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Short Communication

Pharmaceutical biodegradation under three anaerobic redox conditions evaluated by chemical and toxicological analyses

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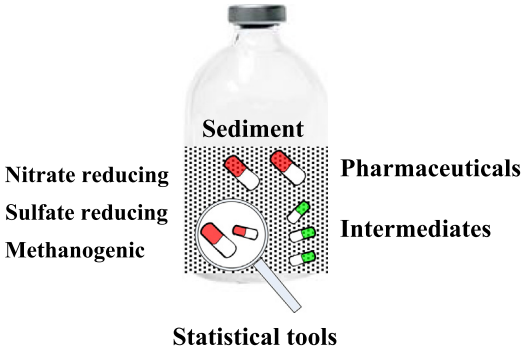
HIGHLIGHTS

- PhACs were biodegraded by a mixed culture enriched from wetland sediment inoculum.
- Different intermediates were observed under different redox conditions.
- Different intermediates were detected in the single and mixed PhACs conditions.
- A statistical tool was used to correlate the chemical and toxicological outcomes.
- Potentially toxic PhACs and their intermediates were indicated.

GRAPHICAL ABSTRACT



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ABSTRACT

Biodegradation of pharmaceutically active compounds (PhACs) in the subsurface layer of constructed wetlands (CWs) under various anaerobic redox conditions is rarely studied. In this study, CW sediment microbial populations were enriched for PhAC biodegrading organisms. Biodegradation effectivity of a mixture of six PhACs (caffeine, CAF; naproxen, NAP; metoprolol, MET; propranolol, PRO; ibuprofen, IBP; carbamazepine, CBZ) and single compounds (CAF, NAP) was investigated under nitrate reducing, sulfate reducing, and methanogenic conditions using chemical and toxicological analyses. Biodegradation efficiencies varied strongly among the six PhACs and three redox conditions chosen. CAF and NAP were completely biodegraded under sulfate reducing and methanogenic conditions whereas biodegradation efficiencies of the other PhACs were much less (MET, PRO < 20%; IBP, CBZ, negligible). CAF and NAP showed significantly lower biodegradation under nitrate reducing conditions than under the other two redox conditions. No difference was found in biodegradation efficiencies of CAF and NAP when present as single compound, or as a mixture with other PhACs. Different intermediates were observed, indicating different biodegradation pathways under different redox conditions and when the PhACs were present as single compound or in a mixture. From toxicological perspective, toxicity of PhACs and/or their intermediates to *Vibrio fischeri* was attenuated during the biodegradation process. Chemical and toxicological data showed positive correlations in principle component analysis, by which potentially toxic PhACs and intermediates are indicated for further ecotoxicological hazard assessment.

1. Introduction

The occurrence and adverse effects of pharmaceutically active compounds (PhACs) in the environment becomes one of the emerging

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environmental problems (Fatta-Kassinos et al., 2011). Wastewater treatment plants (WWTPs) are the main barrier to prevent PhACs from being discharged into surface water (Joss et al., 2006). Unfortunately, conventional WWTPs are not (yet) designed to efficiently remove PhACs (Verlicchi et al., 2012). Thus, residual PhACs and intermediates formed in WWTPs are discharged to aquatic ecosystems. As reported in a recent study, 37 PhACs were detected at 113 ng/L (median) in source water samples from 25 American drinking water treatment plants (Furlong et al., 2017). Although PhACs in the environment are present at low concentrations (ng/L or μg/L) and are unlikely to reach lethal toxicity (Di Nica et al., 2017), their persistent accumulation may lead to observable toxicological effects in key organisms in aquatic or terrestrial ecosystems after long-term exposure (Li and Randak, 2009). For example, the lowest observed effect concentration of propranolol (PRO) affecting reproduction in *Ceriodaphnia dubia* (water flea) was 250 μg/L, and reproduction in *Hyalella azteca* (crustacean) was affected at 100 μg/L after 27 days long-term exposure (Huggett et al., 2002). Although these effects are demonstrated at high concentrations, the effect of cocktails of a multitude of compounds is still unknown. Hence, cost efficient removal of PhACs from WWTP effluents is important and constructed wetlands (CWs) may be attractive for this purpose.

CWs are engineered systems designed to use natural processes to treat contaminants (Vymazal, 2007). It is reported that CW could be a sustainable, cost-efficient and easily maintained and operated system as a post-treatment process for PhAC removal (Kivaisi, 2001). A complicated interplay of physical, chemical and biological processes take place in CWs, such as photodegradation, phytoremediation, sorption and biodegradation. Among those processes, biodegradation plays a main role in the transformation and mineralization of micropollutants including PhACs (Li et al., 2014). Biodegradation of PhACs has been demonstrated and revealed a strong correlation with the availability of electron acceptors and redox conditions (Stottmeister et al., 2003). In the subsurface layer of CWs, due to the limited oxygen concentration, anaerobic processes predominate and different redox conditions take place simultaneously, such as nitrate reducing, sulfate reducing, and methanogenic conditions (Stottmeister et al., 2003).

