Science of the Total Environment 584–585 (2017) 402–413

Contents lists available at ScienceDirect

Science of the Total Environment

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

Removal of pharmaceuticals from municipal wastewaters at laboratory scale by treatment with activated sludge and biostimulation

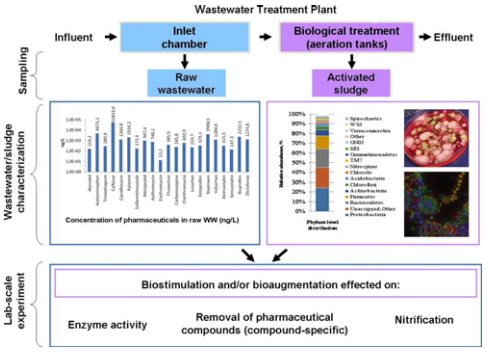
Olga Muter [a,*], Ingus P\erkons [b], Turs Selga [c], Andrejs Berzins [a], Dita Gudra [d], Ilze Radovica-Spalvina [d], Davids Fridmanis [d], Vadims Bartkevics [b]

[a] Institute of Microbiology & Biotechnology, University of Latvia, 1 Jelgavas Str., Riga LV-1004, Latvia
[b] Faculty of Chemistry, University of Latvia, 1 Jelgavas Str., Riga LV-1004, Latvia
[c] Faculty of Biology, University of Latvia, 1 Jelgavas Str., Riga LV-1004, Latvia
[d] Latvian Biomedical Research and Study Center, 1 Ratsupites Str., Riga LV-1067, Latvia

HIGHLIGHTS

- Nutrients stimulated the removal of the majority of pharmaceuticals detected in WW
- 21 pharmaceutical compounds (PCs) ranged from 13.2 ng/L to 51.8 μg/L in WW
- The majority of PCs with concentrations above 1 μg/L belong to NSAID
- Caffeine concentration exceeded other detected PCs by at least one order of magnitude
- Comparatively low rate of ibuprofen and diclofenac removal was observed after 7 days

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 December 2016
Received in revised form 3 January 2017
Accepted 4 January 2017
Available online 24 January 2017

Editor: D. Barcelo

Keywords:
Antimicrobials
Topic:
Biodegradation
Ion-Torrent Next-Generation Sequencing
Pharmaceuticals
Topic:
Wastewater

ABSTRACT

Municipal wastewater containing 21 pharmaceutical compounds, as well as activated sludge obtained from the aeration tank of the same wastewater treatment plant were used in lab-scale biodegradation experiments. The concentrations of pharmaceutical compounds were determined by high-performance liquid chromatography coupled to Orbitrap high-resolution mass spectrometry and ranged from 13.2 ng/L to 51.8 μg/L. Activated sludge was characterized in the terms of phylogenetic and catabolic diversity of microbial community, as well as its morphology. *Proteobacteria* (24.0%) represented the most abundant phylum, followed by *Bacteroidetes* (19.8%) and *Firmicutes* (13.2%). Bioaugmentation of wastewater with activated sludge stimulated the biodegradation process for 14 compounds. The concentration of carbamazepine in non-amended and bioaugmented WW decreased during the first 17 h up to 30% and 70%, respectively. Diclofenac and ibuprofen demonstrated comparatively slow removal. The stimulating effect of the added nutrients was observed for the degradation of almost all pharmaceuticals detected in WW. The most pronounced effect of nutrients was found for erythromycin. The results were compared with those obtained for the full-scale WW treatment process.

* Corresponding author.
E-mail address: olga.muter@inbox.lv (O. Muter).

http://dx.doi.org/10.1016/j.scitotenv.2017.01.023
0048-9697/

1. Introduction

The presence of pharmaceutical compounds (PCs) in the environment has received an increasing attention of researchers (Kümmerer, 2009). PCs are continuously introduced into the environment with personal hygiene products, pharmaceutical industry waste, hospital waste and therapeutic drugs, and are prevalent at small concentrations (Kolpin et al., 2002). Consumption of abused and illicit drugs, e.g., cannabinoids, methadone, etc., is a new concern for water management, posing serious risks to human health and ecosystem integrity (Mastroianni et al., 2013; Repice et al., 2013; Nefau et al., 2013). The potential chronic effects of these compounds associated with long-term ingestion of PCs mixtures through drinking water are still largely unknown (Kümmerer, 2001; Stackelberg et al., 2004; Reungoat et al., 2010; Rivera-Utrilla et al., 2013). Wastewater treatment plants (WWTPs) were considered to be the major source discharging PCs to the environment. Fish ponds are reservoirs of antibiotic resistance genes (ARGs), which also might imply potential risks to human health (Xiong et al., 2015). The concentrations of most PCs were diluted along the river stretches, with the largest decrease found in the smallest river where solutes underwent intense exchange between surface water and the sediments (Li et al., 2016). Seasonal variation in the stability of PCs along the watercourse was reported by (Meierjohann et al., 2016).

Typically, PCs are polar lipophilic molecules with more than one ionizable functional group, and most of them are moderately soluble in water. The molecular weight, structure, the identity of functional groups, and molecular shape can vary widely. Some PCs, such as erythromycin, cyclophosphamide, naproxen, and sulfamethoxazole can persist in the environment for more than a year, while others, such as clofibric acid, can persist for several years and may bioaccumulate to biologically active concentrations (Rivera-Utrilla et al., 2013).

