# Removal of pharmaceuticals from municipal wastewater by aerated submerged attached growth reactors

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**Abstract**
The performance of four aerated submerged attached growth reactors was studied for the removal of three pharmaceuticals (fluoxetine, mefenamic acid and metoprolol) from municipal wastewater. Two packing materials (polyethylene tapes and polyurethane cubes) were compared and the effects of the organic loads (with and without 50 % of effluent recirculation) were investigated. The effects of the organic loads and effluent recirculation were evaluated. The low organic loads, high solid retention times and effluent recirculation enhanced the removal of the three pharmaceutical compounds. The highest removals were achieved at organic load of 3 gCOD $m^{-2}$ $d^{-1}$ and 50 % of effluent recirculation, where the hydraulic residence times were 3.1-4.3 h and the solid retention times were 19-32 d. At this condition, the removals of the fluoxetine, mefenamic acid and metoprolol were up to 95, 82 and 73 % respectively. The reactors with polyurethane cubes showed higher removals compared with the polyethylene tapes.

**Keywords**
Immobilized biomass; wastewater; pharmaceutical

## INTRODUCTION

Pharmaceuticals are a class of micropollutants that may cause acute and chronic effects on aquatic organisms in the concentration range of μg $L^{-1}$ (Escher et al. 2011). Pharmaceuticals have been detected in municipal and hospital wastewater, surface water, groundwater, and even in drinking water (Stuart et al. 2012; Birkholz et al. 2014). Municipal wastewater treatment plants effluents represent one of the main sources of these compounds, because most of these plants are not designed to remove them, as they were built with the principal aim of removing biodegradable carbon, nitrogen and phosphorus compounds (Verlicchi et al. 2012; Luo et al. 2014). For these reasons, it is necessary to improve the removal of pharmaceuticals with high environmental risk. Three pharmaceuticals from different classes of action 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).

The studies on micro-pollutant removal in activated sludge wastewater treatment systems had indicated that high removal rates are achieved at solid retention times (SRT) higher than 10 d (Clara et al. 2005; Suarez et al. 2010. The long SRT allow an enrichment of slow growing bacteria such as nitrifying bacteria and the nitrifying activity contributes to the biotransformation of pharmaceuticals (Dawas et al. 2014; Rattier et al. 2014). Cometabolic biodegradation seems to be responsible for the initial biotransformation due to the action of ammonium monooxygenase enzyme, which catalyzes the first step of nitrification by ammonium oxidizing bacteria (Fernandez-Fontaina et al. 2012). Other experiments have indicated that the heterotrophic degradation rather than autotrophic degradation by ammonium oxidizing microorganisms was the main cause for the removal of several compounds, such as mefenamic acid and metoprolol (Tran el al. 2009; Majewsky et al. 2011; Maeng et al. 2013; Falås et al. 2016). So, the nitrifying bacteria are capable to enhance the biodegradation of pharmaceuticals; nevertheless the role of heterotrophic organisms must be considered.

The attached growth processes offer some advantages over activated sludge processes, such as higher biomass concentration and high solid retention times (SRT) even operating with low hydraulic residence time (HRT), which allows the development of microorganisms with low specific growth rates, so high nitrification rate can be achieved (Luo et al. 2014). Falås et al. (2012) showed that moving bed biofilm carriers (Kaldnes K1 and Biofilm chip) have a pharmaceutical reduction potential superior to the activated sludge one. They gave two potential explanations of the observed difference: higher quantity of slow growing pharmaceutical degrading microorganisms, because of the higher SRT in the biofilm carrier's case, and stratification of the microbial community due to the substrate and redox gradients within the biofilm. The microorganisms adapted to easily degradable organic substrates are located in the outer part of the biofilm and microorganisms adapted to the remaining and hardly degradable organic substrates in the inner part of the biofilm. Later, Falås et al. (2013) observed clear differences between the micro-pollutant removal kinetics obtained with attached and suspended biomass; higher removal rates were found using attached biomass for most of the studied compounds. For example, mefenamic acid was degraded faster by the attached biomass than using suspended biomass, while the degradation pattern was opposite for metoprolol. The nitrification capacity per unit biomass was considerably higher for the attached growth biomass than for the suspended growth one.

