Fate of organic micropollutants during anaerobic digestion of sewage sludge: localization of micropollutants within sludge organic matter pools

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Abstract
Many organic micropolluants enter the environment through wastewaters. Some are partly degraded during wastewater treatment. For others, due to hydrophobic properties, sorption to sludge is the main removal process. Anaerobic digestion is widely used to treat sludge because it produced renewable energy in the form of methane. The digested sludge can be used as organic fertilizer. To evaluate the risk of soil contamination, it is necessary to know if organic pollutants are dissipated during the anaerobic digestion and to determine how residual organic pollutants interact with sludge organic pools during anaerobic digestion. In this study, three sludge matrices were anaerobically digested in three batch system. The distribution of organic micropollutants (polycyclic aromatic hydrocarbons and nonylphenols) between organic compartments of the digested sludge was characterized.

Introduction
Many organic micropollutants are toxic and persistent compounds into the environment. They can bioaccumulate and, even though they are found at low concentration, they may have a huge impact on the environment [1]. Various molecules can be found: polycyclic aromatic hydrocarbons (PAH), nonylphenols (NP), nonylphenols ethoxylates (NPE), polychlorobiphenyls (PCB), etc. [2]. Emerging contaminants appear due to the use of pharmaceutical and personal care products (PPCP), drugs, veterinary products etc. [3].
They enter the environment especially through wastewaters and wastewater treatment plants [1], [2]. During the treatment, they are eliminated from wastewaters by degradation, volatilization or sorption [4–6]. It was shown that almost 65 % of the micropollutants removed during treatment by activated sludge were transferred from the liquid phase to the solid phase of the mixed liquor [6]. The contamination levels in sludge depend on the nature of the molecules: detergents and plasticizers present very high levels of concentration (from g to $mg.kg^{-1}$ Dry Matter (DM)), others (hydrocarbons, pesticides, flame retardants, pharmaceuticals) have low (100 – 1,000 $\mu g.kgDM^{-1}$) to very low (< 100 $\mu g.kgDM^{-1}$) levels of concentration [6].
Anaerobic digestion is one of the most widely used processes for sludge stabilization while treated sludge is very often disposed to the soil or reused for agricultural purpose [7]. The removal of micropollutants during anaerobic digestion depends on two processes: sorption and biodegradation [5], [6]. To minimize the risk of transfer of micropollutants in soil after agricultural use of the treated sludge, we need to improve the removal by increasing sorption to particles (the molecules can be trapped into the organic matrix and then will not transfer to water or biota) or increasing biodegradation (the compounds are metabolized or co-metabolized). To know how to control these processes, it is necessary to better understand the link between evolutions of both the organic matrix and the micropollutants during anaerobic digestion. The particulate phase represents most of the organic matter of the sludge [4] so in this study we focus on this sludge phase.

The aim of this study is to better know the relationship between organic micropollutants and biodegradable (or not) fractions of sludge organic matter. The approach was to combine fractionation method, biochemical analyses and quantification of organic micropollutants.

Material and Methods

Anaerobic incubations

Three matrices were anaerobically digested in batch. The matrices were a mixture of a substrate S and an inoculum X. The S/X ratio was set at 0.5 $gCOD_S.gVM_X^{-1}$ leading to the following proportions: 20 % of dry matter (DM) for S and 80 % of DM for X. The substrate S was the same in all experiments: a thickened secondary sludge from a sewage treatment plant in the center of France. Two inoculi were used: digested sludge from the sewage treatment plant and a granular sludge from a plant treating sugar effluent. The three matrices were then:

- X_1: digested sludge + sewage sludge;
- X_2: digested sludge + sewage sludge + Polycyclic Aromatic Hydrocarbons + Nonylphenols;
- X_3: granular sludge + sewage sludge.

Part of the initial mixture was kept for further analysis (T_0). The rest of the matrix was put in glass flasks (12 replicates). The atmosphere was saturated with molecular nitrogen to apply anaerobic conditions. The flasks were then sealed and placed in a thermostated room at 35°C. Regularly, the production of biogas (quality and quantity) was measured. The endogenic production of biogas was subtracted by using a flask containing only the inoculum. The incubation lasted 32 days (the evolution of cumulated biogas function of time reached a plateau). After incubation, all flasks were pooled and the final matrix (T_f) was used for further analysis.

Fractionation method

The fractionation method was a synthesis of various fractionation methods of organic matter. The aim of the fractionation method was to extract successively less and less accessible constituent of the sludge (exopolymers and humic substance like).

