Science of the Total Environment 463–464 (2013) 355–365

Contents lists available at ScienceDirect

Science of the Total Environment

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

Impact of certain household micropollutants on bacterial behavior. Toxicity tests/study of extracellular polymeric substances in sludge

Laure Pasquini [a,*], Christophe Merlin [b], Lucille Hassenboehler [a], Jean-François Munoz [c], Marie-Noëlle Pons [d], Tatiana Görner [a]

[a] Laboratoire Environnement et Minéralurgie-CNRS, Université de Lorraine, 15 Avenue du Charmois, 54501 Vandœuvre-lès-Nancy Cedex, France
[b] Laboratoire de Chimie, Physique et Microbiologie pour l'Environnement-CNRS, Université de Lorraine, 15 Avenue du Charmois, 54501 Vandœuvre-lès-Nancy Cedex, France
[c] Laboratoire d'Hydrologie de Nancy, ANSES, 40 rue Lionnois, 54000 Nancy, France
[d] Laboratoire Réactions et Génie des Procédés-CNRS, Université de Lorraine, 1 Rue Grandville, 54001 Nancy Cedex, France

HIGHLIGHTS

- Triclosan altered the bacterial growth at concentrations measured in wastewater.
- Some compounds induced an increase of bound EPS in flocs.
- High concentrations of erythromycin or ibuprofen stopped the macropollution removal.
- The studied micropollutants were mainly removed from wastewater by biodegradation.

ARTICLE INFO

Article history:
Received 25 March 2013
Received in revised form 31 May 2013
Accepted 4 June 2013
Available online 2 July 2013

Editor: Damia Barcelo

Keywords:
Household micropollutants
Cytoxicity tests
Sludge extracellular polymeric substances
Micropollutants removal

ABSTRACT

The impact of eight household micropollutants (erythromycin, ofloxacin, ibuprofen, 4-nonylphenol, triclosan, sucralose, PFOA and PFOS (PFAAs)) on the laboratory bacterial strain *Escherichia coli MG1655* and on activated sludge from an urban wastewater treatment plant was studied.
Growth-based toxicity tests on *E. coli* were performed for each micropollutants. The effect of micropollutants on activated sludge (at concentrations usually measured in wastewater up to concentrations disturbing the bacterial growth of *E. coli*) was examined in batch reactors and by comparison to a control reactor (without micropollutants). The bound extracellular polymeric substances (EPS) secreted by the sludge were measured by size exclusion chromatography and their overexpression was considered as an indicator of bacteria sensitivity to environmental changes. The chemical oxygen demand (COD) and the ammonium concentration were monitored to evaluate the biomass ability to remove the macropollution.
Some micropollutants induced an increase of bound EPS in activated sludge flocs at concentrations depending on the micropollutant: erythromycin from 100 μg/L, ofloxacin from 10 μg/L, triclosan from 0.5 μg/L, 4-nonylphenol from 5000 μg/L and PFAAs from 0.1 μg/L. This suggests that the biomass had to cope with new conditions. Moreover, at high concentrations of erythromycin (10 mg/L) and ibuprofen (5 mg/L) bacterial populations were no longer able to carry out the removal of macropollution.
Ibuprofen induced a decrease of bound EPS at all the studied concentrations, probably reflecting a decrease of general bacterial activity. The biomass was not sensitive to sucralose in terms of EPS production, however at very high concentration (1 g/L) it inhibited the COD decrease.
Micropollution removal was also assessed. Ibuprofen, erythromycin, ofloxacin, 4-nonylphenol and triclosan were removed from wastewater, mainly by biodegradation. Sucralose and PFOA were not removed from wastewater at all, and PFOS was slightly eliminated by adsorption on sludge.

1. Introduction

In everyday life we use a large number of pharmaceuticals and complex chemical products in different household activities. These compounds, released in urban wastewater, are conveyed to wastewater treatment plants (WWTPs). WWTPs have been designed to eliminate macropollutants such as organic matter, nitrogen, phosphorus, and suspended solids. The removal of compounds such as pharmaceuticals, personal care products or detergents, present in low concentrations (μg/L, micropollutants) is not commonly investigated because few or no regulations exist for their release into water bodies.

* Corresponding author. Tel.: +33 3 83 59 62 64; fax: +33 3 83 59 62 55.
E-mail address: laure.pasquini@univ-lorraine.fr (L. Pasquini).

0048-9697/$ – see front matter
http://dx.doi.org/10.1016/j.scitotenv.2013.06.018

Scientific interest in their environmental impact or their behavior in WWTPs appeared began ten years ago, as reviewed by Pasquini et al. (2013), and some compounds are already thought to be a possible threat to environmental health and safety (Grice and Goldsmith, 2000; Baquero et al., 2008; Soares et al., 2008). The EU Parliament (2008) has established an initial list of just 41 substances or groups of substances as action priorities to ensure a "satisfactory chemical and biological status for surface and ground waters" by 2015. Some micropollutants, such as antibiotics or biocides present in soaps or in detergents are intended to have an effect on bacteria (Singer et al., 2002; Oo and Shah, 2012). Most of the WWTPs treating urban wastewater employ an activated sludge process as recently reviewed (Verlicchi et al., 2012). Therefore it appears necessary to answer the question whether domestically generated micropollutants (molecules biologically active or not) might have an impact on the biological action of activated sludge and affect the biological treatment efficacy. The possible adverse effects of pollutants towards bacteria are frequently studied using growth inhibition tests (see Schmitz et al. (1998) and Schneider et al. (2009) for instance) some of which are even standard procedures to assess water quality (EN ISO, 10712:1995, 1995). The micropollutant's impact on aquatic life is often studied by tests on algae, crustaceans, fishes… (Lussier et al., 2000; Isidori et al., 2005; Binelli et al., 2009).