The characteristics of the packed materials determine the structure of the biofilms developed in the reactors, Mijaylova et al. (2008) studied the performance of aerobic submerged packed bed reactors for the treatment of domestic wastewater using different kinds of packing materials (ceramic spheres, crushed tezontle, grains of high density polyethylene, polyethylene of low density, polypropylene, cubes of polyurethane and polyethylene tapes). The results showed that the highest SRT (until 39 d) were obtained in the reactors with polyethylene tapes and polyurethane cubes and both reactors presented almost 99% $NH_4$-N removal; that is why polyethylene tapes and polyurethane cubes were chosen as a packing materials in this study. Different kinds of biomass retention can be expected using these materials. Mijaylova et al. (2010) reported that the biofilm developed in the reactors with polyethylene tapes was thin and this favored the diffusivity and mass transfer in the biofilm, while, as indicated by Guo et al. (2010), the biomass on polyurethane cubes is retained in two different forms: biofilm developed onto the cube surfaces, and biomass deposited or entrapped inside the cubes, in the void spaces. A distinctive dissolved oxygen gradient occurred along the sponge inward depth, resulting in anaerobic conditions at deep inside portions of the sponge. As shown, aerated submerged attached growth reactors are an alternative for the removal of pharmaceuticals, however further research is needed to enhance their performance. The objective of this study was to assess the removal of fluoxetine, mefenamic acid and metoprolol from municipal wastewater by aerated submerged attached growth reactors, comparing the performance of two biomass support materials (polyethylene tapes and polyurethane cubes). The effects of the organic loads and effluent recirculation were evaluated.

## MATERIAL AND METHODS

### Experimental set-up and packing materials

The experiments were performed using four aerated submerged attached growth reactors. Each reactor had a cylindrical packed bed zone, a peripheral settling zone and a conical bottom for the extraction of accumulated sludge. Biomass support materials were placed into the cylindrical zone with 0.15 m diameter and a bed height of 0.8 m. Two reactors (PU1 and PU2) were packed with 3,250 polyurethane cubes of 1.5 cm edge length and 10 pores per inch; the other two (PE1 and PE2) were packed with 3,300 polyethylene tapes of 5 cm long and 3 cm wide, the tapes were supported by

a vertical shaft of stainless steel. The specific areas of both packing beds were almost 700 $m^2 m^{-3}$. The schematic diagram of the experimental setup is presented in Figure 1. The reactors were continuously fed with municipal wastewater, the wastewater passed down flow through the packed bed and up flow in the peripheral settling zone. The effluent was collected from the upper part of the settling zone and the sludge accumulated in the conic zone was periodically extracted. The aeration was provided by porous stone diffusers installed at the bottom; the dissolved oxygen levels were kept higher than 3 mg $L^{-1}$.

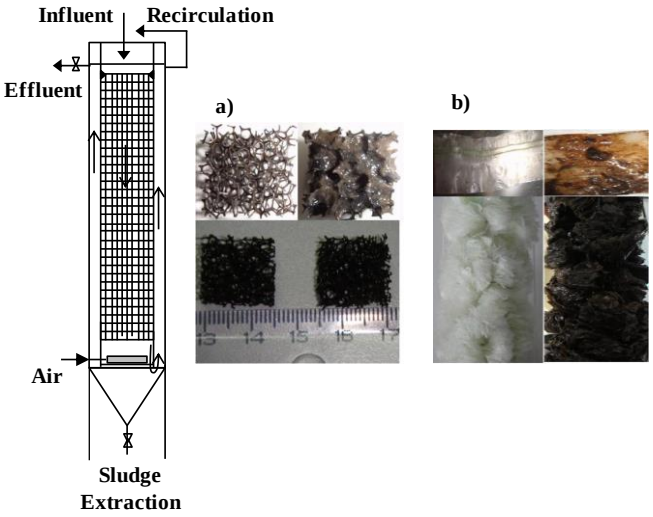



**Figure 1.** Schematic diagram of the bioreactors and general views of the packing materials: *a)* Polyurethane cubes; *b)* Polyethylene tapes.

## Experimental procedure and analysis

The immobilized biomass was developed supplying municipal wastewater at organic load (OL) of 3 gCOD $m^{-2} d^{-1}$, without any special inoculation. The addition of the pharmaceutical compounds began after the process stabilization (80% COD and $NH_4$-N removal). Pharmaceuticals were added to wastewater to obtain 2 μg $L^{-1}$ of fluoxetine and 5 μg $L^{-1}$ for mefenamic acid and metoprolol. After the process stabilization, different operational conditions were evaluated, the reactors were operated with organic loads of 3.0, 6.0, 9.0 and 12 gCOD $m^{-2} d^{-1}$, the effect of 50 % of effluent recirculation was also evaluated for all organic loads; each experimental phase was evaluated during 60 d. The operational parameters are presented in Table 1. The variation of the organic load was made by increasing the flow rate of the influent to the reactors, thus a decrease of HRT occurred when the organic load was increased. The HRT varied between 1.2 and 4.3 h in the reactors PE1 and PU1 and between 0.6 and 1.4 h in the reactors PE2 and PU2

