Water Research 94 (2016) 111–119

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Enhanced sulfamethoxazole degradation through ammonia oxidizing bacteria co-metabolism and fate of transformation products

Elissavet Kassotaki [a], Gianluigi Buttiglieri [a, *], Laura Ferrando-Climent [a, b], Ignasi Rodriguez-Roda [a, c], Maite Pijuan [a]

[a] ICRA, Catalan Institute for Water Research, Scientific and Technological Park of the University of Girona, 17003, Girona, Spain
[b] IFE, Tracer Technology Department, Oil and Gas Section, Institute for Energy Technology, P.O. Box 40, NO-2027, Kjeller, Norway
[c] LEQUiA, Laboratory of Chemical and Environmental Engineering, University of Girona, Campus Montilivi, 17071, Girona, Spain

ARTICLE INFO

Article history:
Received 14 November 2015
Received in revised form
3 February 2016
Accepted 11 February 2016
Available online 15 February 2016

Keywords:
Micropollutants
Sulfamethoxazole degradation
Transformation products
Ammonia oxidizing bacteria
Nitrification rate

ABSTRACT

The occurrence of the widely-used antibiotic sulfamethoxazole (SFX) in wastewaters and surface waters has been reported in a large number of studies. However, the results obtained up-to-date have pointed out disparities in its removal. This manuscript explores the enhanced biodegradation potential of an enriched culture of Ammonia Oxidizing Bacteria (AOB) towards SFX. Several sets of batch tests were conducted to establish a link between SFX degradation and specific ammonia oxidation rate. The occurrence, degradation and generation of SFX and some of its transformation products (4-Nitro SFX, Desamino-SFX and N^4-Acetyl-SFX) was also monitored. A clear link between the degradation of SFX and the nitrification rate was found, resulting in an increased SFX removal at higher specific ammonia oxidation rates. Moreover, experiments conducted under the presence of allylthiourea (ATU) did not present any removal of SFX, suggesting a connection between the AMO enzyme and SFX degradation. Long term experiments (up to 10 weeks) were also conducted adding two different concentrations (10 and 100 μg/L) of SFX in the influent of a partial nitrification sequencing batch reactor, resulting in up to 98% removal. Finally, the formation of transformation products during SFX degradation represented up to 32%, being 4-Nitro-SFX the most abundant.

1. Introduction

In the recent years, the occurrence and fate of pharmaceutically active compounds (PhACs) has become an issue of great environmental concern due to their potential adverse effects on aquatic ecosystems and human health, and in combination with their increasing consumption. They are considered as emerging pollutants in water bodies because they still remain unregulated or are currently undergoing a regularization process, although the directives and legal frameworks are not set-up yet (Rivera-Utrilla et al., 2013).

Once administered, PhACs are metabolised to varying degrees and the excreted metabolites and/or unaltered parent compounds can also undergo further modification due to biological, chemical and physical processes in both sewage transport and treatment facilities, as well as in receiving water bodies (Jelic et al., 2015; Verlicchi et al., 2012). On the other hand, some of these substances can subsequently be transformed back to parent compounds during biological wastewater treatment (Göbel et al., 2005; Radjenović et al., 2007). Current wastewater treatment plants (WWTPs) are not specifically designed to remove these complex and persistent compounds; therefore they have been identified as a main point of discharge of PhACs into the environment (Buttiglieri and Knepper, 2008). Moreover even if parent compounds are not detected after treatment, transformation products (TPs) may still be of concern due to their potential stability or toxicity (Ternes et al., 2007).

Antibiotics, which are a major category of pharmaceuticals, have raised attention mostly because of the risk of a worldwide dispersal of concomitant resistance genes (Müller et al., 2013). Sulfamethoxazole (SFX) is a common antibiotic, which belongs to the class of sulfonamide antibiotics which were the first antimicrobial drugs utilized worldwide. SFX was among the 30 most frequently

* Corresponding author.
E-mail addresses: ekassotaki@icra.cat (E. Kassotaki), gbuttiglieri@icra.cat (G. Buttiglieri), laurafc@ife.no (L. Ferrando-Climent), irodriguezroda@icra.cat (I. Rodriguez-Roda), mpijuan@icra.cat (M. Pijuan).

http://dx.doi.org/10.1016/j.watres.2016.02.022
0043-1354/

detected organic wastewater contaminants as reported by the US Geological Survey (Kolpin et al., 2002), and also among the top 10 high priority pharmaceuticals identified in a European assessment of pharmaceutical and personal care products (De Voogt et al., 2009). In addition, it was among the most refractory pharmaceuticals tested in estuarine and coastal surface water samples with a half-life of 85 to more than 100 days, and it was suggested to be an excellent candidate as a wastewater tracer (Benotti and Brownawell, 2009).

SFX is a low adsorptive, polar sulfonamide, therefore its removal is mainly due to microbial activity (Müller et al., 2013). However, up-to-date investigations pertaining to SFX elimination through biodegradation including both laboratory and full scale studies, are marked by inconsistent results (Drillia et al., 2005; Larcher and Yargeau, 2012; Müller et al., 2013). The removal efficiency of SFX during wastewater treatment ranges from no removal in a conventional activated sludge (CAS) plant (Bendz et al., 2005) to removals greater than 98% in a CAS plant with nitrification (Levine et al., 2006). Conflicting removal efficiencies were also reported in laboratory scale studies. With CAS, Collado et al. (2013) reported SFX removals ranging from 36.5 ± 11.5% under nitrifying and denitrifying conditions. Alvarino et al. (2015) found removals of 57 ± 2% in an autotrophic nitrogen removal reactor (ELAN®) treating the supernatant of an anaerobic sludge digester. Also, Yang et al. (2012) observed SFX removals greater than 99% during batch tests lasting for 14 days and conducted with activated sludge collected from the WWTP of a food manufacturing company under aerobic conditions. Finally, Bouju et al. (2012) isolated five strains, two of these belonging to the phylum Actinobacteria while the other three to Proteobacteria, capable of mineralizing SFX. These discrepancies in reported SFX removals can be attributed to specific factors (e.g. sludge retention time, hydraulic retention time, redox conditions, pH, temperature, and micropollutant biodegradability) (Luo et al., 2014). Moreover they may be also attributed to variations in experimental conditions and especially to the use of different AS inoculum with different microbial communities (Onesios et al., 2009).

