-N, NO_3-N, COD, TSS, and VSS had been followed, and their determination was done according to the standard methods (APHA 2012).

Results and discussion

Enrichment of the microbial consortiums

The biomass performance of the three microbial consortiums and the variation of the VSS in the reactors during the enrichment process are illustrated in Fig. 1. As it can be seen, the concentration of the VSS decreased almost 37–40% in the ammonium- and nitrite-oxidizing reactors during the first 50 days of operation, which can be attributed to the endogenous decay of the heterotrophic population. The ammonium and nitrite removals increased over time in both reactors. The ammonium-nitrite-oxidizing reactor was operated for 85 days. Ammonium removals higher than 91% were obtained after the day 74 from the start-up. The ammonium uptake rate was 0.25 ± 0.03 gNH_4-N $gVSS^{-1}$ d^{-1}, the nitrite and nitrate production rates were 0.04 ± 0.01 gNO_2-N $gSSV^{-1}$ d^{-1} and 0.18 ± 0.01 gNO_3-N $gSSV^{-1}$ d^{-1}, respectively, and VSS concentration was 1233 ± 23 mg L^{-1} during the last 8 days. The nitrite-oxidizing reactor was operated for 82 days; nitrite removals higher than 92% were obtained after the day 59 from the start-up. The nitrite uptake rate was 0.37 ± 0.03 gNO_2-N $gVSS^{-1}$ d^{-1}, the nitrate production rate was 0.29 ± 0.01 gNO_3-N $gSSV^{-1}$ d^{-1}, and the VSS concentration was 1118 ± 33 mg L^{-1} during the last 23 days. The obtained results confirmed the success of the feeding strategy applied for consortium enrichment in both reactors.

The heterotrophic reactor was operated during 42 days (Fig. 1c). The VSS concentration decreased during the first week, after which a gradual increase was observed. Removals of COD higher than 92% were obtained after 2 weeks of operation. The COD and NH_4-N concentration were 1254 ± 28 mg L^{-1} and 40 ± 3 mg L^{-1}, respectively in the fed synthetic water. The COD removal was 96.2 ± 1.8% with a removal rate of 0.68 ± 0.03 gCOD $gSSV^{-1}$ d^{-1} during the days 14–42 from the start-up. More than 99% of the ammonia nitrogen was removed in the reactor. However, nitrites and nitrates were not found in the effluent which indicates that the ammonia was removed through bio-assimilation. The biomass production was calculated of 0.1 $gVSS\ gCOD_{removed}$, lower than the typical values for aerobic bioreactors, which can be explained with a growth limitation caused by the low NH_4-N in the influent. Nitrifying bacteria are very sensitive to pH, *Nitrosomonas* has an optimal pH between 7.0 and 8.0, and the optimum pH range for *Nitrobacter* is 7.5 to 8.0 (EPA 2002). The average pH of the synthetic water fed to the

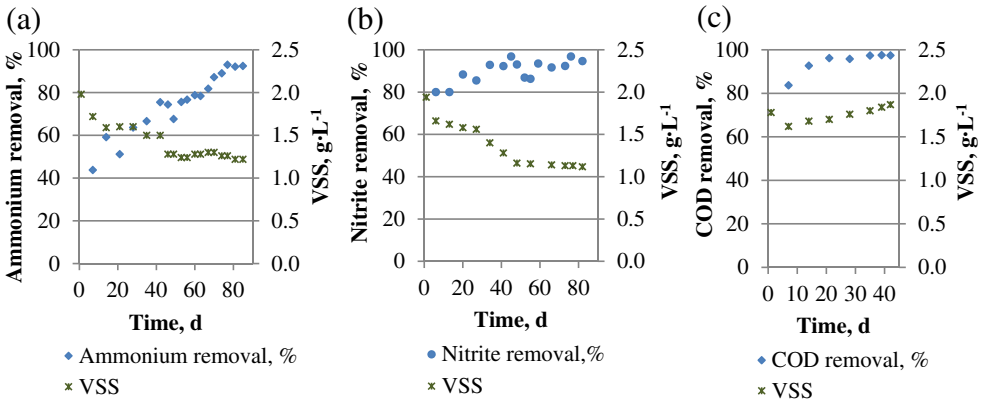


Fig. 1 Biomass performance in the three sequencing batch reactors: **a** Ammonium-nitrite-oxidizing reactor; **b** Nitrite-oxidizing reactor; **c** Heterotrophic reactor

ammonium-nitrite-oxidizing reactor was of 7.54 ± 0.42; pH decreased during the process to a pH of 6.11 ± 0.96. For the nitrite-oxidizing reactor, the pH of the fed synthetic water was of 7.36 ± 0.19 and of 8.21 ± 0.18 in the effluent. There was not a significant change of the pH values during the biodegradation process in the heterotrophic reactor; the averages were of 6.89 ± 0.18 and 6.58 ± 0.19 in the influent and effluent, respectively.

The three reactors were operated at room temperature (21–30 °C). Most strains of nitrifying bacteria grow optimally at temperatures between 25 and 30 °C, and the temperature growth range of *Nitrosomonas* is 5–30 °C and 5–40 °C for *Nitrobacter* (Gerardi 2002). The growth rate of heterotrophic aerobic microorganisms is constant at high temperatures (30–35 °C), and it starts to decline between 35 and 40 °C (Henze et al. 2002).

Biodegradability and kinetics

The fluoxetine, metoprolol, and mefenamic acid concentrations obtained at each degradation time of the biodegradability tests with the three microbial consortiums are presented in Fig. 2, in liquid and solid phases. The global removals of the compounds, attributed to biodegradation and sorption in the biomass, are illustrated in Fig. 3, as well as the removals of NH_4-N, NO_2-N, and COD. As it can be seen, the removals increased over time and the removal rates decreased after 16 days of retention time. The highest removals of metoprolol and mefenamic acid were obtained with the ammonium-nitrite-oxidizing consortium, followed by the heterotrophic one. The fluoxetine removals reached with both consortiums were similar. The lowest removals of the three compounds were obtained with the nitrite-oxidizing consortium.

The specific quantity of the compounds in the biomass was determined in all the tests, and the variations of the values obtained at different retention times were very small. The average of fluoxetine-specific quantity was 153% higher than the mefenamic acid one and 317% higher than the metoprolol one in ammonium-nitrite-oxidizing microbial consortiums. The averages of fluoxetine and mefenamic acid specific quantities were similar for the heterotrophic and nitrite-oxidizing microbial consortiums, but they were almost twice higher compared to the average metoprolol-specific quantities in both consortiums. The obtained results are in accordance with the physical-chemical properties of the pharmaceuticals represented by the reported values of K_{ow}, pK_a, and log D_{lipw} for each compound. The mass balance performed with the obtained results allowed to determine the individual contribution of each process, biodegradation, and sorption, in the global compound removal. The percentages of each process over time are presented in Fig. 4 for the three microbial consortiums.

