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Fate of β-blockers in aquifer material under nitrate reducing conditions: Batch experiments

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HIGHLIGHTS

- Fate of three β-blockers in aquifer material, denitrifying conditions, batch tests.
- Abiotic and bio-activity related processes affecting atenolol.
- Atenolol biotransformed to atenololic acid; quantitative study, zero order kinetics.
- No mineralization for atenolol; atenololic acid stable under the studied conditions.
- Abiotic processes dominating the decline of metoprolol and propranolol.

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ABSTRACT

The fate of the three environmentally relevant β-blockers atenolol, metoprolol and propranolol has been studied in batch experiments involving aquifer material and nitrate reducing conditions. Results from the about 90 d long tests indicate that abiotic processes, most likely sorption, jointly with biotransformation to atenololic acid were responsible for the 65% overall removal observed for atenolol. Zero order kinetics, typical of enzyme-limited reactions, controlled the transformation of this beta blocker to its corresponding carboxylic acid. The mass balance evidences that no mineralization of atenolol occurs in the biotic experiment and that atenololic acid is more stable than its parent compound under the studied conditions. This finding stresses the importance of considering atenololic acid as target compound in the environmental studies on the fate of atenolol. For metoprolol and propranolol the results from the experiment suggest a slower sorption to be the dominant removal process, which led to final decreases in concentrations of 25–30% and 40–45%, respectively. Overall, the removals observed in the experiments suggest that subsurface processes potentially constitute an alternative water treatment for the target beta-blockers, when compared to the removals reported for conventional wastewater treatment plants.

1. Introduction

Beta-blockers (β-adrenergic receptor antagonists) are a class of widely prescribed cardiovascular drugs, used for the treatment of hypertension, cardiac arrhythmias, cardio protection after heart attacks, and anxiety disorders. After excretion, substantial amounts of these drugs get into the wastewater and end up in sewage treatment plants. Conventional wastewater treatments cannot remove them efficiently (Lin et al., 2009; Radjenovic et al., 2009; Gabet-Giraud et al., 2010; Gros et al., 2010). Therefore, β-blockers are discharged into surface waters, where indeed they have been detected at concentrations ranging from ng L^{-1} to μg L^{-1} (Kasprzyk-Hordern et al., 2008; Muñoz et al., 2009; Martínez Bueno et al., 2010; Nödler et al., 2011). Ecotoxicological studies reported β-blockers to affect the aquatic organisms, propranolol being the most harmful one (Fent et al., 2006; Küster et al., 2009). Several studies have thus been devoted to foster the understanding of their behaviour in aquatic-sediment systems. Such studies focus on phototransformation, aerobic biotransformation and sorption to river sediments rich in organic carbon (Andreozzi et al., 2003; Liu and Williams, 2007; Yamamoto et al., 2009; Ramil et al., 2010). However, information on their fate in subsurface environments, especially under reducing conditions, is still scarce. Kibbey et al. (2007) studied the adsorption of nadolol, metoprolol and propranolol to a natural alluvial material, as well as to six individual mineral subcomponents of the sediments in batch experiments. Their results suggested that hydrophobicity serves as a predictor of adsorption even to low carbon sorbents, with propranolol, the most hydrophobic compound studied, adsorbing to the greatest extent. At river

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bank filtration sites in Germany, Schmidt et al. (2007) observed removals of up to more than 70% for atenolol, metoprolol, bisoprolol and sotalol under redox conditions varying from aerobic to strictly anaerobic. Finally, Ternes et al. (2007) reported complete elimination of atenolol, celiprolol, metoprolol, propranolol, and sotalol under unspecified redox conditions during irrigation of an agricultural field with secondary treated sewage, and attributed the removal to biodegradation rather than to sorption.

In this context, and motivated by a managed artificial recharge pond project in the Llobregat River (Barcelona, Spain), we studied the behaviour of three environmentally relevant β-blockers atenolol, metoprolol and propranolol in aquifer material under nitrate reducing conditions. Results from the batch experiments performed are reported in the present paper.