Thus, effective removal of PCs along with other priority pollutants from wastewater (WW) prior to discharge into the environment remains an important issue. The effectiveness of PCs removal depends on the WWTP technology, operating conditions, microbial community composition, methods of disinfection, and other parameters (Khanal et al., 2006; Radjenovic et al., 2009; Suarez et al., 2008; Hedgespeth et al., 2012). Aerobic conditions were shown to have faster degradation kinetics for the majority of PCs, as compared to anaerobic ones. Anaerobic process was characterized as compound-specific and did not depend on operational parameters (Suarez et al., 2010; Gonzalez-Gil et al., 2016). Differences in the micropollutant removal kinetics by attached and suspended biomass were reported by (Falås et al., 2013). Activated carbon adsorption, ozonation and other advanced oxidation processes, as well as membrane filtration are used in the treatment process. Nevertheless, none of these processes can remove all the compounds of concern (Reungoat et al., 2010). Elimination pathways of pharmaceuticals can include sorption, biodegradation, phototransformation, and other processes (Meierjohann et al., 2016).

In this respect, microorganisms associated with the co-metabolic and metabolic degradation of pharmaceuticals in WW treatment process are intensively studied.

The biodegradation rate is dependent on the sludge retention time and sludge characteristics, temperature, pH, redox conditions, and other parameters (Kruglova et al., 2016; Bertelkamp et al., 2016; Cherik and Louhab, 2015). The presence of carboxy groups, hydroxyl groups, and carbonyl groups in the molecule can increase the biodegradation rate, while the presence of ethers, halogens, aliphatic ethers, methyl groups and ring structures can slow this process (Bertelkamp et al., 2016).

During biodegradation, PCs may undergo mineralization or transformation to either more hydrophobic or more hydrophilic derivatives (Halling-Sørensen et al., 1998; Kümmerer, 2003; Zhang et al., 2014). These processes have been shown to depend on the chemical characteristics of PCs, e.g., the presence of secondary, tertiary or quaternary carbon atoms as well as specific functional groups (Imfeld et al., 2009; Zhang et al., 2014). However, even small changes in chemical structure of PCs may change their solubility, polarity, and other properties that govern their environmental fate. In this respect, enzymatic reactions may result in a great variation of biodegradation rates, even within the same therapeutic class (Kümmerer, 2009). Metabolites of PCs also may be persistent and may have similar ecotoxicological effects (Kim et al., 2008; Zhang et al., 2014).

Biodegradation of PCs in WW could be limited due to the relatively high concentrations of other pollutants, which induce the activity of enzymes not relevant to the biodegradation of PCs. Besides, many PCs inhibit the growth or metabolism of microorganisms (Kim et al., 2008; Joss et al., 2005; Zhang et al., 2014). Addition of selected strains/mixed cultures to wastewater reactors is expected to improve the catabolism of specific compounds, including recalcitrant organic compounds (Shah, 2014; Semrany et al., 2012). Nevertheless, bioaugmentation in WWT frequently leads to inconclusive outcomes (Herrero and Stuckey, 2015).

This study was aimed at comparing the microbial activity in municipal WW containing 21 PCs, while applying biostimulation and/or bioaugmentation approaches under laboratory conditions. Activated sludge from the aeration tank of the same WWTP was used for bioaugmentation. The removal of PCs, microbial growth kinetics, enzymatic activity, as well as the concentration of proteins and ammonium (NH_4^+) ions were evaluated.

2. Materials and methods

2.1. Wastewater and activated sludge

WW was collected in May 2016 from the untreated WW basin of the major "Daugavgriva" WWTP near Riga, Latvia. The WW sample had the following characteristics: pH 6.7–6.9; biological and chemical oxygen demand up to 200 mg L^{-1} and 350 mg L^{-1}, respectively. Activated sludge was sampled from the aeration tank of the same WWTP. WW retention time in the aeration tank was 5 days.

WW samples were analyzed applying the HPLC-Q-Orbitrap-HRMS method described in our previous studies (Pugajeva et al., 2017). 21 PCs were included in our study and this selection was based on literature studies of pharmaceutical occurrence in municipal WWs in Europe and other countries. The compounds included in our study have the most frequent occurrence rate throughout the Europe and their concentration levels were also higher in comparison with other compounds. Detailed information on the analytical procedure is given in Supplement 3.

2.2. Biodegradation experiments

Prior to incubation, WW was filtered and aerated for 15 min. Afterwards, a 100 mL aliquot of WW was enriched with microorganisms and/or nutrient composition according to the experiment setup (Table 1). Incubation was performed in 200 mL columns in triplicate, at 24 °C, with periodic agitation (once a day) for 7 days. Sampling was performed after 1 h, 24 h, 48 h, and 168 h incubation. Nutrient composition consisted of 333 μL 30% sugar beet molasses containing 40% w/w sucrose (final concentration 0.1%), previously autoclaved for 20 min at 1 bar, and 500 μL cabbage leaf extract, prepared according to Muter et al. (2008) and sterilized by filtering through hydrophilic Minisart® Syringe Filter (Sartorius, Germany). Sludge-derived culturable bacteria (WB) and fungi (WF) were obtained by plating the activated sludge on Tryptone Glucose Yeast Extract Agar (TGA, Sifin, Germany) and Rose Bengal Agar with Chloramphenicol (Biolife, Italy), respectively. Bacteria and fungi were harvested after 48 h and 72 h, respectively, and the prepared suspensions contained 2.9×10^8 CFU/mL and 3.1×10^7 CFU/mL, respectively.