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Distribution of Polycyclic Aromatic Hydrocarbons (PAHs) in sludge organic matter pools as a driving force of their fate during anaerobic digestion

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ABSTRACT

The fate of organic matter during anaerobic digestion of sewage sludge was studied in batch systems thanks to a sequential chemical fractionation of the particulate phase coupled to fluorescence spectroscopy. Polycyclic Aromatic Hydrocarbons (PAHs) distribution within the organic pools was characterized from their analysis in the residual fraction after each extraction. Both methods were combined to understand the link between PAHs presence in organic pools and their spectral characterization after extraction. Two batch systems (sludge and inoculum mixture) were set up to study the impact of PAHs spiking on their fate and distribution. The sequential fractionation allowed us to extract and characterize about 50% of total Chemical Oxygen Demand. Moreover, fluorescence spectroscopy helped us to understand the organic pools evolution: the most easily extracted pools composed of protein-like molecules were highly degraded meaning that chemical accessibility mimics the bioaccessibility to degrading microorganisms. PAHs were present in all pools of organic matter but native PAHs were mainly present in low accessible (hardly extractable) fractions and during anaerobic digestion, they accumulated in the non-accessible (non extractable) fraction. Spiked PAHs were more dissipated during anaerobic digestion since spiking made them present in more accessible fractions. During the anaerobic digestion, contrary to native PAHs, spiked ones relocated toward less accessible organic fractions confirming the ageing phenomenon. PCA analysis showed that, in spiked mixture, PAHs presence in organic pools is linked to both PAHs physical–chemical properties and quality/quantity of the associated organic pools.

1. Introduction

Animal manures, urban organic wastes including sewage sludge, food processing and other industrial wastes are organic wastes (Westerman and Bicudo, 2005) that are potentially recycled in agriculture as soil amendment or fertilizers, very often after biological treatment such as anaerobic digestion and composting. For example, 41% of the sludge produced in Europe 27 is used in agriculture, 12% being previously composted (Kelessidis and Stasinakis, 2012). Indeed, the recycling of nutrients and organic matter is an economically viable way to reduce the use of mineral fertilizers and to maintain the soil biological activity (Mougin et al., 2006). Moreover due to the use of chemicals and industrial activities, the sewage sludge contains more or less persistent organic micropollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorobiphenyls and nonylphenols (Abad et al., 2005). Emerging contaminants such as pharmaceuticals and personal care products, drugs, veterinary products have been also quantified in environmental matrices including sewage sludge (Teijon et al.,

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Abbreviations: Ant, anthracene; ASE, Accelerated Solvent Extraction; BaA, benzo(a)anthracene; BaP, benzo(a)pyrene; BbF, benzo(b)fluoranthene; BghiP, benzo(ghi)perylene; BkF, benzo(k)fluoranthene; Chry, chrysene; CI, complexity index; COD, Chemical Oxygen Demand (mgO_2/L); DBahA, dibenzo(ah)anthracene; DOM, dissolved organic matter; Flt, fluoranthene; Flu, fluorene; HS-like, humic like substances; Ind, indeno(1,2,3,cd)pyrene; Kow, octanol/water partition coefficient; M1, mixture 1 (native PAHs); M2, mixture 2 (spiked PAHs); NER, non extractable residues; NEX, non extractable organic matter; OMP, organic matter pool; PAHs, Polycyclic Aromatic Hydrocarbons (μg/L); PCA, Principal Components Analysis; Phe, phenanthrene; Pyr, pyrene; RE-EPS, readily extractable exopolymers; S, substrate; S-EPS, soluble exopolymers; T0, before anaerobic digestion; Tf, after anaerobic digestion; TS, total solids (gTS/L); VS, volatile solids (gVS/L); $V_{Zk\text{-}COD}$, fluorescence volume normalized by the extract COD ($k \in [1;7]$, u.a./(gO_2/L); $V_{Zk\text{-}L}$, fluorescence volume normalized by sludge volume ($k \in [1;7]$, u.a./L); WWTP, Waste Water Treatment Plant; X, inoculum; $\%Z_k$, pourcentage of fluorescence volume ($k \in [1;7]$, %).

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2010). Many of these organic micropollutants may have a huge impact on our environment (*e.g.* bioaccumulation in the food chain) even though their concentrations are usually low (Rowsell et al., 2009). The risk that sludge contains these persistent hazardous compounds caused a strong opposition from farmers and food processing industries concerning their use in agriculture fearing that soils could be contaminated and useless for food production (Laturnus and Grøn, 2007). In parallel, it has been shown that biological treatment such as anaerobic digestion used to stabilize sludge could also be used to reduce the concentration of such compounds and minimize the risk of soil contamination. Lately, the removal of organic micropollutants during anaerobic digestion has been shown to rely on their chemical characteristics but also on two processes driving their behavior during treatment: sorption and biodegradation (Barret et al., 2012). The strong sorption and trapping of organic micropollutants into the sludge organic matrix or their biodegradation during sludge treatment minimize the risk of their transfer to water or biota after sludge spreading on soil. Due to their ubiquity in the environment because of their multiple sources and rather large persistence, their various range of physical and chemical properties and their toxicity (mutagenic and carcinogenic properties) and because PAHs are easily analyzed, they can be considered as model molecules to determine how important are the interactions between organic micropollutants and organic matter pools to explain their behavior during biological treatment. PAHs are neutral, apolar and constituted of more than two aromatic rings. They are not intentionally produced by human activities; they come from the incomplete combustion of organic matter (car traffic, industrial process, food preparation, etc.) (Abad et al., 2005; Rogers, 1996) and from natural origin (wildfire, eruptions, etc.).

