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Influence of feed characteristics on the removal of micropollutants during the anaerobic digestion of contaminated sludge

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ABSTRACT

The removal of 13 polycyclic aromatic hydrocarbons, 7 polychlorobiphenyls and nonylphenol was measured during the continuous anaerobic digestion of five different sludge samples. The reactors were fed with one of the following: primary/secondary sludge (PS/SS), thermally treated PS, cellulose-added SS, or SS augmented with dissolved and colloidal matter (DCM). These various feeding conditions induced variable levels of micropollutant bioavailability (assumed to limit their biodegradation) and overall metabolism (supposed to be linked to micropollutant metabolism throughout co-metabolism). On the one hand, overall metabolism was higher with secondary sludge than with primary and the same was observed for micropollutant removal. However, when overall metabolism was enhanced thanks to cellulose addition, a negative influence on micropollutant removal was observed. This suggests that either the co-metabolic synergy would be linked to a specific metabolism or co-metabolism was not the limiting factor in this case. On the other hand, micropollutant bioavailability was presumably diminished by thermal treatment and increased by DCM addition. In both cases, micropollutant removal was reduced. These results suggest that neither overall metabolism nor bioavailability would absolutely limit micropollutant removal. Each phenomenon might alternatively predominate depending on the feed characteristics.

1. Introduction

Organic micropollutants such as polycyclic aromatic hydrocarbons (PAHs) and polychlorobiphenyls (PCBs) can be removed efficiently from wastewater: for example, 75% of PCBs are removed at a WasteWater Treatment Plant (WWTP) in Greece [1] and at a WWTP in Paris, France, 76% and 98% of PCBs and PAHs, respectively [2]. However, biodegradation only accounts for a small part of this removal. In fact, since PAHs and PCBs present very low water solubility and are highly hydrophobic, these properties favour their sorption to organic matter. As a consequence, sorption onto the solid effluent (sludge) has been demonstrated to be the main removal mechanism for PAHs and PCBs [1–4]. Thus, their concentration in sludge reaches 0.001–10 $\mu g_{PAH}/g_{DM}$ [2,5,6] and 0.01–1 $\mu g_{PCB}/g_{DM}$ [1,2,6], depending on the influent and the characteristics of the WWTP.

Nonylphenol (NP) is produced within WWTP as a byproduct of the aerobic/anoxic biodegradation of long-chain ethoxylated nonylphenols [7,8]. Due to its recalcitrance and hydrophobicity, NP accumulates in the biological process and is removed from wastewater through sorption to residual matter and subsequent clarification [9,10]. NP occurs in sludge at a typical concentration of about 100 $\mu g_{NP}/g_{DM}$ [11,12].

Before its final disposal, sludge has to be stabilized. Among the available solutions, anaerobic digestion followed by spreading is the most sustainable option [13]. In addition, the anaerobic consortia involved in this bioprocess have been shown to partially remove PAHs [14–16], PCBs [17–19] and NP [20,21]. However, the comparison of published data reveals considerable variation in the anaerobic removal of micropollutants. Indeed, PCB removal ranged from 12% [19] to 98% [18] in continuous mode while NP removal varied from 0% in continuous mode [22] to 40% in batch mode when operated with same retention times [21]. This variability highlights a lack of understanding of the mechanisms which determine micropollutant removal. Nonetheless, several operational parameters have been shown to influence their removal, such as retention time [18,23] and temperature [15,18,23,24]. In addition to these operating conditions, the microbial population, bioavailability and co-metabolism were also found to influence

Abbreviations: BSA, bovine serum albumin; COD, chemical oxygen demand; CSS, composite sludge prepared from SS and cellulose; DCM, dissolved and colloidal matter; DM, dry matter; Glu, glucose; NP, nonylphenol; OC, organic carbon; OM, organic matter; PAH, polycyclic aromatic hydrocarbon; PCB, polychlorobiphenyl; PEEM, petroleum ether extractible matter; PS, primary sludge; SS, secondary sludge; TTPS, thermally treated primary sludge; VFA, volatile fatty acid.

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micropollutant removal. For example, an adapted inoculum is favourable [15], suggesting that the abundance of micropollutant-degrading microorganisms is a crucial factor. Moreover, the poor bioavailability of micropollutants is usually assumed to limit their biodegradation [19,25]. Thus, when they are transferred to the aqueous phase thanks to surfactants, removal rates are higher [21]. PAHs, PCBs and NP are recalcitrant compounds and their biodegradation is only likely to occur thanks to co-metabolism and syntrophic interaction in conjunction with overall metabolism and the structure of the microbial population. Thus, an additional readily biodegradable carbon source can enhance micropollutant removal [14,21], probably because it stimulates the overall metabolism. Finally, the anaerobic biodegradation of micropollutants is influenced by their physico-chemical properties [23,25,26].