2. Materials and methods

The set up was based on microcosms containing natural sediments, synthetic water and a mixture of organic pollutants at individual initial concentrations of 1 mg L^{-1}. The experiment included biotic and abiotic series to identify the effect of microbial activity. Nitrate reducing conditions were stimulated in the biotic series by adding easily degradable organic compounds as electron donors (sodium acetate and methanol), and an excess of nitrate as specific electron acceptor.

2.1. Materials and experimental procedure

Sediments were obtained from a test site for artificial recharge of groundwater through ponds located in Sant Vicenç dels Horts (Barcelona, Spain). The aquifer consists of quaternary alluvial sediments, mainly gravel and sand with a small fraction of clay. Samples were collected from an oxic unsaturated horizon at about 1 m depth under the bottom of the infiltration pond, prior to the start of water recharge operations. They were sieved to <1 mm and immediately used for assembling the experiments. Their mineralogical and chemical analysis revealed the presence of silicates, carbonates (calcite, dolomite), and some Mn(IV)/Mn(III) and Fe(III) oxides and oxide–hydroxides (associated content of Mn and Fe: 0.07 and 5.8 mg g^{-1} of air dried sediment, respectively). The fraction with grain size <4 μm ranged between 2% and 6%. Total carbon content was 2.5%, with an organic carbon and nitrogen content smaller than 0.2%.

Experiment water was artificially prepared based on the chemical composition of the recharge water (Llobregat river water) at the test site. Magnesium nitrate hexahydrate was used to add 66 mmol L^{-1} of NO_3^-. The resulting water was purged with argon during 1 h to remove dissolved oxygen. Concentrations (mmol L^{-1}) of cations and anions were as follows: 7.8 (Na), 1.0 (K), 3.0 (Ca), 34.3 (Mg), 12.8 (Cl^-), 66.1 (NO_3^-), 2.1 (SO_4^{2-}), 0.5 (Alk), 0.1 (NH_4^+), and 0.02 (PO_4^{3-}). No dissolved organic carbon (DOC) was present in solution at this stage.

Atenolol, metoprolol and propranolol were added to the water by the use of a spiking solution. Atenolol and propranolol were purchased from Fagron (Barsbüttel, Germany), and metoprolol from Sigma–Aldrich (Steinheim, Germany). Atenololic acid was purchased from LGC Promochem (Wesel, Germany). The internal standards (IS) atenolol-D_7 and metoprolol-D_7 were also purchased from Sigma–Aldrich. Individual stock solutions of the analytes were prepared by dissolving each compound in methanol. Working standard mixtures were then prepared at different concentrations by further dilution of the individual stock solutions in methanol, to be used as spiking solution and to prepare the aqueous calibration standards (Section 2.2). Stock and working standard solutions were stored at −18 °C in the dark. The physicochemical characteristics of the three selected β-blockers are shown in Table 1.

The assembling of the batches was carried out inside a glove box, under argon (Ar) atmosphere. The "initial water" was obtained by adding 2.1 mmol of anhydrous sodium acetate and 3 mL of spiking solution (0.333 mg of each pollutant per mL methanol) per L of the synthetic water, yielding an analytical initial DOC of 71.5 mmol L^{-1} and a concentration of 1 mg L^{-1} for each organic pollutant. After sampling the solution for chemical analyses, the microcosms were prepared by filling 0.3 L glass bottles with 120 g of air-dried homogenized sediments, and 240 mL of "initial water". Assembling was concluded by properly sealing the bottles against oxygen with screw-caps plus a PTFE protection seal, and gently shaking. A remaining headspace of 15 mL was left in each bottle. Batches were then removed from the glove box, wrapped in aluminium foil to prevent photodegradation, and incubated at 25 ± 2 °C. On a regular basis, they were gently shaken.

For the abiotic tests, both synthetic water and sediments were sterilized prior to assembling. They were introduced three times (once a day in three consecutive days) into an autoclave at T = 121 °C and $P = P_{atm}$ + 1 atm for 20 min. The glove box was sterilized with UV light before introducing the materials. As an additional precaution, 0.22 mmol L^{-1} of mercury chloride were added (as microbial poison) to the "initial water".