**Table 1.** Operational parameters of the reactors

| Parameter | Phase 1 | Reactors PE1 and PU1 | | | | Reactors PE2 and PU2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Process stabilization | Phase 2 | Phase 3 | Phase 4 | Phase 5 | Phase 2 | Phase 3 | Phase 4 | Phase 5 |
| OL, gCOD $m^{-2} d^{-1}$ | 3.0 | 3.0 | 3.0 | 6.0 | 6.0 | 9.0 | 9.0 | 12.0 | 12.0 |
| Influent flow, L $d^{-1}$ | 96-151 | 83-120 | 79-108 | 200-254 | 199-281 | 248-360 | 236-332 | 401-508 | 398-528 |
| HRT, h | 2.3-3.5 | 2.8-4.1 | 3.1-4.3 | 1.3-1.7 | 1.2-1.7 | 0.9-1.4 | 1.0-1.4 | 0.7-0.8 | 0.6-0.9 |
| Recirculation, % | 0 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 |

The COD, $NH_4$-N, $NO_2$-N and $NO_3$-N were measured in the influent and effluents three times a week. Total solids (TS) and volatile solids (VS) were determined in the packed beds once every two weeks; the samples were obtained from three different heights of the packed bed (upper, central and lower part), the biomass was detached with methanol and 20 minutes of sonication. The biomass in each reactor was determined as average of the dry volatile solids determined at the three heights. In order to determine the SRT, the VS concentrations were measured in the effluents (once a week) and in the extracted sludge (once every two weeks); these parameters were determined according to the standard methods (APHA, 2012). The pharmaceuticals were measured three times a week by Gas Chromatography using Shimadzu TQ8040, fitted with a 30 m DB5-MS fused silica capillary column (30 m x 0.25 mm, 0.25 µm film thickness) and connected to triple quadrupole mass spectrometer.

**Analysis of the pharmaceutical compounds**

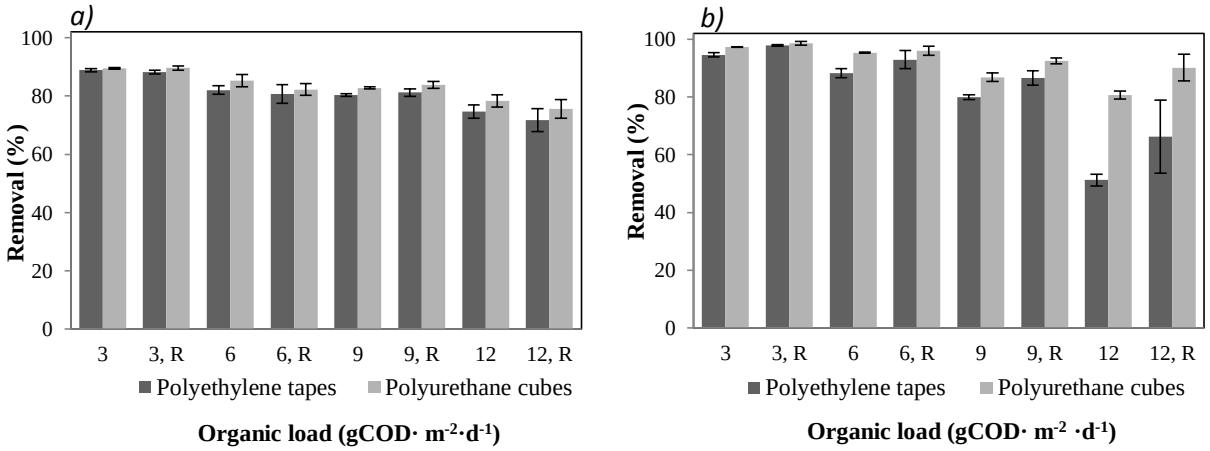
Gas chromatography-mass spectrometry method was developed and validated for the simultaneous detection of the three pharmaceutical compounds (fluoxetine, mefenamic acid and metoprolol) in liquid and solid phases. Solid phase extraction was used to concentrate the pharmaceutical compounds and remove interfering substances, the compounds were extracted on Oasis HLB cartridge 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), 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). Later the cartridges were dried under vacuum during three hours by an air flow to eliminate wetness. 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 derivatizing agent were used and heating at 80°C during 60 min. In the end, after sample drying, it was reconstituted with 1 mL of toluene to be analyzed.