In this study, the removal by anaerobic digestion of PAHs, PCBs and NP was measured for different feed characteristics, in fixed operating conditions (retention time, temperature, inoculum, chemical oxygen demand and micropollutant load). The feed characteristics varied according to sludge origin, thermal treatment and the preparation of sludge composites obtained by mixing with cellulose or sludge supernatant. The objective was to identify both key parameters of the feed sludge along with the properties of the micropollutants which condition their removal and might help to predict their fate.

2. Material and methods

2.1. Chemicals

All solvents were purchased from J.T. Baker. The compounds studied are listed in Table 1. PAH and PCB powders were obtained from Dr Ehrenstorfer GmbH. Each compound was separately dissolved in dichloromethane at 1 g/L. The pure 4-nonylphenol (NP) isomer mixture was purchased from Interchim. It was diluted in hexane to obtain 40 g/L. The spiking mix was prepared from these individual concentrated solutions to the final concentration of 100 mg/L, except for indeno(1,2,3,c,d)pyrene (20 mg/L) and nonylphenol (2 g/L).

The 10 mg/L standard solution of PAHs in acetonitrile, the 10 mg/L standard solutions of PCBs and of tetrachloronaphthalene (TCN) in hexane and the 100 mg/L of NP in hexane were all supplied by Dr Ehrenstorfer GmbH. For quantification, the standard solutions were diluted to obtain 6 calibration levels from 10 to 1000 μg/L in acetonitrile for PAHs, from 100 to 1000 μg/L in dichloromethane for PCBs and from 500 to 5000 μg/L in hexane for NP. Standards were stored at −20 °C.

Table 1
Physico-chemical characteristics of the PAHs, NP and PCB180.

Compound	$\log K_{ow}$	M	$\log H$	n5C	n6C	nCl	nOH
Fluorene	4.18	166	1.64	1	2	0	0
Phenanthrene	4.57	178	1.03	0	3	0	0
Anthracene	4.45	178	2.34	0	3	0	0
Fluoranthene	5.1	202	0.40	1	3	0	0
Pyrene	5.32	202	0.87	0	4	0	0
Benzo(a)anthracene	5.85	228	0.81	0	4	0	0
Chrysene	5.89	228	0.69	0	4	0	0
Benzo(b)fluoranthene	6.57	252	−1.15	1	4	0	0
Benzo(k)fluoranthene	6.84	252	−0.16	1	4	0	0
Benzo(a)pyrene	6.00	252	0.09	0	5	0	0
Dibenzo(a,h)anthracene	6.70	278	−0.08	0	5	0	0
Benzo(g,h,i)perylene	6.73	276	−0.61	0	6	0	0
Indeno(1,2,3,c,d)pyrene	6.60	276	−0.40	1	5	0	0
Nonylphenol	5.76	220	1.04	0	1	0	1
PCB28	5.66	257	1.57	0	2	3	0
PCB52	5.95	292	2.21	0	2	4	0
PCB101	6.38	327	1.71	0	2	5	0
PCB118	6.65	327	1.58	0	2	5	0
PCB138	7.19	364	1.33	0	2	6	0
PCB153	6.86	364	1.77	0	2	6	0
PCB180	7.15	399	1.60	0	2	7	0

M: molar mass (g/mol); H: Henry's law constant (Pa m^3/mol); n5C: number of five carbon rings; n6C: number of six carbon rings; nCl: number of chlorines; nOH: number of hydroxyl substitutions.

2.2. Sludge samples

The experiments were carried out using five different sludge samples. The primary sludge sample (PS) was collected at the outlet of the primary settling tank at a domestic WWTP treating 33 000 PE (Person Equivalent). A part of this sample was hydrolysed at 165 °C for 30 min in a Zipperclave reactor to obtain the thermally treated primary sludge sample (TTPS). The secondary sludge sample (SS) came from the biological aerobic unit of a domestic plant treating 250 000 PE with a very low sludge retention time (0.4 day). The CSS sample was obtained by mixing SS with cellulose particles (20 μm, Sigma–Aldrich) at a chemical oxygen demand (COD) proportion of 50:50.