The first step was a centrifugation at 18,600 g and 4°C during 30 min to separate aqueous phase (dissolved and colloidal matter) from the particulate phase. With the conditions used, the particulate phase represented around 95 % of the total DM.

The fractionation was made on 0.5 g of freeze-dried particulate phase in polyallomer centrifuge tube. 30 mL of fractionation solution was added and the tube was shaken in thermostated incubator (30°C). Then, it was centrifuged at 18,600 g and 4°C during 20 min. The supernatant was filtered with cellulose acetate 0.45 µm filter (Whatman) and its Chemical Oxygen Demand (COD) measured. To extract a compartment, the fractionation step is repeated four times. The filtered liquids were pooled and constituted the compartment. Table I represents the different extraction steps.

Table I: fractionation method steps

Compartment	Solution	Conditions
S-EPS	10 mM NaCl + 4 mM $NaHCO_3$	30 min
RE-EPS	10 mM NaCl + 10 mM NaOH	30 min
HS-RA	0.1 M HCl	1 h
HS-RN	MilliQ water	0 h, pH adjusted at 7 with 1 M NaOH
HS-like	0.1 M NaOH	4 h, atmosphere saturated with N_2

Bioaccessibility is defined as the possible access by microorganisms to the molecule during the process: with sufficient time, a bioaccessible molecule becomes bioavailable (direct access to the molecule to be degraded).

The first two compartments S-EPS (soluble exopolysaccharides) and RE-EPS (easily extractable exopolysaccharides) can be considered as part of the bioaccessible organic fraction. Before further extraction, the residue was freeze-dried. The HS-RA (acidic rinse, once) and HS-RN (neutral rinse, once) eliminated carbonates, sulfates and hydroxides from the sample. As they extracted little COD they were not considered for the rest of the study. It can be considered that HS-like (humic substance like) constitutes a part of non bioaccessible organic matter.

Analyses

Table II presents the different analysis made on the samples gathered during fractionation.

Table II: analyses made on the compartments and the solid phases sacrificed during fractionation

Sample	Analysis
Aqueous compartment	Quantifications of COD, Organic Carbon (OC), Inorganic Carbon (IC), Proteins (P), Sugars (S), 3D spectrofluorimetry
Solid phase sacrificed at each fractionation step	Quantification of Total Carbon (TC), Total Kjeldahl Nitrogen (TKN) and organic micropollutants (PAH & NP), 3D spectrofluorimetry

Organic micropollutants analysis

PAH and NP were extracted with an Accelerated Solvent Extraction using hexane:acetone (50:50, v:v) and analysed with a HPLC coupled with a fluorimeter detector according to Trably et al., 2004 [8]

Results

Methane production

Methane productions (Table III) were compared with ANOVA (Excel 2007). They were the same for X_1 and X_2. The addition of organic micropollutants into the matrix had no effect on the functioning of the microbial ecosystem which led to the production of biogas. The only difference between X_1 and X_2 was the rate of production at the beginning of the incubation. The spiked matrix had slower production at the beginning. It can be due to the fact that between the incubation of X_1 and X_2, digested sludge was kept at 35°C during three weeks. So the microbial ecosystem was not as in good shape as for the first incubation.

The ANOVA result shows that the methane production for X_3 was lower (p value 0.05). This could be explained by the fact that the inoculum was not adapted to the substrate. Indeed, the second inoculum is used to degrade an effluent containing residues of sugar and not a more complex organic matrix like sludge. It was used here to study a matrix completely different from digested sludge.

Table III: methane productions

	X_1	X_2	X_3
$mLCH_4.gCOD_{substrate}^{-1}$	169 ± 9	160 ± 19	142 ± 23
ANOVA p values	X_1/X_2: 0.19	X_2/X_3: 0.07	X_3/X_1: 0.006

As the p value comparing X_2 and X_3 was close to 0.05 and that the difference was significant between X_1 and X_3 and not between X_1 and X_2, we considered that the difference was significant between X_2 and X_3.

Fractionation of the matrices

Less COD was extracted at the end of the anaerobic digestion (T_f, Figure 1): the organic matter was degraded and the non-extractable compartment increased (except for X_1).