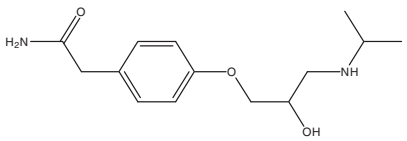
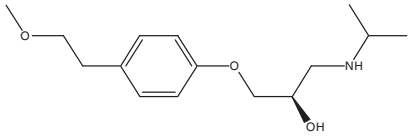
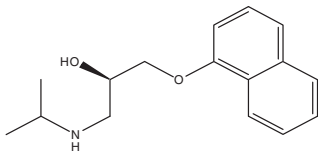
Duplicate bottles were sacrificed according to a pre-defined schedule (at days 2, 5, 10, 15, 25, 41 and 87). One at a time, the two bottles were opened under Ar atmosphere, chemical parameters were measured, and aqueous samples for the analysis of the general chemistry and β-blockers were collected and stored according to laboratory recommendations until analysis. The sterility of the abiotic series was verified twice during the full duration of the tests. To this aim, an aliquot of water from selected samples was spread on tryptic soy agar (TSA) plates and incubated in duplicate at 25 °C under aerobic conditions (during 1 week) and anaerobic conditions (during 2 weeks). None of the plates displayed microorganisms' growth.

2.2. Analytical methods

Biotic samples collected during microcosms' assembling/disassembling for analysing Cl^-, NO_3^-, NO_2^-, SO_4^{2-}, PO_4^{3-}, F^-, NH_4^+ and dissolved organic carbon (DOC) were filtered through 0.45 μm PALL Acrodisc® Sterile Syringe Filters with Supor® membrane and frozen. Anions were analysed by ion chromatography using an ICS-1000 instrument. The analytical error was estimated to be 14% for PO_4^{3-} and 13% for the remaining anions. NH_4^+ concentration was analysed with a selective electrode Orion 9512. DOC was analysed by 680 °C combustion catalytic oxidation/NDIR method using a TOC-V CSH instrument. The estimated analytical error was 20%. Biotic and abiotic samples for analysing chemical oxygen demand (COD) were also filtered 0.45 μm and frozen, to be further analysed by colorimetry with the Spectroquant Nova 60 spectrophotometer. Abiotic samples for Cl^-, NO_3^-, SO_4^{2-} and F^- were frozen and then analysed by ion chromatography using a Dionex DX-320 instrument with conductometric detection, a Dionex AS11-HC (2 × 250 mm) column and 23 mM KOH as eluent (isocratic separation at 30 °C). A flow rate of 0.38 mL min^{-1} was applied. Prior to chromatography, samples were filtered (Whatman Anotop 10 IC, 0.2 μm). The analytical error was estimated to be 8%.

Samples for Fe and Mn, Ca, Mg, Na, K and minor elements were also filtered at 0.45 μm, acidified and stored at 4 °C. They were later analysed by inductively coupled plasma atomic emission spectrometry (ICP-AES) using a Thermo Jarrel-Ash Iris Advantage HS instrument. Detection limits were 100 μg L^{-1} for K and Na, and 50 μg L^{-1} for the rest. The analytical error was estimated below 3%. In the ICP-AES analyses, calibration with three laboratory sets

Table 1
Physicochemical properties of the target β-blockers.

Compound	Structure	CAS number	log K_{ow}[a]	pk_a[a]	Formula
Atenolol		29122-68-7	0.1 ± 0.28	9.2 ± 0.4	$C_{14}H_{22}N_2O_3$
Metoprolol		37350-58-6	1.79 ± 0.4	9.2 ± 0.4	$C_{15}H_{25}NO_3$
Propranolol		525-66-6	3.48[b]	9.42[b]	$C_{16}H_{21}NO_2$

[a] SciFinder predicted values (unless otherwise noted), predominantly positively charged at typical groundwater pH 7.
[b] From SRC database.

of standards was performed every 10 samples, and regression coefficients of the calibration curves exceeded 0.999.

Temperature and pH (Thermo Scientific 9157BN Triode pH electrode, refillable), electrical conductivity (Hanna Instruments, 76302 W conductivity probe), dissolved oxygen (Hanna Instruments, HI 76407/4 DO probe) and alkalinity (drop test kit Taylor K-1726, precision of 0.5 mmol L^{-1}) were measured during the assembling/disassembling procedure.