## RESULTS AND DISCUSSION

**Process performance**

The biomass development and the process stabilization phase lasted 64 d. The COD and $NH_4$-N removals increased gradually reaching 80 % at day 52 from the start up in all the reactors. After 60 d of operation, COD and $NH_4$-N removals were higher than 88% and 91% respectively in all the reactors. After the process stabilization the reactor performance was evaluated applying different OL with and without effluent recirculation. The average COD and $NH_4$-N removals obtained at each experimental phase are presented in Figure 2. The OL increase resulted in a decrease of the COD removals in all the reactors. The highest removal (of 89%) was achieved with OL of 3 gCOD $m^{-2}$ $d^{-1}$, the lowest removals were obtained with OL of 12 gCOD $m^{-2}$ $d^{-1}$. The organic matter influent concentrations (expressed as COD) were between 200-380 mg $L^{-1}$. There were not statistically significant differences between the removals obtained in the reactors with both packing material when they were operated applying the same OL; neither between the removals obtained with and without effluent recirculation. The low organic loads favored the $NH_4$-N removals. Clear increase of $NH_4$-N removal was observed when the reactors were operated with effluent recirculation. This effect can be attributed to the reduction of the organic matter concentration in the reactors which makes the nitrifyers more competitive, and this in turn increases the nitrification efficiency and the dissolved oxygen concentration (EPA 2000). Therefore the highest $NH_4$-N removals (of 98%) were achieved at OL of 3 gCOD $m^{-2}$ $d^{-1}$ and 50% of effluent recirculation in the reactors with both packing material. The $NH_4$-N removals decreased with the OL increasing, the removals at OL of 12 gCOD $m^{-}$

$^{2}$ $d^{-1}$ and 50% of effluent recirculation were less than 66 and 90 % in the reactors PE and PU respectively, so the reactors with polyurethane (PU) cubes achieved higher removals of $NH_4$-N compared with the ones with polyethylene (PE) tapes. The influent concentrations of $NH_4$-N were between 20-60 mg $L^{-1}$. The effluent concentrations of $NO_2$-N and $NO_3$-N confirmed the nitrification process; the concentrations varied with the concentrations of $NH_4$-N in the influent. During all phases, there were lower concentrations of $NO_2$-N in the effluent compared with the $NO_3$-N concentrations. The reactors with PU cubes showed higher production of $NO_3$-N than the reactors PE types. These results indicated a good process performance in the reactors.




R – 50 % effluent recirculation

**Figure 2.** COD *(a)* and $NH_4$-N *(b)* removals during the experimental phases

The amount of attached biomass increased with the increasing of the organic loads, thus the reactors PE1 and PU1 (organic loads of 3 y 6 gCOD $m^{-2}d^{-1}$) accumulated lower amounts of biomass compared with the reactors PE2 and PU2 (organic loads of 9 y 12 gCOD $m^{-2}d^{-1}$). The amount of biomass and the calculated SRT are presented in Table 2. The reactors with polyurethane cubes allowed higher biomass accumulation during all the phases. The biomass quantity was 5.8–10.5 gVS $m^{-2}$ in these reactors and 4.9-9.0 gVS $m^{-2}$ in the reactors with polyethylene tapes.

**Table 2.** Biomass amount and solid retention times in the reactors

| Organic load gCOD $m^{-2}$ $d^{-1}$ | Polyethylene tapes | | Polyurethane cubes | |
|---|---|---|---|---|
| | Biomass, $g_{VS}$ $m^{-2}$ | SRT, d | Biomass, $g_{VS}$ $m^{-2}$ | SRT, d |
| 3.0 (Stabilization) | 5.9-6.2 | 28-31 | 6.8-7.0 | 33-36 |
| 3.0 (without recirculation) | 6.3-6.6 | 26-34 | 7.3-7.9 | 27-37 |
| 3.0 (with recirculation) | 5.9-6.0 | 19-28 | 6.3-7.1 | 26-32 |
| 6.0 (without recirculation) | 6.0-6.1 | 14-20 | 6.5-6.8 | 20-25 |
| 6.0 (with recirculation) | 4.9-7.0 | 12-18 | 5.8-6.6 | 15-18 |
| 9.0 (without recirculation) | 8.9-9.0 | 11-13 | 9.8-10.3 | 13-14 |
| 9.0 (with recirculation) | 7.4-8.1 | 10-13 | 9.0-10.2 | 11-16 |
| 12.0 (without recirculation) | 8.2-8.6 | 8-9 | 10.3-10.5 | 9-10 |
| 12.0 (with recirculation) | 6.8-8.3 | 4-6 | 8.8-9.6 | 4-6 |

The startup of the effluent recirculation was accompanied by a reduction in biomass quantity, because the recirculation increases the flow and velocities through the reactor, causing greater detachment of excess biofilm. The SRT were between 12-34 and 15-37 d in the reactors PE1 and PU1 respectively, whereas SRT were between 4-13 and 4-16 d in the reactors PE2 and PU2. The low

organic loads favored the high solid retention times, so that the highest SRT were found during the phase 2 of the reactors PE1 and PU1 (OL of 3 gCOD $m^{-2}d^{-1}$), the highest values were determined in the reactors with polyurethane cubes.