PAHs are present in water and sewage sludge due to an easy diffusion as soon as they are produced: atmosphere carrying, rain deposit and leaching to sewers (Carrère et al., 2006). Due to their low solubility and high octanol/water partition coefficient (Kow), these hydrophobic compounds are sorbed onto suspended solids during wastewater treatment (Clarke and Smith, 2011). For example, nearly 64% of PAHs are sorbed on sludge during primary settling of sludge (Petrasek et al., 1983). PAHs elimination during wastewater treatment can reach 90% but due to their hydrophobic properties, they are concentrated in sewage sludge (Aparicio et al., 2009).

Aerobic PAHs dissipation is quite well documented and usually observed during composting (Brändli et al., 2005, 2007). By contrast, anaerobic digestion is less described and removal performances are lower than under aerobic conditions (Haritash and Kaushik, 2009). The limitation of degradation is always linked to the low availability or accessibility of such molecules (Barret et al., 2010a). In literature, PAHs are usually measured on total sludge or only in the aqueous phase. The common hypothesis is that PAHs dissipation is due to the degradation in the aqueous phase of sludge considered as the only available pool (Barret et al., 2012). But the dissipated PAHs quantity can exceed the aqueous pool meaning part of PAHs present in the particulate phase is available or accessible and may be degraded (Barret et al., 2010a) or forme strong association to the particulate phase and became unextractable (non extractable residues – NER). Modeling was used to confirm the hypothesis that the aqueous pool is available (Delgadillo-Mirquez et al., 2011). But as previously mentioned, the quantity of PAHs initially present in this pool cannot explain the overall removal of PAHs; the particulate phase may release PAHs in this pool to explain the removal. It is thus important to better describe the interactions between PAHs and the organic matrix and their influence on PAHs behavior during anaerobic digestion of sewage sludge. As far as the authors know, no study has been made before on the fractionation of the particulate phase of sludge for both PAHs and organic matter. In this paper, we combined a new method of sequential chemical fractionation with PAHs analysis to characterize organic matter in the particulate phase of sludge and to localize PAHs into the defined compartments before and after anaerobic digestion. The goal of this study was to better define which PAHs pools were accessible for degradation and how these pools evolve during the anaerobic degradation of organic matter. This will allow us to better understand the mechanisms that control the fate of PAHs during anaerobic digestion of sewage sludge and then to propose strategies to manage their elimination. In order to fulfill this goal, we decided to work on a model organic matrix (secondary sludge) and a model process (anaerobic digestion) in batch systems (Biochemical Methane Potential based on Angelidaki et al. (2009)). The impact of PAHs spiking on both organic matter and PAHs behavior was also studied.

2. Material and methods

2.1. Anaerobic batch systems

Two mixtures (400 mL) composed of secondary sludge (substrate S) and digested sludge (inoculum X) were introduced into bottles ($n = 12$) flushed with nitrogen for anaerobic conditions, sealed and placed in a thermostated room at 35°C. The ratio substrate/inoculum (S/X) was set at 0.5 g COD_S/gVS_X (substrate chemical oxygen demand/inoculum volatile solids) with VS_X at 22.5 g/L. The secondary sludge came from a wastewater treatment plant (WWTP) located in the center of France with a capacity of 285,000 population equivalents, previously frozen to stop its transformation. The inoculum was a digested sludge sampled in the same WWTP and kept at 35°C. The first mixture was incubated without addition of PAHs (mixture M1). The second mixture (mixture M2) was incubated three weeks after the M1 mixture and was spiked with 13 PAHs (60 μg/L for all PAHs except indeno(1,2,3,cd) pyrene at 15 μg/L). The concentration of each PAH in M2 was measured just after spiking. Indeed, the sludge already contained PAHs and the concentration could be thus higher than the predicted concentration. Furthermore, losses could occur during spiking and errors due to low weighing during the preparation of the spiking solution could reduce or increase the predicted PAHs concentrations. As the sorption on organic matter is almost instantaneous (Barret et al., 2011), the contact time between sludge and spiking solution was set at 3 h. For each mixture, 12 bottles were set up. The quantity of produced biogas was measured regularly by water displacement and its composition was determined by gas chromatography with a Perkin Elmer GC Clarus 480 (see Appendix A). After 32 days of incubation, the experiment was stopped and the content of the 12 bottles for each mixture was pooled to obtain an average mixture on which analyses were made.

2.2. Fractionation method

A part (1,500 mL) of the initial and final mixtures (T0 and Tf respectively) was centrifuged at 18,600 g during 30 min at 4°C to separate the aqueous phase (dissolved organic matter, DOM) from the particulate phase. The total mixture and the particulate phase were freeze dried and grinded with a mortar before further analysis. The particulate phase was divided into different pools using successive chemical extractions based on the methodology proposed by Jimenez et al. (2014). For both particulate phases, 8 centrifuged tubes were prepared, 2 tubes being sacrificed per step of fractionation. In each centrifuge tube, 0.5 g of freeze dried and grinded particulate phase was mixed with 30 mL of extraction reageant and shaken at 300 rpm in an incubator at 30°C (Fig. 1). Then,

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