Samples for the analysis of β-blockers were kept frozen until analysis. Atenolol and metoprolol were quantified without preconcentration by an HPLC/ESI-MS–MS method as described by Nödler et al. (2010). The individual MS–MS parameters of atenololic acid, propranolol and metoprolol-D_7 were as follows: for atenololic acid, the quantifier and qualifier transitions in positive electrospray (+ESI) were 268 → 145 and 268 → 191, respectively. The respective collision energies were −17.5 V and −12 V, and the capillary voltage was set to 60 V. For propranolol, the quantifier and qualifier transitions in +ESI were 260 → 116 and 260 → 183, respectively. The applied collision energy was −8.5 V for both transitions and the capillary voltage was set to 55 V. For metoprolol-D_7, the quantifier transition in +ESI was 275 → 123, the applied collision energy was −11.5 V, and the capillary voltage was 55 V. Prior to analysis, the samples were diluted (v/v) 1:2 with aqueous 5 mM ammonium acetate solution, containing 4% methanol. Atenolol-D_7 was used for the quantification of atenolol and propranolol. Metoprolol-D_7 was used for that of metoprolol. 100 ng mL^{-1} of each IS was added. For additional matrix compensation, calibration standards were prepared in inorganic matrix according to 50% of the experimental water concentration. Before analysis, all samples and standard solutions were centrifuged at 1500 rpm (Christ RVC 2-18, purchased from Fisher Scientific, Schwerte, Germany) for 30 min at room temperature. Six concentration levels (1-500 ng mL^{-1}) were used for the calibration and the correlation coefficients exceeded 0.99.

3. Discussion of results

Results are presented below in terms of averages of data from the duplicate batches. For the organic pollutants, concentrations are presented in relative terms (C/C_0, where C_0 is the measured initial concentration) in order to remove systematic errors from the analyses. Error bars were calculated by combining the analytical errors and the difference between results of duplicate batches.

3.1. Bulk water chemistry

The chemical evolution of the main water chemistry in the biotic experiment is presented in Fig. 1. Results from duplicate batches showed a satisfactory reproducibility at all sampling times. When plotting the data plus the error bars from each batch, there was always some overlap. Thus, only the average of results from the duplicate bottles is reported in the figure.

DOC and nitrate decreased over the whole experiment, starting (appreciably) after day 5 and being still present at day 87 with final concentrations of 27.2 mmol L^{-1} and 7.5 mmol L^{-1}, respectively. Alkalinity increased continuously after day 2, from 0.8 mmol L^{-1} to 22 mmol L^{-1}. Dissolved oxygen was not detected at any time. Nitrite was already detectable at day 2 but its concentration only began to increase appreciably after 2 d, reaching its maximum at day 41 and becoming completely depleted by day 87. Dissolved manganese and iron were not detected, sulphate remained constant throughout the experiment (results not shown), and pH ranged between 7.3 and 8.3.

The previous observations, consistent with the expected evolution of the redox sensitive species, suggest that nitrate reducing conditions were established within less than 2 d of microbial adaptation, and dominated the system during the whole test. The occurrence of nitrite reflects the actual denitrification pathway, with nitrite being an intermediate product between nitrate and nitrogen. Its depletion between day 41 and 87, when nitrate

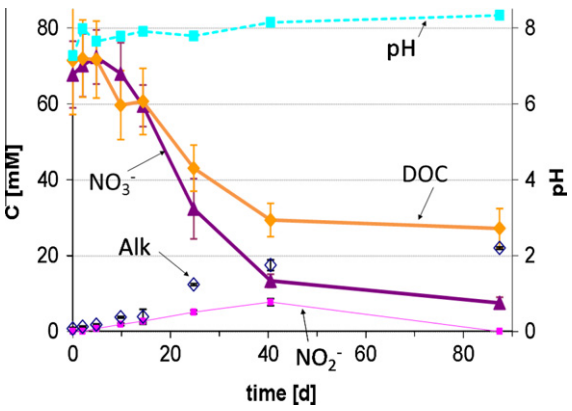


Fig. 1. Evolution of bulk water chemistry in the biotic experiment.

reduction was still occurring, can be attributed to nitrite reduction toward nitrogen being faster than the production of nitrite from nitrate during this time.