**Pharmaceutical compounds removal**

The addition and determination of the pharmaceutical compounds began at day 65. The concentrations of the pharmaceutical compounds determined in the influent and effluents from all reactors are presented on Figure 3. Low pharmaceutical removals were observed in the reactors PE1 and PU1 at the beginning of the evaluation phase 2. The fluoxetine, mefenamic acid and metoprolol concentrations in the effluents progressively decreased until they became almost constant during the last 14 days of this phase. The average removals of fluoxetine, mefenamic acid and metoprolol at OL of 3 gCOD $m^{-2}$ $d^{-1}$, HRT of 2.8-4.1 h and SRT of 26-37 d, were 77.5±1.2, 41.4±3.9 and 59.2±2.9 % respectively in the reactors with PE tapes, meanwhile they were 83.4±1.0%, 60.4±2.7% and 60.6±4.1 % respectively in the reactors with PU cubes. The average removals of the pharmaceutical compounds during the experimental phases are presented on Figure 4. The highest average removals of the three pharmaceuticals were achieved applying OL of 3.0 gCOD $m^{-2}$ $d^{-1}$ and 50 % of recirculation in the reactors with both support materials. The effluent concentrations of fluoxetine, mefenamic acid and metoprolol were 0.14±0.01, 1.43±0.14 and 1.76±0.24 µg $L^{-1}$ respectively for the reactor with PE tapes, obtaining removals of 94.0±0.3, 77.6±2.7 and 67.5±4.3 % respectively during the last 14 days. The highest removals of mefenamic acid were achieved in the reactor with PU cubes, the effluent concentration was 1.17±0.22 µg $L^{-1}$, achieving removals of 81.7±3.5%. While the fluoxetine removal was 94.9±0.8%, similar to the one obtained in the reactor with PE types, and the fluoxetine concentration was of 0.11±0.01 µg $L^{-1}$ in the effluent. The metoprolol removal was 72.7±5.1%, higher than the one obtained in the reactor with PE types, with concentrations of 1.47±0.3 µg $L^{-1}$ effluent. The performance of the reactors with both packing materials was very good during the experimental phase with OL of 3.0 gCOD $m^{-2}$ $d^{-1}$ and 50 % of recirculation, the COD and $NH_4$-N removals were more than 88 % and 97 % respectively. The average $NO_3$-N concentrations were 11.1±2.7 and 19.0±3.6 mg·$L^{-1}$ in the effluents of the reactors with PE types and PU cubes respectively. Although the recirculation involved a decrease of the SRT to 19-32 d, due to the greater detachment of the excess biomass, it also reduces the resistance to mass transfer (EPA 2000) and allowed the removal efficiency increasing in the reactors.

The OL increase to 6.0 gCOD $m^{-2}d^{-1}$ (without recirculation) caused an increase of the pharmaceutical concentration in the effluents of the reactors with both packing materials. This effect could be attributed to the change of the operational conditions, the influent flow increase caused HRT decrease to 1.3-1.7 h and the SRT was reduced to 14-25 d. Stabilization period of 30-40 d was required to reach again relatively constant pharmaceutical concentrations in the effluents. Removals of fluoxetine, mefenamic acid and metoprolol of 86.5±0.9, 40±1.4 and 58±4.3% respectively were determined in the reactor with PE types, however higher removals of 90.4±0.5, 57.3±1 and 64.2±3.5% respectively were obtained in the reactor with PU cubes. The reactors with PU cubes had higher SRT and $NH_4$-N removals compared with the obtained for the reactor with PE tapes. During the next experimental stage, the OL was maintained of 6.0 gCOD $m^{-2}d^{-1}$ but effluent recirculation of 50% was implemented. These operational conditions caused a decrease of the SRT to 12-18 d, nevertheless the pharmaceutical removals increased in the reactors with both packing materials. The removals of fluoxetine, mefenamic acid and metoprolol were 91.6±0.3, 59.2±2.1 and 68.3±1% respectively in the reactor with PU cubes, higher than those determined in the reactors with PE tapes (88±0.9, 50.3±1.7 and 59.8±1.8 % respectively), which can be attributed to the higher SRT and $NH_4$-N removal obtained in this reactor.