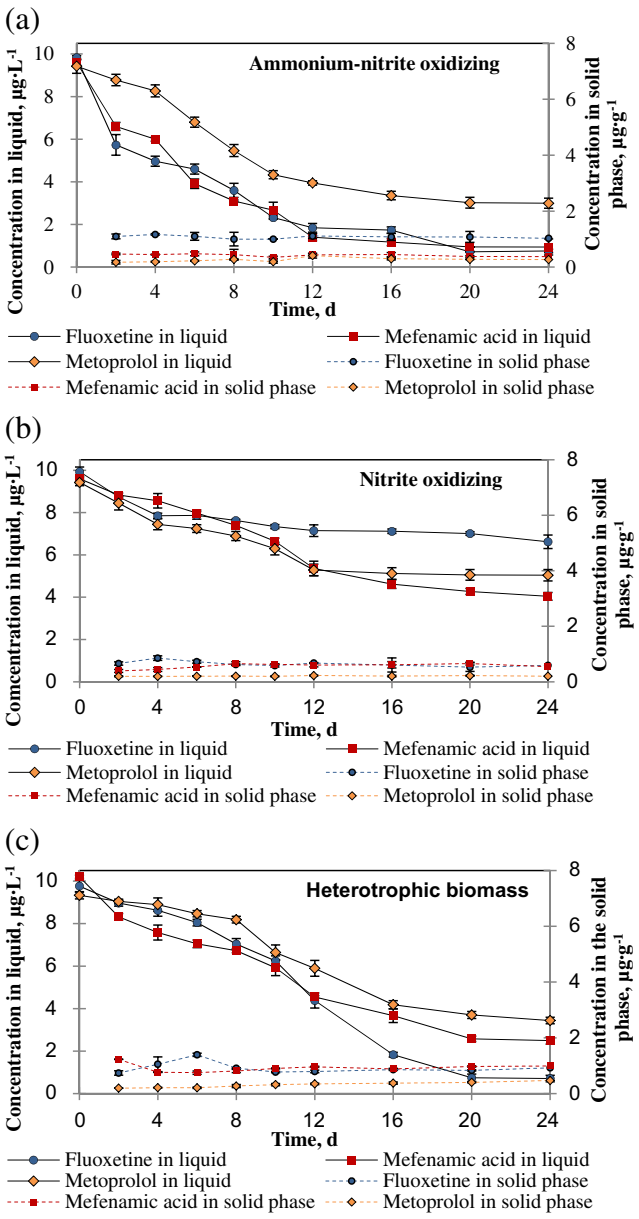


Fig. 2 Concentrations of the pharmaceutical compounds in liquid (µg L^{-1}) and in solid phases (µg g^{-1}) during the tests with: **a** Ammonium-nitrite-oxidizing, **b** Nitrite-oxidizing, and **c** Heterotrophic biomass

The ammonium-nitrite-oxidizing consortium presented the highest biodegradation and the lowest sorption in the biomass of mefenamic acid and metoprolol (Fig. 4); biodegradations of 85% (18.36 µg $gVSS^{-1}$) and 64.5% (13.68 µg $gVSS^{-1}$), respectively, were reached, while the sorption percentages were relatively low, of 5.8 ± 0.6% and 3.6 ± 1%, respectively. The NH_4-N removal at the retention time of 24 days was 59.5% (0.22 g $gVSS^{-1}$), and the NO_2-N and NO_3-N productions were 0.03 and 0.15 g $gVSS^{-1}$, respectively, which indicated a good nitrification activity of the biomass. The ammonium-nitrite-oxidizing consortium was also capable to biodegrade 78.6% of fluoxetine (17.38 µg $gVSS^{-1}$); however, the

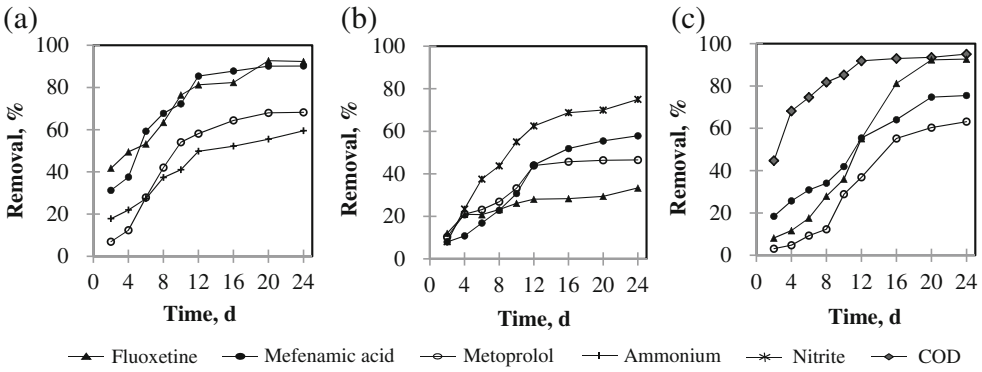


Fig. 3 Removals of pharmaceuticals obtained using different microbial consortiums: **a** Ammonium-nitrite-oxidizing biomass; **b** Nitrite-oxidizing biomass; **c** Heterotrophic biomass

sorption in the biomass was relatively high (14.3 ± 0.7%). Ammonium-nitrite-oxidizing consortium exhibited relatively fast removal of the three compounds during the first 12 days, and the removal rates decreased when the removal rate of NH_4-N decreased (Fig.5a). These results are consistent with the previous publications, and Tran et al. (2009) found that nitrification enhanced the biotransformation of pharmaceuticals, and that the removal of persistent pharmaceuticals was improved with the increase of the nitrification activity of the biomass. Fernandez-Fontaina et al. (2012) found that good-nitrifying activities increased the biodegradation rate of fluoxetine with ammonium monooxygenase enzyme as the main responsible of the cometabolic biodegradation; however, no correlation was seen between biodegradation kinetic constant (k_{biol}) of fluoxetine and specific nitrification rate. In the case of mefenamic acid, Falås et al. (2012) observed a clearly positive trend between the nitrification capacity and the rate constants in carrier reactors; this correlation was restricted to the carrier biomass, and the low rate constants in nitrifying activated sludge suggest a heterotrophic degradation of mefenamic acid.