Quantitative consistency of the expected degradation processes was checked by a mass balance of electrons and major elements. Assuming that the only process that can change nitrate concentration was reduction caused by the oxidation of organic matter (i.e. acetate and methanol in the present experiment), we considered the overall stoichiometries:

$$CH_3OH + 1.2NO_3^- + 0.2H^+ \rightarrow HCO_3^- + 0.6N_2 + 1.6H_2O \quad \text{(Reaction 1)}$$

$$CH_3COO^- + 1.6NO_3^- + 0.6H^+ \rightarrow 2HCO_3^- + 0.8N_2 + 0.8H_2O \quad \text{(Reaction 2)}$$

plus an additional reaction:

$$CH_3OH + 0.4NO_3^- + 0.4H^+ \rightarrow CH_2O + 0.2N_2 + 1.2H_2O \quad \text{(Reaction 3)}$$

which takes into account that in the case of methanol (oxidation number of carbon = −2) the formation of biomass (CH_2O used as a simplified formula) requires a partial oxidation (bulk biomass has a redox state of 0). By using such reactions, it was calculated that 5–10% of the organic carbon consumed during the experiment was converted into biomass, while the remaining part was mineralized. By the overall measured decrease of calcium and magnesium (not shown) it was then estimated that about 24.3 mmol L^{-1} of the generated inorganic carbon precipitated as carbonates. Accounting for inorganic carbon transfer to the gas phase in the headspace of the bottles, alkalinity build up and precision of alkalinity measurements, the overall inorganic carbon mass balance could be finally closed with an error of about 10%.

Regarding the abiotic experiment, hydrochemistry remained practically constant for the whole time, as expected (results not shown).

3.2. Fate of the selected β-blockers

3.2.1. Fate of atenolol and metoprolol

Results for atenolol in the biotic and abiotic control series (Fig. 2) display a similar trend during the first 5–10 d, suggesting that the initial disappearance of atenolol (of about 14%) was dominated by abiotic processes. This abiotic removal can be attributed to sorption affinity e.g. on the negative charged surface of clay minerals because atenolol, with a pK_a of 9.2, is predominantly positively charged in the pH range of these experiments. Taking into account the error bars, no further removal could be observed in the abiotic series after day 10. Since atenolol $\log K_{ow}$ is very low (~0.1), no additional sorption onto biofilm should be expected during the biotic experiment. From day 10 on, therefore, the removal of atenolol in the denitrifying series must be related to microbial activity. Only 35% of the initial atenolol was left at the end of the biotic experiment (day 87).

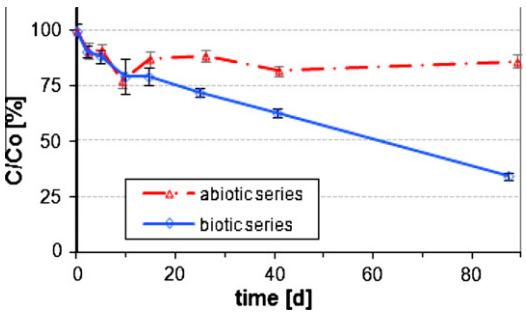


Fig. 2. Evolution of atenolol average concentration (normalized to the initial concentration Co) during the biotic experiment and its abiotic control.

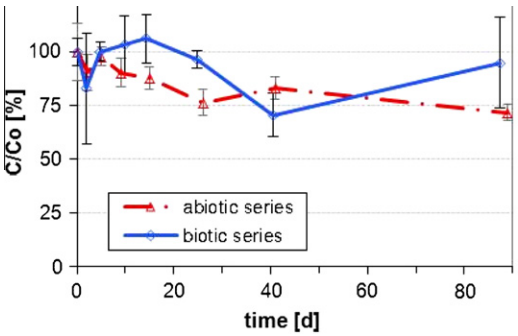


Fig. 3. Evolution of metoprolol average concentration (normalized to the initial concentration Co) during the biotic experiment and its abiotic control.