Higher removal rates of some pharmaceutical compounds have been reported in nitrifying systems (Fernandez-Fontaina et al., 2012; Tran et al., 2009). This enhanced biodegradation seems to be related to the activity of ammonia oxidizing bacteria (AOB) which could co-metabolize these compounds using one of its key enzymes, ammonia monooxygenase (AMO), responsible for ammonia oxidation (Forrez et al., 2011). It is known that AMO has a broad substrate range and it is capable of oxidizing a large variety of pollutants, simultaneously to the oxidation of ammonia, potentially being able to play a key role on several micropollutants' biodegradation (Tran et al., 2013). In the case of SFX, the results are again contradictory. Both Drillia et al. (2005) and Müller et al. (2013) found that nitrogen deficiency (no addition) enhanced SFX removal, at mg/L concentration, in lab-scale experiments that were executed under aerobic conditions with sludge withdrawn from a CAS system. These results suggest that SFX biodegradation may not be undertaken simultaneously with nitrification (process called co-metabolism) and that SFX at mg/L concentrations can serve either as organic carbon and/or nitrogen source, with autotrophic nitrifying bacteria potentially being the responsible group for SFX biodegradation in the latter case. On the other hand Fernandez-Fontaina et al. (2014) reported that SFX was slowly biodegradable (kbiol<1 L/gVSSd) at μg/L concentration with autotrophic nitrifying biomass. SFX removal was, nonetheless, enhanced at higher specific nitrification rates, this behavior being in accordance with the co-metabolic hypothesis and the fact that biotransformation of some compounds can rely on specific microbial populations. The observed SFX removal variability, combined with the extreme shortage of data on production and removal of its transformation products (TPs), which as reported by Majewsky et al. (2014) can present similar or higher ecotoxicological effects than SFX, highlights the necessity of more in depth exploration.

Up to our knowledge studies focusing on SFX removal by enriched AOB cultures while simultaneously exploring the occurrence of its TPs are currently lacking. The aim of the present work was to explore the biodegradation capacity of an enriched AOB culture (more than 80% of the microbial population belonging to the AOB group) towards SFX, under different conditions. Batch experiments were performed to evaluate i) the effect of the nitrification rate measured as specific ammonium oxidation rate (SAOR), ii) the role of AMO enzyme and iii) the effect of adding a carbon source in the nitrifying culture, on SFX degradation. In addition, the formation of two TPs of SFX: 4-Nitro-SFX and Desamino-SFX (Barbieri et al., 2012; Nödler et al., 2012) as well as of a human metabolite: N^4-Acetyl-SFX (Larcher and Yargeau, 2012), and their correlation with the parent compound were investigated. Finally, long term experiments on SFX removal, at 10 and 100 μg/L influent concentrations, were carried out in a partial nitrification sequencing batch reactor (SBR) treating synthetic reject wastewater, so as to investigate a longer hydraulic retention time (HRT) and possible acclimation factors.

2. Materials and methods

2.1. SBR operation for the enrichment of the AOB population

An 8L SBR was inoculated with activated sludge from a domestic wastewater treatment plant (WWTP) located in Girona (Spain). The reactor was operated in cycles of 6 h, consisting of feed-1 (1 min), aeration-1 (120 min), feed-2 (1 min), aeration-2 (120 min), waste (2 min), settling (103 min) and decanting (15 min). 1 L of synthetic reject wastewater (wastewater that simulates the effluent of an anaerobic digester in terms of ammonia and bicarbonate concentrations and is prepared in the laboratory) with a concentration of 1 g NH_4^+-N/L was added in each feeding period, while during the "waste" phase 2 L of the clarified supernatant were withdrawn. The HRT of the system was 24 h. The mixed liquor temperature was controlled at 30 °C using a water jacket, to mimic the common temperature conditions of reactors treating reject wastewater. Dissolved oxygen (DO) was controlled with a programmable logic controller (PLC) between 0.5 and 3 mg O_2/L and a minimum pH level was maintained at 6.7 by adding 1 M $NaHCO_3$ solution.

The synthetic wastewater composition was modified from Kuai and Verstraete (1998): 5.64 g/L of NH_4HCO_3 (1 g NH_4^+-N/L), 0.088 g/L of KH_2PO_4, 0.11 g/L K_2HPO_4 and 2 mL of trace element stock solution. The trace element stock solution was previously described elsewhere (Rodriguez-Caballero and Pijuan, 2013). Cycle studies were performed on a weekly basis to monitor the nitrification activity, where samples for the analyses of NH_4^+, NO_2^- and NO_3^- were taken along the cycle and immediately filtered through disposable Millipore filter units (0.22 μm pore size). The experiments detailed in this manuscript were conducted after more than 1 year of reactor operation, with a stable AOB population (more than 80% of the total microbial community) and with stable nitritation performance (95% of NH_4^+ converted to NO_2^- and no NO_3^- detected in the effluent).

2.2. Batch experiments

2.2.1. Batch reactor experimental setup

All batch tests were performed using a 1 L lab-scale Applikon stirred tank reactor coupled with a proportional-integral-derivative (PID) controller. Enriched AOB biomass was withdrawn from the nitritation SBR during the settling phase previously sparged with