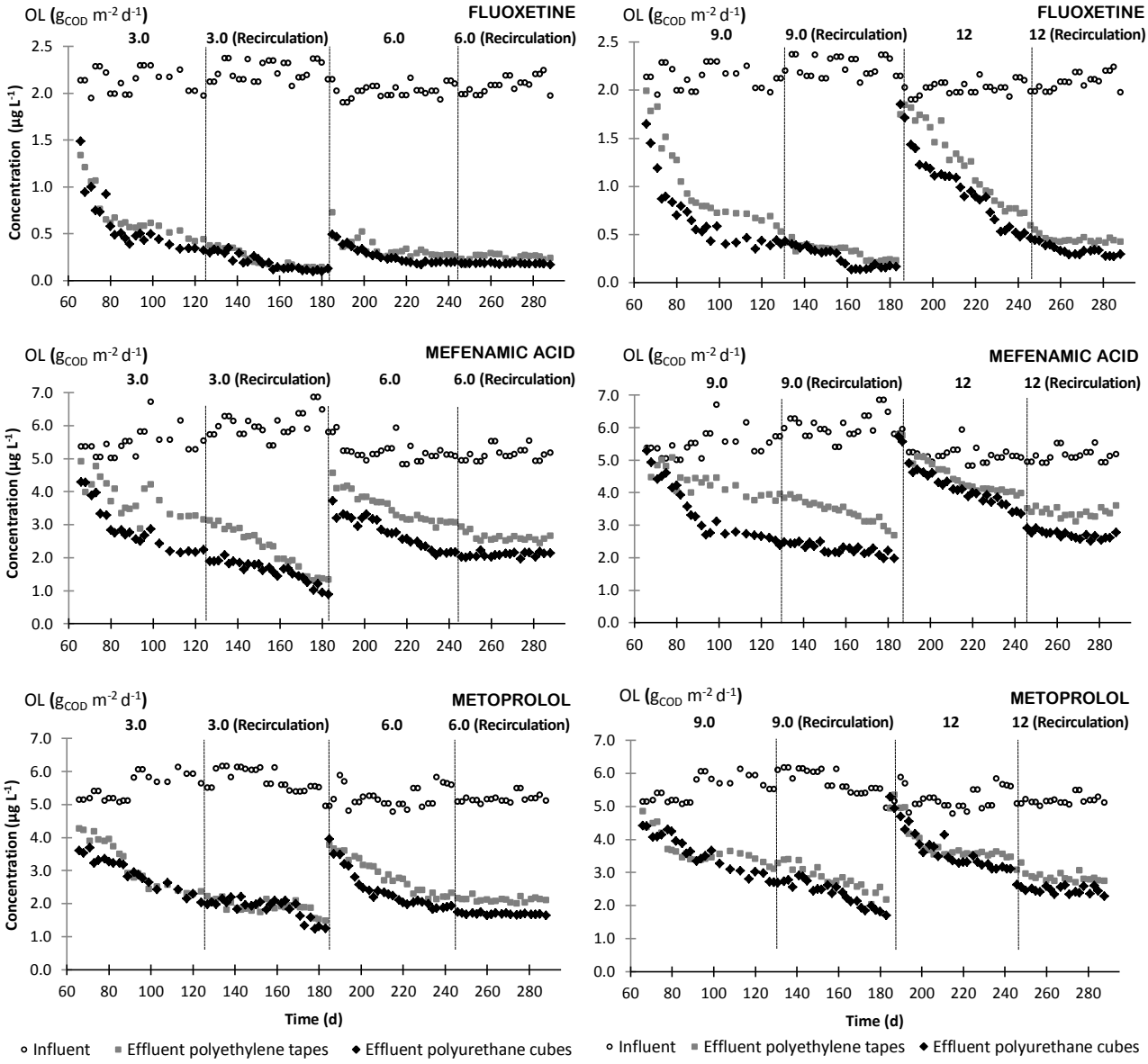



**Figure 3.** Pharmaceutical compounds concentrations during the experimental phases

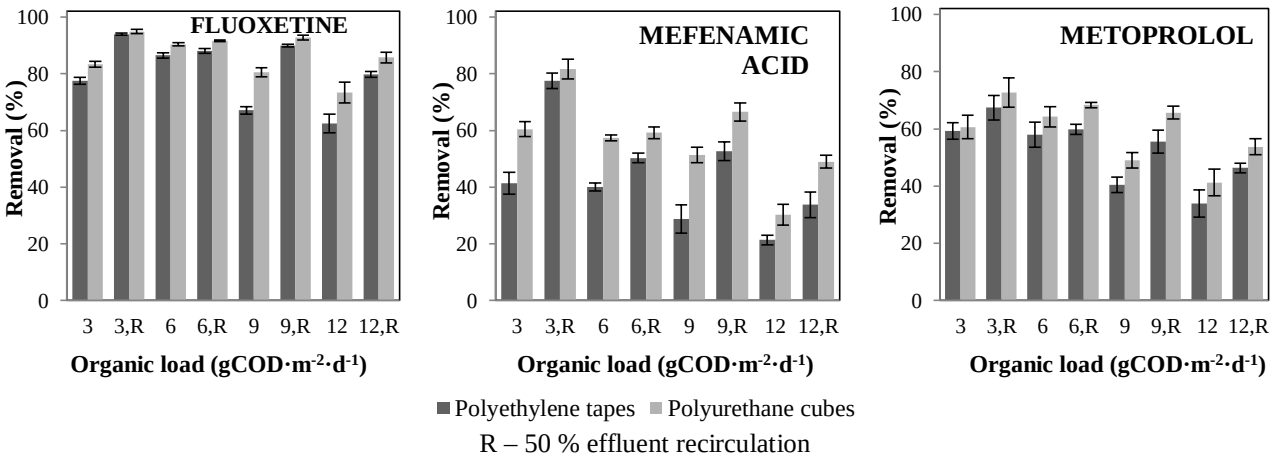



**Figure 4.** Pharmaceutical compounds removals during the experimental phases

The reactors PE2 and PU2 have been operated applying OL of 3 gCOD $m^{-2}$ $d^{-1}$ for biomass development and process stabilization for 65 d and after this the addition of the pharmaceuticals was started. The OL increase to 9.0 gCOD $m^{-2}d^{-1}$ produced instability at the beginning of the evaluation phase and 30-40 d were required to obtain relatively constant pharmaceutical concentrations in the effluents. The HRT was of 0.9-1.4 h and SRT of 11-14 d was determined for this experimental phase. The effluent concentrations of fluoxetine, mefenamic acid and metoprolol were 0.69±0.03, 3.95±0.17 and 3.49±0.13 µg $L^{-1}$ respectively for the reactor with PE tapes (removals of 67.1±1.3, 28.8±5 and 40.4±2.7 % respectively). The effluent pharmaceutical concentrations were 0.41±0.04, 2.7±0.07 and 2.99±0.11 µg $L^{-1}$ respectively for the reactor with PU cubes and the removals were calculated of 80.4±1.6, 51.4±2.8 and 49±2.8% respectively. So the PU cubes showed higher removals of fluoxetine and mefenamic acid than the PE tapes. When effluent recirculation was applied maintaining the same OL of 9.0 gCOD $m^{-2}d^{-1}$ to, the removals of fluoxetine, mefenamic acid and metoprolol increased in the reactors with both packing materials. Removals of fluoxetine, mefenamic acid and metoprolol of 89.9±0.46, 52.7±3.3 and 55.6±4.0% respectively were determined in the reactor with PE types, however higher removals of 92.8±0.8, 66.6±3.2 and 65.7±2.3% respectively were obtained in the reactor with PU cubes. The reactors with PU cubes had higher SRT and $NH_4$-N removals compared with the obtained for the reactor with PE tapes. The organic load increase to 12 gCOD $m^{-2}d^{-1}$ performed on day 184 from the startup of the reactors PE2 and PU2 caused a strong inhibition of the pharmaceuticals degradation (Figure 3), which can be attributed to the change of the operational conditions; the HRT was of 0.7-0.8 h and the SRT of 8-10 d. The effluent pharmaceutical concentrations decreased over the time, however almost 50 d were required to reach stability for the pharmaceutical removals in the reactors. The average removals of the fluoxetine, mefenamic acid and metoprolol were 62.5±3.3, 21.4±1.7 and 33.9±4.8% respectively in the reactor PE types, and they were 73.3±3.6, 30.2.