To better understand the fate of atenolol, all samples were analysed for atenololic acid. This acid was identified by Radjenovic et al. (2008) as a microbial transformation product of atenolol, generated by hydrolysis of its amide bond. Indeed, atenololic acid could be detected in our microcosms. The fact that it could only be found in water from the biotic series confirmed the relationship of its formation with microbial processes. Actually the human and animal metabolite of metoprolol, metoprolol acid, is identical to atenololic acid (Lennard, 1985 and references therein; Fang et al., 2004). Therefore, it might be conjectured that the observed atenololic acid in our samples results from the biotransformation of both atenolol and metoprolol. However, the evolution of metoprolol in the biotic and abiotic series (Fig. 3) suggests abiotic processes to be responsible of the overall removal of this compound, of 25–30% at day 87. Thus, no biotransformation of metoprolol occurred in our tests and the atenololic acid solely originates from atenolol transformation.

The time evolutions of the concentrations of atenolol and atenololic acid are presented together in Fig. 4. In order to close the mass balance of atenolol in the biotic experiment we also plot the sum of the two compounds.

By the plot it could be observed that, even if some atenololic acid was already being formed, the sum of the two concentrations decreases during the first 10 d of the biotic experiment, most likely reflecting the predominance of atenolol sorption during this period. Afterwards, the sum remains constant, i.e. the amount of atenolol disappearing is consistent with atenololic acid build-up. Therefore, we conclude that from day 10 up to day 87 of the biotic

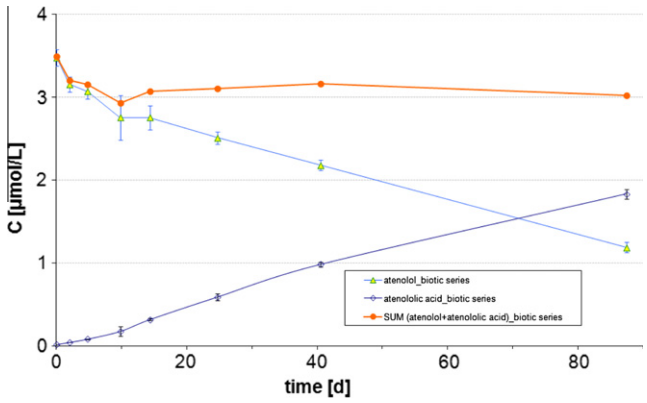


Fig. 4. Evolution of atenolol (blue), atenololic acid (purple) and the sum (red) concentrations with time. Symbols indicate sampling times and lines are plot for visualization aid. Concentrations are expressed as µmol L^{-1}. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

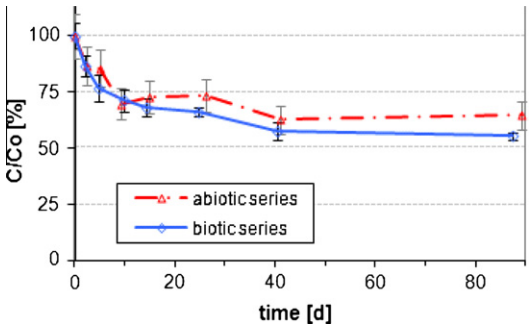


Fig. 5. Evolution of propranolol average concentration (normalized to the initial concentration Co) during the biotic experiment and its abiotic control.

experiment under denitrifying conditions, atenolol is predominantly being biotransformed to atenololic acid. Overall, the mass balance evidences no mineralization of atenolol during the whole experiment and the formed atenololic acid is stable under the conditions studied.

The likelihood of the process of amide hydrolysis, leading to the production of atenolol corresponding carboxylic acid, is consistent with the findings of Radjenovic et al. (2008), who performed biodegradation tests in aerated batch reactors using sewage sludge as inoculum. In a study on several amide-containing compounds, Helbling et al. (2010) observed hydrolysis to be a preferential biotransformation pathway for primary amides and also confirmed atenolol to be hydrolysed to atenololic acid. They furthermore proposed a mechanism for such enzyme-catalysed reaction, indicating amidases and proteolytic enzyme as the catalysts possibly involved. Such enzymes are ubiquitous in nature, and could obtain a quite high bioconversion yield by reactions involving C–N bond-containing substrates like amides (Fournand and Arnaud, 2001; Sharma et al., 2009).

