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Is anaerobic digestion effective for the removal of organic micropollutants and biological activities from sewage sludge?

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ABSTRACT

The occurrence of emerging organic micropollutants (OMPs) in sewage sludge has been widely reported; nevertheless, their fate during sludge treatment remains unclear. The objective of this work was to study the fate of OMPs during mesophilic and thermophilic anaerobic digestion (AD), the most common processes used for sludge stabilization, by using raw sewage sludge without spiking OMPs. Moreover, the results of analytical chemistry were complemented with biological assays in order to verify the possible adverse effects (estrogenic and genotoxic) on the environment and human health in view of an agricultural (re)use of digested sludge. Musk fragrances (AHTN, HHCB), ibuprofen (IBP) and triclosan (TCS) were the most abundant compounds detected in sewage sludge. In general, the efficiency of the AD process was not dependent on operational parameters but compound-specific: some OMPs were highly biotransformed (e.g. sulfamethoxazole and naproxen), while others were only slightly affected (e.g. IBP and TCS) or even unaltered (e.g. AHTN and HHCB). The MCF-7 assay evidenced that estrogenicity removal was driven by temperature. The Ames test did not show point mutation in *Salmonella typhimurium* while the Comet test exhibited a genotoxic effect on human leukocytes attenuated by AD. This study highlights the importance of combining chemical analysis and biological activities in order to establish appropriate operational strategies for a safer disposal of sewage sludge. Actually, it was demonstrated that temperature has an insignificant effect on the disappearance of the parent compounds while it is crucial to decrease estrogenicity.

1. Introduction

A great number of organic micropollutants (OMPs) enters sewage treatment plants (STPs), including pharmaceuticals, personal care products, steroid hormones, industrial chemicals, pesticides and many others (Luo et al., 2014). Sludge is the endpoint of most hydrophobic pollutants through sorption (Carballa et al., 2008), but also of an important fraction of hydrophilic OMPs not biotransformed during the wastewater treatment. The concentrations of OMPs in sewage sludge are much dependent on their physicochemical characteristics and usage rates, varying strongly among countries or even the STPs. In general, hydrophobic substances, such as triclosan (TCS) and musk fragrances, are detected at important concentrations (up to 10,000 μg/kg), while much lower levels (10–100 μg/kg) are measured for hydrophilic pharmaceuticals, as diclofenac (DCF), trimethoprim (TMP), ibuprofen (IBP), naproxen (NPX), carbamazepine (CBZ) or sulfamethoxazole (SMX) (Stasinakis, 2012).

The presence of emerging pollutants in aquatic environments has already been considered by the Water Framework Directive, which establishes a "Watch List" and a priority list of substances. In this sense, some OMPs, such as DCF, 17α-ethinylestradiol (EE2), 17β-

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estradiol (E2), estrone (E1) and erythromycin (ERY), only have to be monitored, while the concentration of priority pollutants, such as nonylphenol (NP) and octylphenol (OP), is limited. On the contrary, the European regulation on sewage sludge use in agriculture (Directive 86/278/EEC) disregards the presence of most OMPs. Only 7 countries of the EU have included limits for some OMPs in sludge in their national legislation, although the maximum values and the target compounds (usually, halogenated organic compounds, linear alkyl benzene sulphonates, polychlorinated biphenyls, dibenzo-dioxins/dibenzofurans, phthalates, NPs and polycyclic aromatic hydrocarbons) vary among countries (Kelessidis and Stasinakis, 2012). Consequently, important environmental and human risks were already reported due to the accumulation of OMPs in biosolid-amended soils (Chen et al., 2014) and due to the leaching of these substances into groundwater after rainfalls (Barron et al., 2010). However, the upcoming regulatory trends for land application of biosolids (Inglezakis et al., 2014) are going to bridge this gap, so that the occurrence of OMPs in sludge is expected to become a "hot topic" for STP managers.

Anaerobic digestion (AD) is the most widely employed process for sludge stabilization. Some studies have already evaluated the fate of OMPs during sludge AD (Carballa et al., 2007; Malmborg and Magnér, 2015; Paterakis et al., 2012; Samaras et al., 2014) but the results are not always conclusive. All of them agreed that the temperature effect (mesophilic and thermophilic conditions) can be neglected, except for NP and their ethoxylates (NPE), and that CBZ is slightly affected by AD, while SMX, NPX, and TMP are highly removed. In contrast, the removal efficiencies of other OMPs are quite controversial; some studies reported low (<25%) or no removal of hormones (E1, E2, EE2) (des Mes et al., 2008; Malmborg and Magnér, 2015), musk fragrances (AHTN, HHCB) (Alvarino et al., 2014; Clara et al., 2011), DCF (Malmborg and Magnér, 2015; Narumiya et al., 2013), IBP (Alvarino et al., 2014; Malmborg and Magnér, 2015) and TCS (Narumiya et al., 2013), while other authors disagree. For instance, Carballa et al. (2007) found that musk fragrances and hormones were removed up to 95% and 70%, respectively; as well, Samaras et al. (2014) stated eliminations above 90% for DCF and IBP and between 60 and 80% for TCS. The causes of these discrepancies remain unclear.