±3.7 and 41.3±4.6% in the reactor with PU cubes. Better pharmaceuticals removals were obtained in the reactor with the PU cubes compared with the one with PE tapes, however it is important to mention that the lowest removals of $NH_4$-N removals (lower than 80 %) were observed in the reactors during this experimental phase. The pharmaceuticals removals increased when the effluent recirculation was applied maintaining the same OL of 12 gCOD $m^{-2}d^{-1}$. The fluoxetine, mefenamic acid and metoprolol concentrations were 0.43±0.02, 3.42±0.12 and 2.83±0.11 µg $L^{-1}$ respectively and removals of 79.8±1, 33.8±4.5 and 46.3±1.7% respectively were achieved in the reactor with PE types. In the case of the reactor with PU cubes, the concentrations were 0.30±0.03, 2.64±0.09 and 2.44±0.12 µg $L^{-1}$ respectively and the removals were 85.8±1.9, 49±2.2, and 53.7±2.8 % respectively. The differences of the reactor performances were pronounced, better performance was obtained again in the reactor with the PU cubes, which can be associated with the high SRT. Additionally it can be observed that the removals obtained with OL of 12 gCOD $m^{-2}$ $d^{-1}$ and 50% recirculation were higher than the determined with OL of 9gCOD $m^{-2}$ $d^{-1}$ without recirculation in the reactors with both packing materials. The most biodegradable compound was the fluoxetine; high removals of this compound were obtained in the reactors with both packing materials (Figure 4). The highest fluoxetine removal of 95% was obtained with SRT of 26-32 d and HRT of 3.1-4.3 h (OL of 3.0 gCOD $m^{-2}d^{-1}$ and 50% recirculation). This removal is higher than the reported by Radjenović et al. (2009), of 33% using SRT of 10 d and HRT of 11.5 h in activated sludge wastewater system and higher than the reported by Suarez et al. (2010), of 92% using SRT>50 d in nitrifying activated sludge. Radjenović et al. (2009) evaluated also two pilot-scale membrane bioreactors and they found fluoxetine removals of 98 % which were obtained with HRT>7 h, much greater than the used in this study. There were not significant differences between the fluoxetine removals obtained in the reactors packed with PE types and PU cubes when the effluent recirculation was applied despite the higher removals of $NH_4$-N and SRT in the reactors with PU cubes. This finding is in accordance with the reported by Fernandez-Fontaina et al. (2012); they indicated that no correlation between biodegradation kinetic constants and specific nitrification rate had been found for fluoxetine. The greatest differences between the removals in the reactors packed