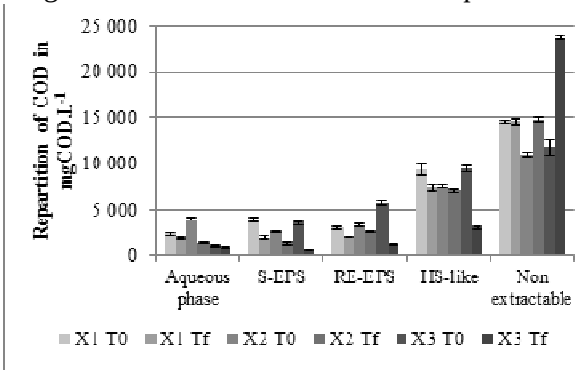

Figure 1: evolution of each compartment

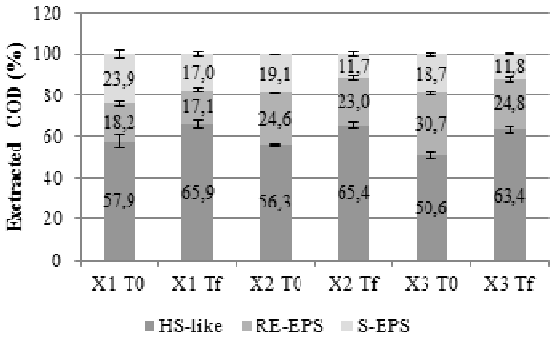

Figure 2: repartition of extracted COD

All compartments except the non-extractable one were degraded during anaerobic digestion. Nevertheless, the most available compartments *i.e.* S-EPS and RE-EPS were more degraded than HS-like compartment except for X_3. It seems that the inoculum had strong impact on the matrix evolution. The repartition of extracted COD showed an increase of less available compartment HS-like and a decrease of biodegradable compartments S-EPS and RE-EPS (Figure 2). Indeed, anaerobic digestion led to a more stable matrix after degradation of the more degradable organic matter in the initial substrate. The distribution of organic matter differed in the aged digested sludge X_2 compared to X_1. Nevertheless, it did not impact the inoculum capacity to degrade the substrate and produce methane.

Characterization of X_1 compartments before anaerobic digestion

Figure 3 and Figure 4 show the biochemical characterization of each compartment and the amount of total PAH in each compartment for X_1 T_0 matrix.

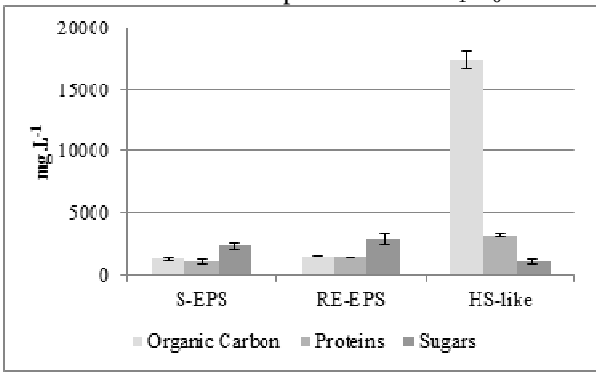

Figure 3: Organic Carbon, Proteins and Sugars in each extract of X_1 matrix at T_0

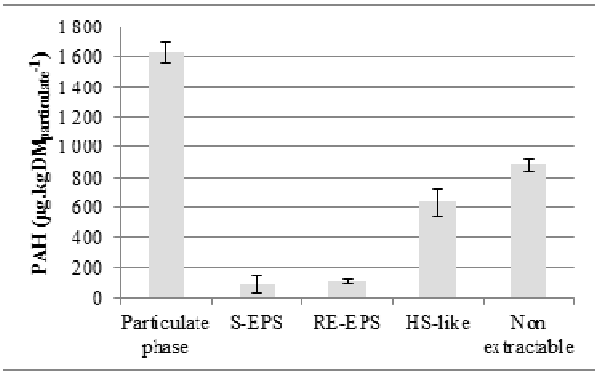

Figure 4: presence of PAH in each extract of X_1 matrix at T_0

PAH were present into HS-like and non-extractable compartments (Figure 4). This was in accordance with the biochemical characterization of each compartment. Indeed, 86 % of extracted OC was in HS-like and 28 % and 44 % of total COD were respectively in HS-like and non-extractable compartments. This shows that PAH have a high affinity for non bioaccessible fractions more complex than S-EPS and RE-EPS compartments.

Conclusion

The three matrices evolved differently during anaerobic digestion. Part of COD was removed during the incubation which led to methane production. The compartments evolved during the treatment and their compositions were different for each matrix. The biochemical characterization of the matrices and the localization of the contaminants will be used in a Partial Least Square regression to create a model which will characterize the affinity between a compound and compartments defined by their biochemical composition.

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