As it can be seen in Figs. 3 and 4, the heterotrophic consortium had also a good performance; this consortium exhibited the highest ability to biodegrade fluoxetine reaching 84.7% (18.61 μg $gVSS^{-1}$), while the sorption percentage was 8.8 ± 1.9%. The heterotrophic bacteria were also capable of biodegrading 66.4 and 58.4% of mefenamic acid and metoprolol, respectively, and the sorption percentages were 8.5 ± 1.3% and 3.1 ± 1%, respectively. At the end of the test, the removal of organic matter as COD was 95.1%. The removal rates of the pharmaceuticals and the COD obtained using the heterotrophic bacteria are presented in (Fig. 5c). In contrast to the observed correlation with the ammonium removal rate, the pharmaceutical removal rates increased when the organic matter had been consumed by the heterotrophic bacteria (retention time of 12 days), and the COD removal rates decreased. When the primary substrate is available in low quantities, the transformation rate of the secondary substrates (the micropollutants) and the active cells in the biomass remains constant and does not yield energy for growth. Under these conditions, the degradation of the secondary substrates is essentially due to cometabolism (Rieger et al. 2002). The heterotrophic microorganisms are also capable to use pharmaceutical compounds as the sole carbon or energy sources (Tran et al. 2013).

The nitrite-oxidizing consortium presented the lowest biodegradation of the three pharmaceuticals (less than 48%), although the removal of NO_2-N was 75% (0.75 g $gVSS^{-1}$) and the NO_3-N production was 0.71 g $gVSS^{-1}$, which indicated a good biomass activity. The biodegradation percentages obtained for mefenamic acid and metoprolol were 47.6% (10.3 μg $gVSS^{-1}$) and 42.7% (9.1 μg $gVSS^{-1}$), respectively,

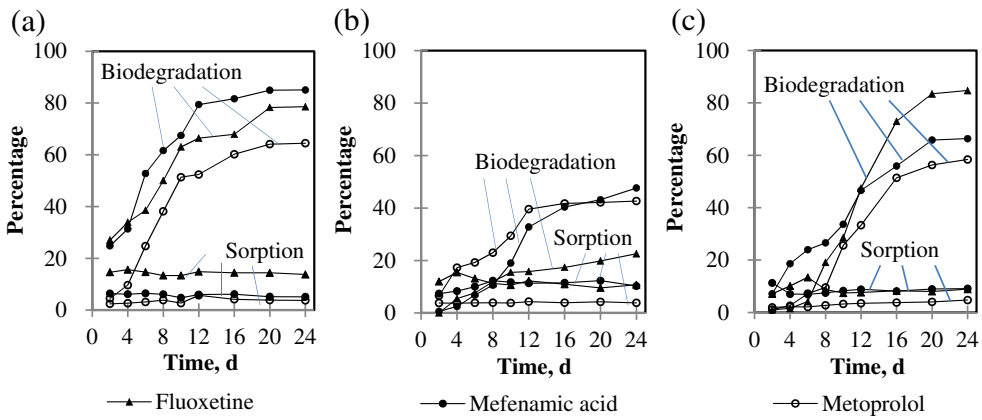


Fig. 4 Biodegradation and sorption percentages obtained using the different microbial consortiums: **a** Ammonium-nitrite-oxidizing biomass; **b** Nitrite-oxidizing biomass; **c** Heterotrophic biomass

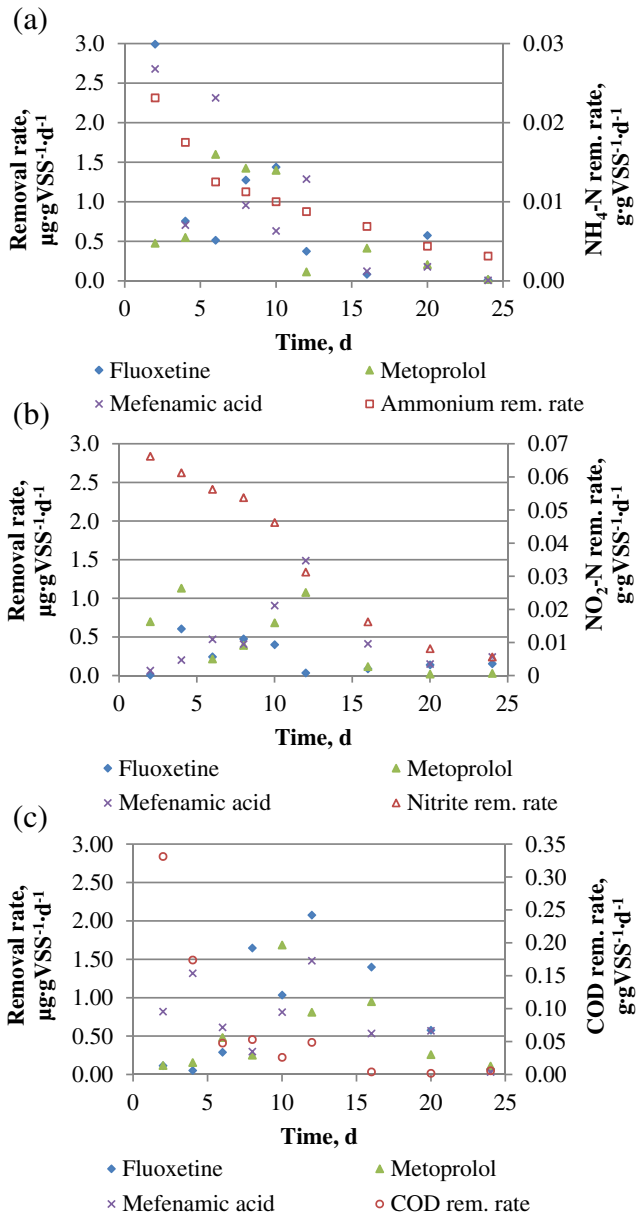


Fig. 5 Pharmaceutical removal rates obtained with the different consortiums and comparison with the ammonium, nitrite, and COD removal rates: **a** Ammonium-nitrite-oxidizing biomass; **b** Nitrite-oxidizing biomass; **c** Heterotrophic biomass