with different materials were observed for mefenamic acid. Metoprolol presented higher removals than the obtained for mefenamic acid in the reactors with PE tapes during all experimental phases. This relationship was also obtained for PU reactors operated with OL of 6 and 12 gCOD $m^{-2}d^{-1}$ with and without recirculation, almost same removals were obtained at OL of 3 gCOD $m^{-2}$ $d^{-1}$ without recirculation and at OL of 9 gCOD $m^{-2}$ $d^{-1}$ with and without recirculation; however an inverse relationship was obtained at OL of 3 gCOD $m^{-2}$ $d^{-1}$ with recirculation. With reference to mefenamic acid, Radjenović et al. (2009) found removals of 40 and 35% in two membrane bioreactors (HRT>7 h). The reactors with PE tapes and PU cubes allowed higher removals applying OL of 3 and 6 gCOD $m^{-2}d^{-1}$, using or not effluent recirculation, with SRT of 15-37 d and HRT of 1.2-4.3 h. The reactors with PU cubes reached higher removals of mefenamic acid during all the experimental phases. The highest mefenamic acid removal of 82% was obtained with SRT of 26-32 d and HRT of 3.1-4.3 h (OL of 3.0 gCOD $m^{-2}d^{-1}$ and 50% recirculation) in the reactor with PU cubes. Kovalova et al. (2012) reported removals of 92 % for a membrane bioreactor operated with SRT of 30-50 d and HRT of 98 h, much greater than the ones used in this study. Falås et al. *(*2012) demonstrated that the reactors with Kaldnes K1 biofilm carriers and Biofilm Chip reached higher removal of mefenamic acid compared to nitrifying activated sludge processes. Despite the activated sludge biomass showed significantly higher nitrification rates than the carrier biomass. It indicates that the difference in mefenamic acid removal was due to a difference in the heterotrophic microbial community, while a clearly positive trend between the nitrification capacity and the rate constants was observed in the carrier reactors. Radjenović et al. (2009) found metoprolol removals of 24% (SRT of 10 d; HRT of 11.5 h) in activated sludge and removals of 44 and 29% in two membrane bioreactors (HRT>7h). Higher metoprolol removals were obtained in this study during all the experimental phases. Later, Kovalova et al. (2012) reported metoprolol removals of 55±13 % in a membrane bioreactor at SRT of 30-50 d and HRT of 98 h. The reactors with PE tapes and PU cubes allowed removals higher than 55% applying OL of 3, 6 and 9 gCOD $m^{-2}d^{-1}$, using or not effluent recirculation, with SRT of 11-37 d and HRT of 1.0-4.3 h. The reactors with PU cubes reached higher removals of metoprolol during all the experimental phases. The highest metoprolol removal of 73% was obtained with SRT of 26-32 d and HRT of 3.1-4.3 h (OL of 3.0 gCOD $m^{-2}d^{-1}$ and 50% recirculation) in the reactor with PU cubes. Vieno et al. 2007 found that there was no clear correlation between the SRT applied in sewage treatment plants and the elimination of the pharmaceuticals like metoprolol. According to another study, metoprolol was degraded faster by aerobic suspended biomass than attached biomass, where the nitrification was higher for the attached growth than for the suspended growth (Falås et al. 2013).

## CONCLUSIONS

The aerated submerged attached growth reactors with two biomass support materials (polyethylene tapes and polyurethane cubes) were able to remove fluoxetine, mefenamic acid and metoprolol from municipal wastewater up to 95, 82 and 73 % respectively. The reactors packed with polyurethane cubes showed a better performance compared with the ones with polyethylene tapes. This difference was considerably greater for the mefenamic acid, which can be attributed to the higher solid retention times obtained in the reactors with polyurethane cubes. The low organic loads, high solid retention times and the use of effluent recirculation enhanced the removals of the pharmaceutical compounds. When recirculation was applied, an increase of $NH_4$-N removals and nitrification activity were observed, despite the reduction of the SRT. The highest removals of fluoxetine, mefenamic acid and metoprolol were achieved at organic load of 3.0 gCOD $m^{-2}$ $d^{-1}$ with 50 % effluent recirculation (HRT of 3.1-4.3 h; SRT of 19-32 d). The removals were 94.0±0.3, 77.6±2.7 and 67.5±4.3% respectively in the reactor with polyethylene tapes and 94.9±0.8, 81.7±3.5, and 72.7±5.1% respectively in the reactors with polyurethane cubes. The highest $NH_4$-N removals and nitrification activity were obtained at this operational condition.

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