Centrifugation of SS (10 000 × g, 20 min), followed by filtration at 1.2 μm (Whatman GF/C filter), was carried out to separate the particles from the supernatant, this latter containing the dissolved and colloidal matter (DCM). Finally, SS was diluted with its own supernatant at volumic proportions of 3:1 (sludge: supernatant) to provide the fifth sludge sample SupSS.

Prior to their direct use or to composite preparation, PS and SS were stored at −20 °C.

All these samples were then diluted with deionised water to obtain 24 ± 5 g_{COD}/L and spiked at 5 μg/g_{DM} for each PCB and PAH, except for indeno(1,2,3,c,d)pyrene (1 μg/g_{DM}), and for NP (100 μg/g_{DM}), so that the spiked concentrations were similar to actual contamination levels.

2.3. Experimental setup

The anaerobic digestion of PS, TTPS, SS, CSS and SupSS was performed in stirred lab-scale reactors of 5L. Temperature was regulated at 35 °C thanks to hot water circulation in the external jacket. The feed, stored at 4 °C, was pumped six times per day into the reactor straight after the pumping out of the digested sludge. This latter was collected in tanks at 4 °C. Hence, the reactors were operated with a retention time of 20 days and an organic load of 1.2 ± 0.2 g_{COD}/L/day. For the start-up, they were filled with methanogenic sludge coming from an anaerobic mesophilic reactor adapted to PAH-polluted sludge, and directly fed at the normal operating conditions. The reactors were run during 4–5 retention times.

The pH and the volumetric production of biogas were monitored on-line. Once a week, 7-day composite samples were taken from the feeding tank, the outlet tank and the gaseous phase. Removals were calculated when a steady state was achieved.

A sorbent cartridge (ORBO, Supelco) was placed at the gaseous outlet of the reactor fed with SupSS for 7 days, to quantify the volatilisation of micropollutants.

2.4. Analytical methods

The phase containing dissolved and colloidal matter was separated from the total sludge samples by centrifugation (10 000 × g, 20 min) followed by filtration at 1.2 μm. The dry matter (DM, g_{DM}/L) in total sludge and in the dissolved/colloidal phase was measured by weighing the sample after heating at 105 °C during 24 h. The proteins and carbohydrates in total sludge samples were analysed according to the Lowry [27] and anthrone [28] methods, respectively. The standard curves 20–100 mg/L were obtained with bovine

serum albumin (BSA) for proteins (expressed in g_{eqBSA}/L) and with glucose for carbohydrates (g_{eqGlu}/L). The chemical oxygen demand in total sludge (COD, g_{O_2}/L) was determined using Merck Spectroquant kits, in accordance with ISO 15 705. The samples were diluted to be in the range 150–1500 mg_{COD}/L. To determine the concentrations of volatile fatty acids (VFA) acetate, propionate, iso-butyrate, butyrate, iso-valerate and valerate in the dissolved/colloidal phase, 0.5 μL were injected at 250 °C into a capillary column Econocap FFAP (Altech), Varian 3900 gas chromatograph. The carrier gas was composed of N_2 (25 bar), H_2 (50 bar) and air (100 bar). The detection by flame ionisation was carried out at 275 °C.

A fraction of each sludge sample was freeze-dried and ground in order to quantify the lipids and the micropollutants. The lipid content was defined as the matter extractible with petroleum ether (PEEM). The extraction was performed with an Accelerated Solvent Extractor (Dionex) operating at 70 bar and 105 °C. PAHs, PCBs and NP were extracted from the ORBO cartridge using a Soxhlet setup operating for 16 h at 60 °C, with 200 mL of hexane/acetone (50:50, v:v). Extraction from inlet and outlet sludge samples and quantification in all extracts were performed in accordance with Barret et al. [29].

The composition of biogas was measured by gas chromatography (Shimadzu GC-8A), with argon as the carrier gas (2.8 bar). A 1 mL volume was injected at 100 °C. Separation was performed using two columns: a Hayesep Q (Altech CTRI) and a 5 Å molecular sieve (Altech CTRI), both maintained at 30 °C. CO_2, CH_4, O_2, N_2 and H_2 were quantified with a catharometer operating at 80 mA.

2.5. PLS regression

The partial least-squares (PLS) technique is based on constructing PLS factors by minimizing the covariance between the dependant variable (Y block) and the explicative variables (X block). The prediction of Y block is then calculated with a multivariable linear regression on the X block using the software R version 1.2.2 for Windows and by using PLS functions developed elsewhere [30]. The first PLS factor accounts for the highest percentage of variance and the following factors for decreasing amounts of variance. The number of PLS factors (dimension, dim) of the models was determined by minimizing the mean squared predictions error (predicted residual sum of squares, PRESS) through a cross-validation procedure.