which is 15–37% lower than those found with the rest of the studied consortiums at a retention time of 24 days. Sorption percentages were 10.6 ± 1.7% and 3.9 ± 0.2%, respectively. Fluoxetine biodegradation was very scarce with the nitrite-oxidizing consortium, of only 22.6% (5.0 μg $gVSS^{-1}$), and the sorption percentage was 11.8 ± 1.7%. The removal rates presented in (Fig. 5b) indicated that there was not a clear correlation between the pharmaceutical removal rates and nitrite oxidation rates, and that the pharmaceutical removal rates are significantly lower than those obtained with the other two consortiums. The pharmaceuticals fluoxetine and mefenamic acid were more biodegradable than the metoprolol; 85% of biodegradation was reached for both compounds using heterotrophic consortium for the fluoxetine and ammonium-nitrite-oxidizing consortium for the mefenamic acid. The highest biodegradation percentage for metoprolol, of 64%, was obtained with the ammonium-nitrite-oxidizing consortium. The biodegradation of pharmaceuticals can be attributed to cometabolic activities of both autotrophic and heterotrophic microorganisms. The heterotrophic microorganisms degrade pharmaceuticals via metabolism and/or cometabolism, and the metabolism is possible at high pharmaceutical concentration levels. When the pharmaceuticals are present at trace concentration levels, the biodegradation can be attributed to cometabolic activities. Unlike heterotrophs, the autotrophic microorganisms can only cometabolize the pharmaceutical compounds in the presence of a growth substrate. Autotrophic ammonia-oxidizing bacteria (AOB) cometabolize a variety of pharmaceuticals via the non-specific enzymes, such as ammonium monooxygenase enzyme (AMO) (Tran et al. 2013). In the presence of oxygen, ammonium nitrogen is transformed into hydroxylamine (NH_2OH) by the action of AMO through a hydroxylation reaction. This step requires the reducing power that is regenerated as NH_2OH is oxidized to NO_2 by the hydroxylamine oxidoreductase enzyme. So, both enzymes are codependent, since they generate the substrate and electrons, respectively. In the same way, AMO is able to utilize elemental oxygen to introduce the hydroxyl function in the pharmaceutical compounds (Yi and Harper 2007). In the absence of ammonium nitrogen, hydroxylamine is not produced and the reductant is provided by endogenous respiration. Endogenous respiration of AOB could provide enough reductant to support the biotransformation of organic micropollutants in the absence of ammonium. However, on the long term, ammonium nitrogen must be provided to support turnover of microorganisms (Fernandez-Fontaina et al. 2016). The studies have shown that the main transformation products of pharmaceuticals like 17α-ethinylestradiol and ibuprofen are products of hydroxylation (Quintana et al. 2005; Yi and Harper 2007; Ferrando-Climent et al. 2012). The attack by the reactive oxygen of AMO mostly occurs against an N–H bond, a C–H bond, or a double bond. Two other reactions have been observed: a dehydrogenation/oxidation and a reductive dehalogenation (Forrez et al. 2011).

The hydroxylated intermediaries may serve as a substrate for the heterotrophic organisms, and further degradation of some micropollutants could be done if previous hydroxylation has been occurred (Yi and Harper 2007). The biotransformation by heterotrophic bacteria can involve multiple enzymatic reactions, including ring cleavage and mineralization (Khunjar et al. 2011). The studies have suggested the transformation of mefenamic acid to mono-hydroxylated mefenamic acid (3′-hydroxymethylmefenamic acid, 3′-carboxymefenamic acid) and di-hydroxylated mefenamic acid (3′-hydroxymethyl-5′-hydroxymefenamic acid, 3′-hydroxymethyl-6′-hydroxymefenamic acid) (Hata et al. 2010). In the

11case of metoprolol, two transformation products of hydroxylation have been identified under aerobic conditions: 4-2-hydroxy-3-(isopropylamino) propoxy) phenol and 1-(4-(2-hydroxy-3-(isopropylamino) propoxy) phenyl)-2-methoxyethanone. The fragmentation pathways of these transformation products include the N-dealkylation corresponding to a loss of the isopropyl group, oxidation to benzaldehyde, dehydrogenation and ether cleavage, and producing a phenol derivative and an aldehyde. The metoprolol acid (oxidation to carboxylic acid) and O-desmethylmetoprolol (O-demethylation) are another transformation products (Rubirola et al. 2014). It is known that the aerobic degradation of compounds with an aromatic-aliphatic ether fragment like fluoxetine and metoprolol can proceed by ether cleavage (O-dealkylation) (Tadkaew et al. 2011).

The fluoxetine, mefenamic acid, and metoprolol posses50s a secondary amine. Two principal initial oxidative reactions at the amine functional group have been observed for the activated sludge system, namely, (1) N-oxidation and (2) α-C-hydroxylation followed by N-dealkylation, oxidation to amide, or dehydration to iminium species. Additionally, several N-acylation reactions, namely, N-formylation, N-acetylation, N-propionylation, N-malonylation, N-succinylation, and presumably desaturation to N-fumarylated products have been observed. The N-oxidation reactions involve the direct introduction of an oxygen atom on the nitrogen of the amine functional group, resulting in hydroxylamine products for secondary amines. In addition, the N-hydroxylated products of secondary amines are known to be further transformed to products such as nitrones. It was suggested that especially the N-acetylation, N-formylation, and N-succinylation transformation products are formed directly. For the secondary amine of fluoxetine, the N-dealkylation, N-acetylation, or the combination of N-demethylation, N-propionylation, and N-succinylation reactions are possible. The reactions that do not involve the amine functional groups are O-demethylation, hydroxylation at groups other than the amine-N or the α-C, desaturation, oxidation of a hydroxylated product to a carbonyl or carboxylic acid product, dehydration of an aliphatic alcohol to an alkene, and combinations of several reactions including the decarboxylation involving the amine functional group (Gulde et al. 2016). The sorption percentages determined for the studied pharmaceuticals in the three consortiums were in accordance with the values for log K_{ow} and log D_{lipw} reported for these compounds (Jones et al. 2002; Ternes and Joss 2006; Escher et al. 2011; Kovalova et al. 2012; Grossberger et al. 2014). The lowest sorption percentages (3.1–3.9%) were determined for metoprolol, which has the lowest log K_{ow} and log D_{lipw} values; unfortunately, this was also the less degradable pharmaceutical compound. The sorption percentages were 5.8–14.3% for fluoxetine and mefenamic acid, and the sorption was lower in the consortiums which allowed higher biodegradation rates. The results of the abiotic control experiments indicated that the concentrations of the three pharmaceuticals remained relatively constant over the experimental period of 24 days (the decreases were less than 4%), and that the biomass activity was not inhibited by the presence of pharmaceuticals. Finally, the sorption controls using inactive biomass showed that less than 20% of the three pharmaceuticals were sorbed. Linear regression was applied using the data for biodegraded compound quantities to adjust the kinetics to pseudo-first-order model. The calculated R^2 values were in the range of 0.91–0.98, and the kinetic biodegradation constants were obtained dividing the slope by the biomass concentration. The results obtained for each consortium are presented in Table 4. The highest biodegradation constant (k_{biol}) for fluoxetine was obtained using heterotrophic biomass, followed by the one using ammonium-nitrite-oxidizing biomass. An inverse relationship was obtained for mefenamic acid and metoprolol, and the highest k_{biol} were determined with ammonium-nitrite-oxidizing consortium. The lowest biodegradation constants for the three pharmaceuticals were found using the nitrite-oxidizing consortium.