In addition, to check the characteristics of the sludge before land-spreading and to set regulatory limits for OMPs, it is not enough to measure the disappearance of the parent pollutant via chemical analysis. In fact, it is essential to perform bioassays that assess the biological effect of the final discharge containing not only the residual parent compound but also the transformation products and other unknown compounds in view of their danger to the ecosystem and to humans (Escher and Leusch, 2012). Once released into the environment, some of them can exert their toxic effect directly interfering with the DNA of living organisms (mutagenic-carcinogenic risks) or with the endocrine system compromising reproductive and development functions in humans and wildlife species (de Jesus Gaffney et al., 2015; World Health Organization, 2006). Chemical analyses reveal adverse impacts with a compound-based approach, while bioassays provide an effect-based view, so both methods are complementary. Notwithstanding, the application of bio-analytical tools to sewage sludge is still very limited and most studies aimed at characterizing the sludge after composting (Kapanen et al., 2013; Patureau et al., 2012). In contrast, there is a very limited experience (Citulski and Farahbakhsh, 2012; Furlong et al., 2010) describing the effect of AD on specific modes of toxic action.

The main aim of this work was to combine chemical and biological methods in order to evaluate the fate of OMPs and the removal of estrogenic and genotoxic activities during mesophilic and thermophilic sludge digestion, at environmentally relevant concentrations (no OMPs spike was performed). To the best of our knowledge, this is the first study conducting an integrated assessment of the biotransformation of OMPs and these specific toxicities to evaluate the effectiveness of different AD strategies.

2. Materials and methods

2.1. Organic micropollutants

20 compounds commonly used in daily life were considered in this study: three musk fragrances, galaxolide (HHCB), tonalide (AHTN) and celestolide (ADBI); three anti-inflammatories, ibuprofen (IBP), naproxen (NPX) and diclofenac (DCF); four antibiotics, sulfamethoxazole (SMX), trimethoprim (TMP), erythromycin (ERY) and roxithromycin (ROX); four neurodrugs, fluoxetine (FLX), carbamazepine (CBZ), diazepam (DZP) and citalopram (CTL); three endocrine disrupting compounds, triclosan (TCS), 4-octylphenol (OP) and 4-nonylphenol (NP); and three hormones, estrone (E1), 17β-estradiol (E2) and 17α-ethinylestradiol (EE2).

2.2. Sewage sludge

A mixture of primary and secondary sludge (70/30, v/v), coming from the thickener and the activated sludge flotator of a nearby STP in Santiago de Compostela (Spain), was used. The STP is designed for 184,000 population equivalent with an average wastewater flowrate of approximately 55,000 m^3/d, which is mainly composed by domestic wastewater (hospital discharges represent 1–2% of the total flowrate). The characteristics of the mixed sewage sludge were almost stable along the experimental period (330 d). The average pH was 5.4 ± 0.3, the total and soluble chemical oxygen demands (COD) were correspondingly 34.5 ± 5.9 g/L and 2.9 ± 1.0 g/L, the average concentration of total (TS) and volatile (VS) solids were 28.8 ± 5.5 g/L and 22.3 ± 4.1 g/L respectively, and the content of volatile fatty acids (VFA) was 2.1 ± 0.9 g/L. More differences would be expected regarding the season, because of rainfalls, but it seems that the main factor affecting the sludge characteristics was the operation of the STP. The measured values are in accordance with previously reported data for sewage sludge coming from the same STP (Carballa et al., 2007).

2.3. Lab-scale anaerobic digesters and monitoring campaigns

Two continuously stirred (IKA RW20, 150 rpm) tank reactors with a total volume of 15 L (liquid volume of 13 L) were operated in parallel conditions, except for the temperature. One digester was mesophilic (MAD, 37 °C) and the other one thermophilic (TAD, 55 °C). The reactors were inoculated with biomass from a STP mesophilic anaerobic digester (sludge retention time (SRT) of 25–30 d) and operated semi-continuously by feeding the sludge mixture manually every day. The operation of the digesters can be divided into three periods: start-up (days 0–15) with an organic loading rate (OLR) of below 1 g COD/L d and a SRT of 40 d; Period I (days 15–90) was characterized by a SRT of 30 d and an average OLR of 1.1 g COD/L d; and Period II (days 90–330), with a SRT of 20 d and an OLR of around 1.8 g COD/L d. Conventional parameters of raw and digested sludge were analysed twice a week to check the performance of both reactors.

In order to evaluate the fate of OMPs and the estrogenic and the genotoxic activities during different AD conditions, two monitoring campaigns were conducted: one during Period I (days 71–77, spring 2014) and the second one during Period II (days 267–273, autumn 2014). Two samples of sewage sludge and digestates from MAD and TAD were taken in different days of each sampling campaign. The samples were immediately centrifuged at 3500 rpm