This technique was used to model the removal of PAHs, PCB180 and NP in the five reactors. X block was composed of micropollutant characteristics (Table 1) and of inlet sludge characteristics (Table 2), whereas Y block consisted of the removal values as percentage.

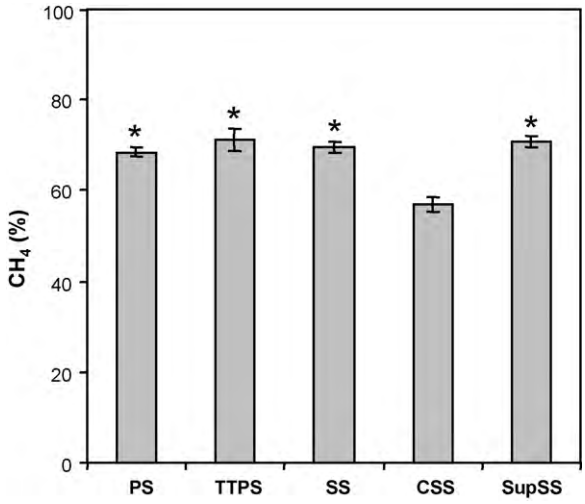

Fig. 1. Average methane percentage of the biogas produced under the different feedings. Means labelled with a star are not significantly different (p-value = 0.05).

3. Results and discussion

3.1. Suitability of the operating conditions

VFAs did not accumulate within the five reactors (Table 2), indicating that the biodegradation was complete and that the organic load was appropriate. Moreover, the methane content of the biogas was about 70%, except for the reactor fed with CSS which will be discussed later (Fig. 1). 70% of methane in the biogas is typical for the anaerobic digestion of sludge [31,32]. The reactors thus ran in favourable methanogenic conditions.

3.2. Apparent removal vs. biodegradation of micropollutants

The removals of PAHs, NP and PCBs are presented in Fig. 2. Since PAH volatilisation was undetectable in the reactor fed with SupSS, the calculated removals include two main mechanisms: biodegradation and sequestration. An analysis of variance was performed for each micropollutant between the removals measured in the 3 or 5 reactors (PCB removal was not measured in reactors fed with CSS and SupSS). For most compounds, removals in the different reactors were statistically different.

Slightly substituted PCBs exhibited lower removals (Fig. 2). Indeed, PCB anaerobic degradation is initiated by reductive dechlo-

Table 2
Average value and standard deviation of the feed characteristics and removals measured for the five operated reactors (calculated from 5 measurements performed at steady state). For each parameter, the p-value between the 5 sets of measurements is indicated in italic. Characteristics and removals can be considered as statistically different for p-value below 0.05.

Feeding	COD g_{O_2}/L	DM g_{DM}/L	DCM % of total DM	Proteins g_{eqBSA}/g_{DM}	Carbohydrates (from cellulose) g_{eqGlu}/g_{DM}	Lipids g_{PEEM}/g_{DM}	VFA g_{VFA}/g_{DM}
Feed characteristics							
PS	28 ± 4	24 ± 2	5 ± 1	0,27 ± 0.03	0.29 (0.00) ± 0.09	0.13 ± 0.01	0.03 ± 0.02
TTPS	27 ± 3	20 ± 2	11 ± 2	0.32 ± 0.10	0.29 (0.00) ± 0.15	0.15 ± 0.02	0.04 ± 0.01
SS	22 ± 2	19 ± 1	4 ± 4	0.25 ± 0.02	0.30 (0.00) ± 0.05	0.10 ± 0.01	0.04 ± 0.03
CSS	27 ± 2	21 ± 4	8 ± 7	0.16 ± 0.03	0.66 (0.46) ± 0.15	0.06 ± 0.04	0.02 ± 0.04
SupSS	19 ± 2	16 ± 2	14 ± 2	0.29 ± 0.05	0.26 (0.00) ± 0.06	0.10 ± 0.05	0.10 ± 0.02
p-Value	*0.0004*	*0.0002*	*0.0003*	*<0.0001*	*<0.0001*	*0.001*	*0.03*
Removal (%)							
PS	57 ± 7	49 ± 9	–	38 ± 12	88 ± 1	79 ± 7	93 ± 3
TTPS	64 ± 10	60 ± 10	–	51 ± 15	84 ± 10	83 ± 8	100 ± 5
SS	67 ± 7	58 ± 10	–	41 ± 16	81 ± 14	79 ± 5	97 ± 3
CSS	83 ± 4	79 ± 5	–	53 ± 11	98 ± 1	nm	100 ± 5
SupSS	62 ± 5	52 ± 11	–	49 ± 9	90 ± 3	nm	100 ± 4
p-Value	*0.0001*	*0.0002*	–	*0.35*	*0.02*	–	*0.01*

nm: not measured.