It is worth mentioning that the arithmetic scale evolution of atenolol in the biotic series is virtually linear (removal rate of 0.021 μmol L^{-1} d^{-1}) after day 5–10 (Fig. 4). Such a zero order kinetic law indicates that the removal of atenolol associated to bioactivity was controlled by factors other than nitrate, DOC or atenolol concentration. The evolution of atenololic acid exhibited also a linear trend (Fig. 4), similar and opposite in sign to that of atenolol. A production rate of 0.021 μmol L^{-1} d^{-1} could be estimated, matching the rate of atenolol removal. In light of the microbial nature of the process, the observed zero order kinetics could be explained by an enzyme-limited transformation of atenolol to atenololic acid, i.e. a biotransformation in which the enzyme concentration represented the rate-limiting factor.

3.2.2. Fate of propranolol

The evolution of propranolol in the biotic and abiotic series (Fig. 5) presented almost the same trend over time. This implies that some slow abiotic process dominated the concentration decline for this compound, leading to an overall removal of 40–45% at day 87. We conjecture that it can be attributed to sorption affinity, e.g. of the cationic portion of propranolol on the negatively charged surface of clay minerals and of the neutral portion of the compound onto the small amount of organic matter existing in the sediments (pK_a 9.42, pH during the experiments between 7.3 and 8.3, and a significant $\log K_{ow}$ of 3.48).

4. Conclusions

Results from the about 90 d long batch experiments involving aquifer material and characterised by denitrifying conditions indicate that:

- Abiotic processes, most likely sorption of the positively charged species to clay minerals, dominated the first 5–10 d of the experiment in the case of atenolol, yielding a removal of about 14%. This beta blocker exhibited also an additional bioactivity-related removal in the biotic series. A total of 65% of atenolol has disappeared in such experiment by day 87.
- The detection of atenololic acid in the biotic, but not in the abiotic, series suggests that atenolol is biotransformed during the denitrifying experiment by hydrolysis of its amide bond, according to the mechanism proposed by Radjenovic et al. (2008). The temporal evolution of the concentration of both compounds reflects zero order kinetics, likely indicating an enzyme-limited biotransformation of atenolol into its corresponding carboxylic acid. It is relevant to notice that overall the mass balance evidences no measurable mineralization of atenolol during the whole experiment. In fact, its transformation product atenololic acid appears to be stable under the studied conditions, as it does not appear to be degraded nor sorb onto either minerals or biofilms. This finding stresses the importance of including atenololic acid as target compound in environmental studies on the fate of atenolol.
- There was no evidence for biotransformation or biologically mediated removal of metoprolol and propranolol. Some slow abiotic processes were responsible of the concentration decline for these blood pressure regulators, leading to overall removals of 25–30% and 40–45%, respectively, by day 87. Beside a potential sorption of the cationic species of both compounds e.g. to clay minerals, the higher removal observed for propranolol could be likely explained by a higher sorption affinity of its neutral species to the small amount of organic matter present in the sediments. Indeed, propranolol $\log K_{ow}$ (3.48) is significantly higher than the respective $\log K_{ow}$ of the two other target beta-blockers, atenolol and metoprolol.

It has to be added that the average removal reported for the three target β-blockers in conventional wastewater treatments plants ranges from 58% to 80% for atenolol, 20–40% for metoprolol, and 20–60% for propranolol (Lin et al., 2009; Radjenovic et al., 2009; Gabet-Giraud et al., 2010; Gros et al., 2010). These are comparable to the overall removals observed in our experiments (i.e. approximately 65% removal for atenolol, 25–30% for metoprolol, and 40–45% for propranolol). Therefore, we conclude that aquifer processes constitute an alternative water treatment for the studied beta-blockers. These processes can lead to similar or even higher elimination percentages than conventional wastewater treatments. The longer time needed to reach such removals may be ensured by the long residence times in aquifers. Further investigation is needed to confirm these findings at lower concentrations of the target pollutants.

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