Joss et al. (2006) and Poseidon (2004) proposed to classify the organic micropollutants in three groups according to their

Table 4 Biodegradation constants in the different microbial consortiums

Compound	k_{biol} (L gVSS^{-1} d^{-1}) Biomass			k_{biol} Literature (L gSS^{-1} d^{-1})
	Ammonium-nitrite-oxidizing	Nitrite-oxidizing	Heterotrophic	
Fluoxetine	0.188	0.03	0.244	0.6[e]–9.0[b]
Mefenamic acid	0.254	0.085	0.137	0.01–5.3[c, d]
Metoprolol	0.147	0.075	0.117	0.25–0.76[a, b, d]

[a] Wick et al. 2009
[b] Suarez et al. 2010
[c] Falås et al. 2012
[d] Falås et al. 2013
[e] Fernandez-Fontaina et al. 2014

biodegradation constant: $k_{biol} < 0.1$ L gSS^{-1} d^{-1} - no substantial removal by degradation (<20%) can be obtained; however, the removal may be greater for strongly sorbing compounds with $K_d > 1$ L gSS^{-1} due to transfer to sludge; $0.1 < k_{biol} < 10$ L gSS^{-1} d^{-1} -partial removal (between 20 and 90%), the degree of removal is strongly dependent on reactor configuration; $k_{biol} > 10$ L gSS^{-1} d^{-1} -more than 90% removal by biological degradation. The values of k_{biol} for fluoxetine, mefenamic acid, and metoprolol determined using the ammonium-nitrite-oxidizing consortium, and heterotrophic bacteria belong to the second category range, so partial removals by biodegradation could be expected. The k_{biol} values of the three compounds with the nitrite-oxidizing consortium were less than 0.1 L gSS^{-1} d^{-1}, so no substantial removal can be expected by biodegradation. The highest k_{biol} was determined for mefenamic acid when ammonium-nitrite-oxidizing consortium was used; the value of k_{biol} was 46% lower in the heterotrophic consortium. The highest k_{biol} for fluoxetine was determined using the heterotrophic biomass, and it was only 4% lower than the highest value for mefenamic acid. The value of k_{biol} for fluoxetine determined using ammonium-nitrite-oxidizing consortium was 23% lower than the one obtained with the heterotrophic consortium. The highest k_{biol} was determined for metoprolol using ammonium-nitrite-oxidizing consortium; the k_{biol} value was 20% lower in the heterotrophic consortium. The highest k_{biol} for metoprolol was 42% lower than the highest value for mefenamic acid.

The k_{biol} values reported in the literature for fluoxetine, mefenamic acid, and metoprolol were similar or higher than the values determined in this study. Falås et al. (2012) compared the removal rates of mefenamic acid in nitrifying activated sludge and biofilm carriers and found that the biofilm carriers demonstrated considerably higher removal rates of mefenamic acid per unit biomass (0.06–0.17 L $g_{biomass}^{-1}$ d^{-1} compared to 0–0.04 L $g_{biomass}^{-1}$ d^{-1} for activated sludge), and that the nitrification capacity of the biofilm was lower than the one determined in activated sludge. According to Falås et al. (2013), mefenamic acid was degraded faster by the attached biomass than the suspended biomass, where the nitrification capacity per unit biomass was higher for the attached growth than for the suspended growth, and the rate constants obtained for mefenamic acid were 3.9–5.3 L $g^{-1}_{biomass}$ d^{-1} with the attached biomass and 0.9–1.1 L $g_{biomass}^{-1}$ d^{-1} for the oxic sludge. In contrast, the rate constants obtained for metoprolol were 0.25–0.31 L $g_{biomass}^{-1}$ d^{-1} with the attached biomass and 0.69–0.76 L $g_{biomass}^{-1}$ d^{-1} for the oxic sludge. The k_{biol} values of metoprolol determined in activated sludge by Wick et al. (2009) were 0.35–0.4 L gSS^{-1} d^{-1}. Suarez et al. (2010) obtained k_{biol} of 9 L gSS^{-1} d^{-1} for fluoxetine in a nitrifying reactor, while Fernandez-Fontaina et al. 2014 reported k_{biol} of 0.6 and 1.3 L gSS^{-1} d^{-1} in highly enriched nitrifying activated sludge reactors, and these values are higher than the ones obtained in this study.

The results indicate that the biodegradation of pharmaceuticals does not only depend on the chemical characteristics but also depends on the microbial species involved. Some studies differ from our biodegradation constants, Joss et al. (2006) and Tran et al. (2009) suggest that part of the difference may be explained by the different concentrations of pharmaceuticals, the characteristics of biomass (fraction of active biomass, floc size, and microbial diversity), the wastewater composition, and the experimental conditions.

Sorption coefficients

The equilibrium concentrations in the balance were reached after 24 h for the three compounds. Figure 6 shows the sorption isotherms, a good adjustment to the linear Freundlich model was found, and the slope of the isotherms represents the coefficient K_d (L kg^{-1}). The sorption coefficients of each kind of inactive biomass and the sorption coefficients reported in other studies are summarized in Table 5. The sorption coefficients for inactive heterotrophic biomass were slightly higher than the values obtained for the other two consortiums, which might be attributed to the different morphology of the enriched biomasses. Low value of a solid liquid partition coefficient ($K_d < 500$ L kg^{-1} or log $K_d < 2.7$) implies poor sorption onto sludge, which results in their presence mainly in the aqueous phase (Ternes et al. 2004; Poseidon 2004). The lowest sorption coefficients were determined for metoprolol, the compound with the lowest log K_{ow} and log D_{lipw} values, which as well is in accordance with the obtained low sorption percentages in the performed biodegradability tests. The values of log K_d for mefenamic acid were between 2.56 and 2.58, slightly lower than 2.7, and for fluoxetine, they were slightly higher than 2.7 indicating the potential trend of these pharmaceuticals to sorb onto biomass and particles.