Microbial community composition is dictated by the availability of electron acceptors that vary with specific redox conditions, and select for specific biodegradation processes (Faulwetter et al., 2009). Most of the studies addressing PhAC biodegradation focused on the aerobic biodegradation and the indigenous microbial populations present in the sediment and soil (Li et al., 2013; Lin and Gan, 2011; Yu et al., 2013; Zhang et al., 2013). In comparison, investigations of PhAC biodegradation under specific anaerobic redox conditions are scarce, and rarely focus on redox specific microbial populations in CW sediment. When looking at the effect of redox conditions on PhAC biodegradation, the limited studies mainly discuss the effect by comparing the biodegradation rates of parent compounds (Conkle et al., 2012). However, different microbial communities can be selected by different redox conditions, which may result in differences not only in biodegradation rates but also in biodegradation pathways. As a previous study observed, different intermediates of ^{14}C-radiolabelled sulfamethoxazole were produced under aerobic, anoxic, and anaerobic conditions (Alvarino et al., 2016). Toxicity of those varied intermediates is much less unknown compared with the parent PhACs (Celiz et al., 2009). Therefore, it is essential to gain more knowledge of PhAC anaerobic biodegradation under specific redox conditions by chemical and toxicological analyses of parent PhACs and their intermediates.

In this study, batch experiments were performed to explore the effect of specific anaerobic redox conditions on PhAC biodegradation by chemical and toxicological analyses. Six widely consumed PhACs with different physiochemical properties were selected as the target compounds. As PhACs are often present in a variety of mixture, biodegradation of PhACs as single compounds or as a mixture with other PhACs were compared to investigate the interaction between PhACs. This research thus aims to demonstrate the fate of PhACs under specific anaerobic redox conditions. This research is a first step towards increasing our understanding of PhAC biodegradation processes in the subsurface layers of CWs, and their contribution to reduce hazardous effects of PhACs and intermediates to aquatic ecosystems.

2. Materials and methods

2.1. Chemicals and reagents

PhACs were purchased from Sigma-Aldrich (USA): metoprolol (MET), caffeine (CAF), PRO, carbamazepine (CBZ), naproxen (NAP), ibuprofen (IBP), and fenoprofen calcium salt (internal standard). Mixed PhAC stock (20 mg/L) was prepared with MilliQ water (Millipore, USA). Acetonitrile with 0.1% formic acid, water with 0.1% formic acid, and methanol (Biosolve B.V., the Netherlands) were used for ultra-performance liquid chromatography (UPLC) analysis. All the other chemicals used are of analytical grade.

2.2. Batch experiment

Batch experiments were conducted to study PhAC biodegradation at different redox conditions with individual PhACs and a mixture of PhACs. The batch experiment included three phases: (1) cultivation of sediment microbial culture (15 months); (2) transfer of sediment inoculum to obtain liquid inoculum (6 weeks); (3) cultivation of enriched liquid microbial culture (10 weeks). Three types of liquid media were prepared according to previous works for nitrate reducing (Evans et al., 1991), sulfate reducing (Langenhoff, 1997), and methanogenic conditions (Holliger et al., 1993). The compositions of media for the three redox conditions are shown in Table S1 in the Supplementary materials. PhAC stock was prepared in Milli Q water. The gas phase in the batch bottles was CO_2/N_2 (20/80, v/v). Batch bottles were incubated on a shaker (120 rmp) at 20 °C. To avoid photodegradation of PhACs, all bottles were covered with aluminium foil.

In phase 1, the sediment for the inoculum was collected from two CWs that are in use for post-treatment processes of two Dutch WWTPs. The raw sediment was cultivated with a PhAC mixture (1 mg/L each) under three redox conditions for 15 months. The culture was amended with PhAC mixture every 2–3 months during cultivation. In phase 2, after these 15 months, 10% of the enrichment cultures from three redox conditions were transferred twice in 6 weeks (3 weeks incubation per transfer). Microorganisms were active before being transferred by removing >95% of CAF and NAP (Table S2). By transferring, a liquid enrichment culture without sediment was obtained and more active microorganisms for PhAC biodegradation were expected to be selected.

In phase 3, for each redox condition, three batch groups were designed with either a mix of six PhACs, or single CAF or NAP, which makes nine groups in total. CAF and NAP were selected to investigate the biodegradation when present as single or mixed resulting from their higher biodegradation compared with other PhACs during the enrichment. In each group, biotic batches were conducted in triplicate and abiotic controls in duplicate. Abiotic batches were autoclaved at 120 °C for 20 min with 4 times continuous repetition. Additionally, chemical inhibitors were added to further inhibit microbial activity in abiotic controls (0.3 g/L $HgCl_2$ and 1.3 g/L NaN_3). In each batch bottle, 10 ml liquid inoculum was mixed with 110 ml medium spiked with a mixture of six PhACs (1 mg/L each), or only CAF or NAP (1 mg/L). Blank controls with only the three types of media were prepared. Chromatography peaks of PhACs and their intermediates were obtained by excluding the peaks detected in the media blank controls from the peaks detected in the nine batch groups. The batch experiments lasted for 10 weeks and samples were taken at week 0, 3, 6, 8, 10 for chemical and toxicological analyses. Since there were PhACs incompletely biodegraded in the enrichment period, the residual PhACs and their intermediates were

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