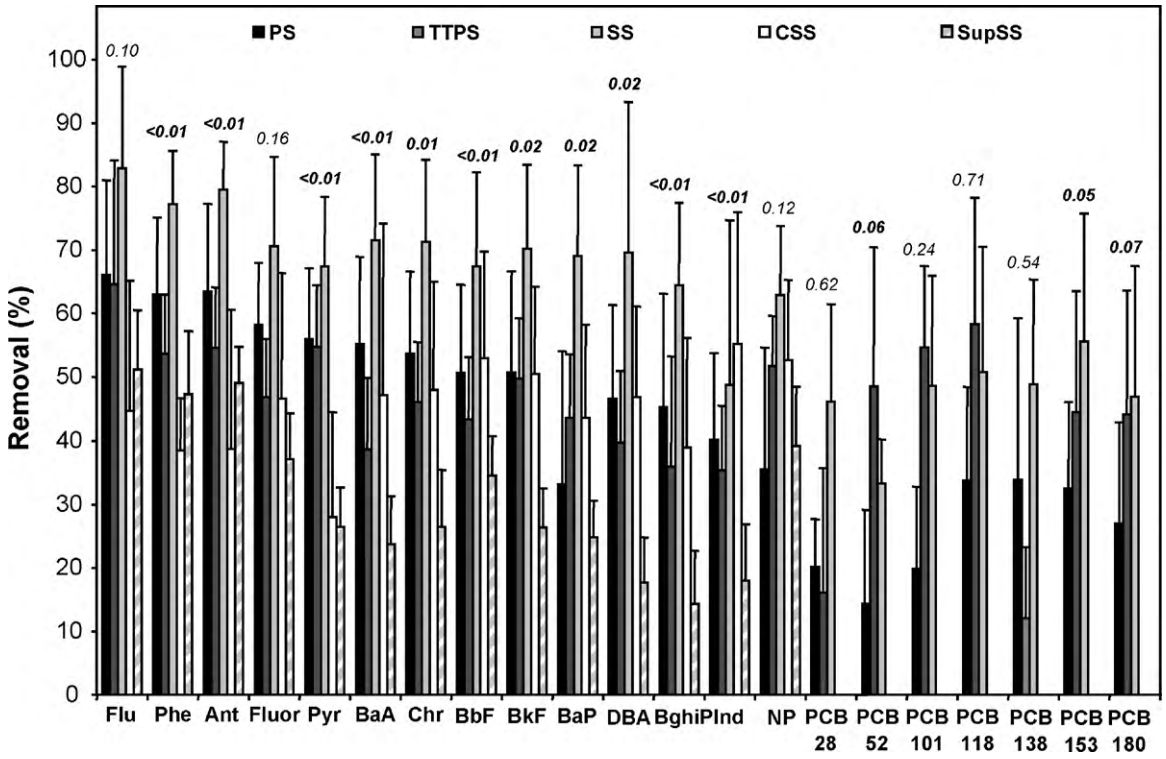

Fig. 2. Micropollutant removal with PS, TTPS, SS, CSS and SupSS feeds. For each compound, the *p*-value between the 5 sets of measurements (3 for PCBs) is indicated in italics. Removal can be considered as statistically different for a *p*-value below 0.05.

rination [33]. Hence, the apparent removal of slightly substituted PCBs may result from their degradation and from their formation by dechlorination of highly substituted PCBs. Two cascade reactions could have occurred:

PCB180 → PCB153 → PCB101 → PCB52

PCB180 → PCB138 → PCB118 → PCB28

As we were not able to determine whether such reactions actually occurred, we only considered the fate of PCB180.