Hörsing et al. (2011) presented sorption isotherms of fluoxetine to secondary sludge with K_d of 5700–6000 L kg^{-1} (log K_d of 3.76–3.78), and the coefficients were higher than the ones calculated in this study. The K_d values obtained for mefenamic acid in this study were similar to those calculated by Radjenovic et al. (2009), which reported K_d values from 434 to 537 L kg^{-1} (log K_d of 2.64–2.73) in activated sludge and membrane bioreactors, indicating that sorption could be a relevant removal pathway. They suggested that the discrepancies in the literature data could be due to the differences in sludge age, as well as the composition of sludge and wastewater. Finally, the sorption coefficients measured for metoprolol were in the same range as those observed by Ramil et al. (2010), as they obtained log K_d of 1.75 on natural river sediments. Similarly, the K_d values for metoprolol in activated sludge by Scheurer et al. (2010) were between 50 and 90 L kg^{-1} (log K_d of 1.7–1.95).

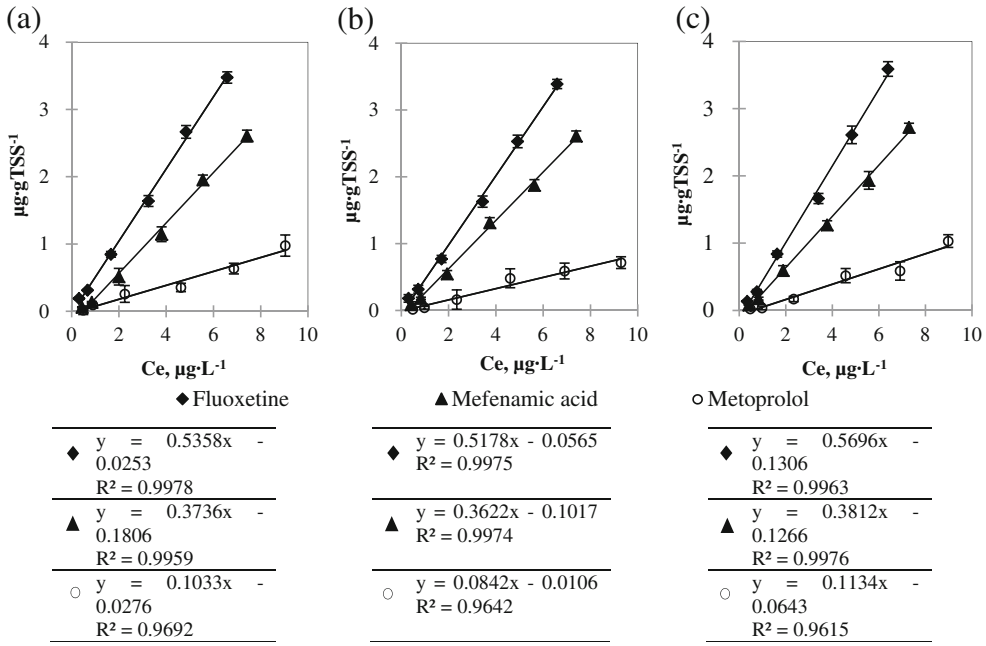


Fig. 6 Sorption isotherms of pharmaceuticals in the different microbial consortiums: **a** Ammonium-nitrite-oxidizing biomass; **b** Nitrite-oxidizing biomass; **c** Heterotrophic biomass

Conclusions

The ammonium-nitrite-oxidizing and the aerobic heterotrophic consortiums are able to biodegrade fluoxetine, mefenamic acid, and metoprolol. The determination of the selected pharmaceuticals in the dissolved phase and in the biomass, as well as the performed mass balances indicated that biodegradation was the major removal mechanism of the three compounds. The biodegradation rates are proportional to the nitrifying activity in the ammonium-nitrite-oxidizing consortium, and they increase after the depletion of the primary substrate in the heterotrophic consortiums, suggesting that the biodegradation is through cometabolism in both consortiums. The nitrite-oxidizing consortium presented the lowest biodegradation rates of the three compounds.

The pharmaceuticals fluoxetine and mefenamic acid were more biodegradable than the metoprolol; 85% of biodegradation was reached for both compounds using heterotrophic consortium for the fluoxetine and ammonium-nitrite-oxidizing consortium for the mefenamic acid after 24 days of incubation. The highest biodegradation percentage for metoprolol, of 64%, was obtained with the ammonium-nitrite-oxidizing consortium.

The biodegradation kinetics were successfully adjusted to pseudo-first-order for the three pharmaceuticals. The highest biodegradation constant for fluoxetine was obtained using heterotrophic biomass, followed by the one using ammonium-nitrite-oxidizing biomass, while the highest values of the biodegradation constant for mefenamic acid and metoprolol were determined with ammonium-nitrite-oxidizing consortium. The highest biodegradation constant for metoprolol was 40–42% lower than the highest values for fluoxetine and mefenamic acid. The values of the biodegradation constants for fluoxetine, mefenamic acid, and metoprolol determined using the ammonium-nitrite-oxidizing and heterotrophic consortium indicate that both consortiums allow

Table 5 Sorption coefficients in the different microbial consortiums

Compound	log K_d			log K_d (based on reported data in the literature)
	Ammonium-nitrite-oxidizing consortium	Nitrite-oxidizing consortium	Heterotrophic consortium	
Fluoxetine	2.73	2.71	2.76	3.76–3.78[d]
Mefenamic acid	2.57	2.56	2.58	2.64–2.73[a]
Metoprolol	2.01	1.93	2.05	1.7–1.95[b, c]

[a] Radjenovic et al. 2009

[b] Ramil et al. 2010

[c] Scheurer et al. 2010

[d] Hörsing et al. 2011

partial biodegradation of the compounds, while no substantial biodegradation can be expected using nitrite-oxidizing consortium.

The sorption onto biomass had a significant contribution (6–14%) in the removal of fluoxetine and mefenamic acid; the values of the sorption coefficients for fluoxetine were higher than the ones for mefenamic acid in the three consortiums. The lowest sorption coefficients were determined for metoprolol indicating that the sorption onto biomass is poor (3–4%), and the contribution of this process to the global removal can be neglected.