The sludge from WWTP is a consortium of microorganisms and to our knowledge, only few studies have been performed on the impact of micropollutants on it. The WWTP biomass is a complex dynamic structure of bacteria embedded in a matrix of extracellular polymeric substances (EPS), composed of up to 90% of proteins and polysaccharides and secreted by microorganism metabolism (Wingender et al., 1999). Therefore the WWTP biomass behavior towards micropollutants can be examined by considering the EPS which constitute up to 70% of the sludge (Nielsen et al., 1997). This polymeric network enables microorganisms to live at high cell densities and to ensure their survival, adsorbing pollutants, nutrients and minerals (Finlayson et al., 1998; Flemming and Wingender, 2001). For example, bacteria in a biofilm were found to be thousand times more resistant to antibiotics than in liquid suspension (Everts, 2006). Microorganisms also release soluble microbial products (SMP) and both EPS and SMP have an excellent buffering action towards external changes (Huang et al., 2009). An overexpression of EPS or SMP can be considered as a response to stressful situations (Wingender et al., 1999), and an indicator of bacterial sensitivity to environmental changes (Aquino and Stuckey, 2004; Avella et al., 2010). This property has even been exploited to enhance production of EPS (with potentially interestingly biotechnological applications) by exposing bacteria to pollutants (Onbasli and Aslim, 2009). The study of EPS provides original information about the behavior of the biomass towards the presence of micropollutants, even at very low concentrations.

The phenomenon of overexpression of EPS or/and SMP was used here to investigate, in batch reactors, the impact of eight household micropollutants on the biomass from an urban WWTP. Preliminarily, cytoxicity tests were made on the laboratory bacterial strain *Escherichia coli MG1655* to check a relevant concentration range for the study of the target micropollutants. Two antibiotics (erythromycin and ofloxacin), an anti-inflammatory drug (ibuprofen), a biocide used in personal care products (triclosan), a detergent degradation product (4-nonylphenol), a widely used and persistent sweetener (sucralose) and two perfluoroalkyl acids (perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS)) were studied. Table 1 presents their concentrations reported in the influents of various wastewater treatment plants (WWTPs). These compounds were chosen because they are commonly used in a domestic context and for their specific physical and chemical properties (octanol/water partition coefficient log K_{ow}, solubility in water and pKa) (Table 1) in order to observe their fate and possible partitioning in water and sludge in WWTP process. One of the molecules (4-NP) is on the Water Framework Directive (EU Parliament, 2008) priority list; perfluorinated compounds are currently discussed to be part of the new priority list; other molecules are relevant on a social and research level for the preservation of the natural environment.

2. Materials and methods

2.1. Cytotoxicity tests

Bacteria were routinely cultured at 37 °C with shaking (160 rpm) in 250 mL conical flasks filled with 50 mL of Lysogenic-Broth (LB Broth, Miller, Difco™). Growth inhibition tests were carried out on *E. coli MG1655* as previously described (Schneider et al., 2009). Bacteria were pre-grown in LB medium without micropollutant until the cultures reached mid-log phase (optical density at 600 nm (OD_{600}) of approximately 0.2). Cultures were then diluted by one-tenth in pre-warmed LB medium spiked with a range of micropollutant concentrations, and the OD_{600} was measured at intervals. For each growth, the doubling times of exponentially growing cultures were calculated from the slope of a regression curve, and compared to the one obtained for control cultures in the same medium without micropollutants (20 ± 2 min (n = 16) for *E. coli MG1655*).

The toxicity of each compound was examined individually. The minimum concentrations were chosen so as to be close to those found in WWTP influents and effluents. Higher concentration levels (chosen based on micropollutant solubility) were also tested to estimate the toxicity threshold of the target compounds.

Although mixture effects can be significant according to some authors (Celander, 2011; Wunder et al., 2013), they were not examined in our study.

The experiments with poor water soluble micropollutants required the use of an alcohol based solvent (ethanol or methanol). In these cases, the effects of the solvents were also checked. However no significant differences were observed when compared with a standard culture.

2.2. Sewage sludge behavior in batch reactors

The biological response of the sludge sampled in a municipal WWTP to the presence of micropollutants was examined in batch reactors. The experiments were based on the protocol of Avella et al. (2010). The concentrations were chosen to be under toxicity levels previously established by toxicity growth tests on *E. coli*. The studied concentrations ranged from those usually measured in municipal wastewater to much higher concentrations in order to observe any possible impact. The study was performed over 24 h and several parameters, reflecting biomass activity, were measured:

- The production of EPS
- The capacity of the biomass to degrade macropollution (measured by the chemical oxygen demand, COD and ammonium concentration).
- Micropollution removal estimated by the quantification of micropollutants in liquid and solid phases.

Wastewater and sludge came from the WWTP of Nancy-Maxéville (France, 500,000 population equivalent). Grab samples of the wastewater (2 L) were collected in the WWTP tank recovering wastewater from all the catchments of the agglomeration and grab samples of the sludge (1 L) were collected in the recycling line (where the sludge is more concentrated). Wastewater and sludge were mixed in order to obtain the same biomass concentration as those of the WWTP aeration tank (about 2.5 g/L). Four batch reactors (4 L) operated simultaneously for 24 h with the biological mixture. They were aerated with an air diffuser on their bottom (4–5 mg/L of dissolved oxygen) and no nutrients were added during the experiments. One reactor was used as a control (without micropollutants added) and three reactors