References

APHA (2012) Standard methods for the examination of water and wastewater. 22nd ed. American Public Health Association (APHA-AWWA-WEF), Washington, DC

Birkholz DA, Stilson SM, Elliot HS (2014) Analysis of emerging contaminants in drinking water—a review. Comprehensive Water Quality and Purification 2:212–229

Chen F, Ying G, Kong L, Wang L, Zhao J, Zhou L, Zhang L (2011) Distribution and accumulation of endocrine-disrupting chemicals and pharmaceuticals in wastewater irrigated soils in Hebei, China. Environ Pollut 159:1490–1498

Clara M, Kreuzinger N, Strenn B, Gans O, Kroiss H (2005) The solids retention time—a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. Water Res 39:97–106

Daughton CG, Ternes TA (1999) Pharmaceutical and personal care products in the environment: agents of subtle change? Environ Health Perspect 107(Supplement 6):907–938

Dawas A, Gur-Reznik S, Lerman S, Sabbah I, Desoretz C (2014) Co-metabolic oxidation of pharmaceutical compounds by a nitrifying bacterial enrichment. Bioresour Technol 167:336–342

Deblonde T, Cossu-Leguille C, Hartemann P (2011) Emerging pollutants in wastewater: a review of the literature. International Journal of Hygiene and Environmental Healt 214:442–448

EPA (2002) Nitrification. Office of Water (4601 M), Washington DC

Escher BI, Baumgartner R, Koller M, Treyer K, Lienert J, McArdell CS (2011) Environmental toxicology and risk assessment of pharmaceuticals from hospital wastewater. Water Res 45:75–92

Falås P, Baillon-Dhumez A, Andersen HR, Ledin A, la Cour JJ (2012) Suspended biofilm carrier and activated sludge removal of acidic pharmaceuticals. Water Res 46:1167–1175

Falås P, Longrée P, Cour Jansen J, Siegrist H, Hollender J (2013) Micropollutant removal by attached and suspended growth in a hybrid biofilm-activated sludge process. Water Res 47:4498–4506

Fernandez-Fontaina E, Omil F, Lema JM, Carballa M (2012) Influence of nitrifying conditions on the biodegradation and sorption of emerging micropollutants. Water Res 46:5434–5444

Fernandez-Fontaina E, Carballa M, Omil F, Lema JM (2014) Modelling cometabolic biotransformation of organic micropollutants in nitrifying reactors. Water Res 65:371–383

Fernandez-Fontaina E, Gomes IB, Aga DS, Omil F, Lema JM, Carballa M (2016) Biotransformation of pharmaceuticals under nitrification, nitratation and heterotrophic conditions. Sci Total Environ 541: 1439–1447

Ferrando-Climent L, Collado N, Buttiglieri G, Gros M, Rodriguez-Roda I, Rodriguez-Mozaz S, Barceló D (2012) Comprehensive study of ibuprofen and its metabolites in activated sludge batch experiments and aquatic environment. Sci Total Environ 438:404–413

Forrez I, Boon N, Verstraete W, Carballa M (2011) Biodegradation of micropollutants and prospects for water and wastewater biotreatment. Comprehensive Biotechnology 6:485–494

Gerardi MH (2002) Nitrification and denitrification in the activated sludge process. Environmental Protection. Willey Interscience, Nueva York

Grossberger A, Hadar Y, Borch T, Chefetz B (2014) Biodegradability of pharmaceutical compounds in agricultural soils irrigated with treated wastewater. Environ Pollut 185:168–177

Gulde R, Meier U, Schymanski EL, Kohler HE, Helbling DE, Derrer S, Rentsch D, Fenner K (2016) Systematic exploration of biotransformation reactions of amine containing micropollutants in activated sludge. Environ Sci Technol 50:2908–2920

Hata T, Kawai S, Okamura H, Nishida T (2010) Removal of diclofenac and mefenamic acid by the white rot fungus Phanerochaete sordida YK-624 and identification of their metabolites after fungal transformation. Biodegradation 21:681–689

Helbling DE, Johnson DR, Honti M, Fenner K (2012) Micropollutant biotransformation kinetics associate with WWTP process parameters and microbial community characteristics. Environ Sci Technol 46:10579–10588

Henze M, Harremoës P, Arvin E, Jansen J (2002) Wastewater treatment: biological and chemical processes, Third edn. Springer, Heidelberg

Hörsing M, Ledin A, Grabic R, Fick J, Tysklind M, Jansen J, Andersen HR (2011) Determination of sorption of seventy-five pharmaceuticals in sewage sludge. Water Res 45:4470–4482

Jones OAH, Voulvoulis N, Lester JN (2002) Aquatic environmental assessment of the top 25 English prescription pharmaceuticals. Water Res 36:5013–5022

Joss A, Keller E, Alder AC, Göbel A, McArdell CS, Ternes T, Siegrist H (2005) Removal of pharmaceuticals and fragrances in biological wastewater treatment. Water Res 39:3139–3152

Joss A, Zabczynski S, Göbel A, Hoffmann B, Löffer D, McArdell CS, Ternes TA, Thomsen A, Siegrist H (2006) Biological degradation of pharmaceuticals in municipal wastewater treatment: proposing a classification scheme. Water Res 40:1686–1696

Khunjar WO, Mackintosh SA, Skotnicka-Pitak J, Baik S, Aga DS, Love NG (2011) Elucidating the relative roles of ammonia oxidizing and heterotrophic bacteria during the biotransformation of 17α-ethinylestradiol and trimethoprim. Environ Sci Technol 45:3605–3612

Kibbey TCG, Paruchuri R, Sabatini DA, Chen L (2007) Adsorption of beta blockers to environmental surfaces. Environ Sci Technol 41: 5349–5356

Kovalova L, Siegrist H, Singer H, Wittmer A, McArdell CS (2012) Hospital wastewater treatment by membrane bioreactor: performance and efficiency for organic micropollutant elimination. Environmental Science & Technology 46:1536–1545

Li J, Bishop PL (2004) Time course observations of nitrifying biofilm development using microelectrodes. J Environ Eng Sci 2:523–528

Luo Y, Guo W, Hao H, Duc Nghiem L, Ibney F, Zhang J, Liang S, Wang X (2014) A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci Total Environ 473-474:619–641

Maeng SK, Choi BB, Lee KT, Song KG (2013) Influences of solid retention time, nitrification and microbial activity on the attenuation of pharmaceuticals and estrogens in membrane bioreactors. Water Res 47:3151–3162

Majewsky M, Gallé T, Yargeau V, Fischer K (2011) Active heterotrophic biomass and sludge retention time (SRT) as determining factors for biodegradation kinetics of pharmaceuticals in activated sludge. Bioresour Technol 102:7415–7421

Mansour F, Al-Hindi M, Saad W, Salam D (2016) Environmental risk analysis and prioritization of pharmaceuticals in a developing world context. Sci Total Environ 557-558:31–43

Martínez MJ, Gomez MJ, Herrera S, Hernando MD, Agüera A, Fernández AR (2012) Occurrence and persistence of organic emerging contaminants and priority pollutants in five sewage treatment plants of Spain: two years pilot survey monitoring. Environ Pollut 164:267–273

Miège C, Choubert JM, Ribeiro L, Eusèbe M, Coquery M (2009) Fate of pharmaceuticals and personal care products in wastewater treatment plants—conception of a database and first results. Environ Pollut 157:1721–1726

Monteiro SC, Boxall ABA (2010) Occurrence and fate of human pharmaceuticals in the environment. Rev Environ Contam Toxicol 202: 53–154

Nogueira R, Melo LF (2006) Competition between Nitrospira spp. and Nitrobacter spp. in nitrite-oxidizing bioreactors. Biotechnol Bioeng 95:169–175

Poseidon, detailed report related to the overall duration (2004) Assessment of technologies for the removal of pharmaceuticals and personal care products in sewage, and drinking water facilities to improve the indirect potable water reuse

Quintana JB, Weiss S, Reemtsna T (2005) Pathways and metabolites of microbial degradation of selected acidic pharmaceutical and their occurrence in municipal wastewater treated by a membrane bioreactor. Water Res 39:2654–2664

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

Ramil M, El Aref T, Kink G, Scheurer M, Ternes TA (2010) Fate of beta blockers in aquatic-sediment systems: sorption and biotransformation. Environmental Science & Technology 44:962–970

Rattier M, Reungoat J, Keller J, Gernjak W (2014) Removal of micropollutants during tertiary wastewater treatment by biofiltration: role of nitrifiers and removal mechanisms. Water Res 54:89–99

Rieger PG, Meier HM, Gerle M, Vogt U, Groth T, Knackmuss HJ (2002) Xenobiotics in the environment: present and future strategies to obviate the problem of biological persistence. J Biotechnol 94(1): 101–123

Rogers HR (1996) Sources, behaviour and fate of organic contaminants during sewage treatment and in sewage sludges. Sci Total Environ 185:3–26

Roos V, Gunnarsson L, Fick J, Larsson DGJ, Rudén C (2012) Prioritising pharmaceuticals for environmental risk assessment: towards adequate and feasible first-tier selection. Sci Total Environ 421-422: 102–110

Rosal R, Rodríguez A, Perdigón-Melón JA, Petre A, García-Calvo E, Gómez J, Agüera A, Fernández-Alba AR (2010) Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation. Water Res 44:578–588

Rubirola A, Llorca M, Rodriguez-Mozaz S, Casas N, Rodriguez-Roda I, Barceló D, Buttiglieri G (2014) Characterization of metoprolol biodegradation and its transformation products generated in activated sludge batch experiments and in full scale WWTPs. Water Res 63: 21–32

Sathyamoorthy S, Ramsburg CA (2013) Assessment of quantitative structural property relationships for prediction of pharmaceutical sorption during biological wastewater treatment. Chemosphere 92: 639–646

Scheurer M, Ramil M, Metcalfe CD, Groh S, Ternes TA (2010) The challenge of analyzing beta-blocker drugs in sludge and wastewater. Anal Bioanal Chem 396:845–856

Spieck E, Bock E (2005) The lithoautotrophic nitrite-oxidizing bacteria. Bergey's Manual® of Systematic Bacteriology 2:149–153

Spieck E, Lipski A (2011) Cultivation, growth physiology, and chemotaxonomy of nitrite-oxidizing bacteria. Methods Enzymol 486:109–130

Stuart M, Lapworth D, Crene E, Hart A (2012) Review of risk from potential emerging contaminants in UK groundwater. Sci Total Environ 416:1–21

Suarez S, Lema JM, Omil F (2010) Removal of pharmaceutical and personal care products (PPCPs) under nitrifying and denitrifying conditions. Water Res 44:3214–3224

Sui Q, Huang J, Deng S, Yu G, Fan Q (2010) Occurrence and removal of pharmaceuticals, caffeine and DEET in wastewater treatment plants of Beijing, China. Water Res 44:417–426

Tadkaew N, Hai FI, McDonakd JA, Khan SJ, Nghiem LD (2011) Removal of trace organics by MBR treatment: the role of molecular properties. Water Res 45:2439–2451

Tauxe-Wuersch A, De Alencastro LF, Grandjean D, Tarradellas J (2005) Occurrence of several acidic drugs in sewage treatment plants in Switzerland and risk assessment. Water Res 39:1761–1772

Ternes T (1998) Occurrence of drugs in German sewage treatment plants and rivers. Water Res 32:3245–3260

Ternes T, Joss A (2006) Human pharmaceuticals, hormones and fragrances: the challenge of micropollutants in urban water management. IWA Publishing. UK

Ternes TA, Janex-Habibi MJ, Knacker T, Kreuzinger N, Siegrist H (2004) Assessment of technologies for the removal of pharmaceuticals and personal care products in sewage and drinking water facilities to improve the indirect potable water reuse. Project acronym. POSEIDON. Contract No. EVKI-CT-2000-00047

Torres AX, Buitrón G (2012) Biodegradation of nonylphenols using nitrifying sludge, 4-chlorophenol-adapted consortia and activated sludge in liquid and solid phases. Environ Technol 33:1727–1737

Tran NH, Urase T, Kusakabe O (2009) The characteristics of enriched nitrifier culture in the degradation of selected pharmaceutically active compounds. J Hazard Mater 171:1051–1057

Tran NH, Urase T, Ngo HH, Hu J, Ong SL (2013) Insight into metabolic and cometabolic activities of autotrophic and heterotrophic microorganisms in the biodegradation of emerging trace organic contaminants. Bioresour Technol 146:721–731

Trenholm RA, Vanderford BJ, Holady JC, Rexing DJ, Snyder SA (2006) Broad range analysis of endocrine disruptors and pharmaceuticals using gas chromatography and liquid chromatography tandem mass spectrometry. Chemosphere 65:1990–1998

Urase T, Kikuta T (2005) Separate estimation of adsorption and degradation of pharmaceutical substances and estrogens in the activated sludge process. Water Res 39:1289–1300

Verlicchi P, Aukidy MA, Zambello E (2012) Occurrence of pharmaceutical compounds in urban wastewater: removal, mass load and environmental risk after a secondary treatment—a review. Sci Total Environ 429:123–155

Vieno N, Tuhkanen T, Kronberg L (2007) Elimination of pharmaceuticals in sewage treatment plants in Finland. Water Res 41:1001–1012

Vulliet E, Cren-Olivé C (2011) Screening of pharmaceuticals and hormones at the regional scale, in surface and groundwaters intended to human consumption. Environ Pollut 159:2929–2934

Wick A, Fink G, Joss A, Siegrist H, Ternes TA (2009) Fate of beta blockers and psycho-active drugs in conventional wastewater treatment. Water Res 43:1060–1074

Yi T, Harper WF (2007) The link between nitrification and biotransformation of 17α-ethinylestradiol. Environ Sci Technol 41:4311–4316

Zhao J, Li Y, Zhang C, Zeng Q, Zhou Q (2008) Sorption and degradation of bisfenol A by aerobic activated sludge. J Hazard Mater 155:305–311