3.3. Influence of sludge origin on micropollutant removal

Both primary sludge (PS) and secondary sludge (SS) presented a similar composition (Table 2). As the SS was sampled in an aerated tank with a very low retention time, it was not stabilized, which explains the slight differences with the PS. PS and SS differed slightly in protein and lipid content: both were higher in the PS. As microbial cells and exopolymeric substances structuring sludge flocs are mainly constituted of proteins [34], the measurement of proteins may be an indicator of poorly biodegradable matter. Higher lipid content suggests a higher proportion of hydrophobic material, poorly accessible during anaerobic digestion. These slight differences are probably responsible for the lower COD and DM removal with the PS feed. The removal of all micropollutants was also lower with the PS feed. Benabdallah El-Hadj et al. [35] observed contradictory results for NP degradation using primary and secondary sludge feed, with respective removals of 27% and 20%. However, information given by the authors about the origin and characteristics of their sludge samples was not complete. If their secondary sludge sample originated from a WWTP with the most common retention time (several days), its biodegradability would be lower than the SS used in this study [32], and also lower than PS [36], which would explain the lower NP removal observed in secondary sludge than in primary sludge. Hence, a link may exist between the biodegradation of sludge matter and micropollutant removal. Nevertheless, the origin of sludge is not the determining factor: the characteristics of the sludge matter and its biodegradability are likely to be more reliable indicators.

3.4. Influence of thermal treatment of sludge on micropollutant removal

To investigate more thoroughly the link between sludge characteristics, sludge degradation and micropollutant removal, thermal treatment was applied to the PS to obtain TTPS. This pre-treatment improved the removal of dry matter from 49% to 60% (Table 2), which is in accordance with results in the literature. Indeed, thermal pre-treatment, as well as ozone and ultrasound pre-treatments, have all been previously shown to solubilise sludge matter and to enhance sludge biodegradability and methane production [36–39]. Bougrier et al. [32] established that the higher the initial biodegradability, the lower the pre-treatment effect. This may explain the positive but limited effect of thermal treatment in our case (11% enhancement), since the raw PS exhibited a high removal rate.

In contrast, micropollutant removal was slightly reduced by thermal pre-treatment (Fig. 2). Studies concerning the impact of such pre-treatment on the fate of organic micropollutants are scarce. Whereas ozonation was demonstrated to solubilise PAHs [35], probably because of PAH sorption to the solubilised dissolved and colloidal matter, no PAH transfer into the aqueous phase was detected after thermal treatment [35], or the aqueous fraction was found to decrease in both feed sludge and digested sludge on account of the denaturing of the dissolved and colloidal matter [29]. Usually, the aqueous fraction of micropollutants is assumed to be bioavailable to microorganisms [14,40,41]. According to this hypothesis, the bioavailability of micropollutants should decrease with thermal pre-treatment.

Furthermore, the second widely postulated hypothesis is that the biodegradation of micropollutants is limited by their bioavailability [19,25]. According to this view, thermal pre-treatment should decrease micropollutant biodegradation, as a result of a decrease in the bioavailability. The fact that no impact or, indeed, a negative impact was previously detected on NP [35], pharmaceuticals and personal care products [26] and linear alkylbenzene sulphonates [42] tend to corroborate the hypotheses. The poorer removal of micropollutants after thermal pre-treatment observed in this study is thus consistent with the previously published data and might be explained by lower bioavailability after such pre-treatment.

In addition to bioavailability, co-metabolism is known to influence micropollutants metabolism. However, in spite of higher overall metabolism, as proven by higher DM removal (Table 2), micropollutant removal was not improved, indicating that the co-metabolic interactions were not linked to the overall metabolism. Either a specific metabolism is involved in co-metabolic interactions or the biodegradation of micropollutants is limited by other phenoma such as bioavailability.

3.5. Influence of the addition of cellulose on micropollutant removal

As a readily biodegradable particulate compound, cellulose was added to SS in order to obtain a high level of overall co-metabolism.

Cellulose is a polymer of glucose. The mass balance of monomeric pattern conversion [43] predicts the production of a biogas composed of 50% of methane during cellulose degradation:

$$C_6H_{10}O_5 + H_2O \rightarrow 3CH_4 + 3CO_2$$

This explains why the methane content in the reactor fed with CSS decreased ($57 \pm 2\%$) in comparison to the reactor fed with SS ($69 \pm 1\%$, Fig. 1). The extent of the enhancement of COD and DM removal (Table 2), as well as the low carbohydrate content in digested CSS (not statistically different from digested SS), suggests that all the cellulose was metabolized (100% removal). The degradation rates for cellulose and sludge matter could thus be calculated. In fact, the overall metabolism was found to be stimulated with CSS (1.1 g_{COD}/L/day removed with CSS, in contrast to 0.7 g_{COD}/L/day with SS), whereas the metabolism of the specific sludge matter was divided by a 2-fold factor compared to the SS feed (0.4 g_{COD}/L/day with CSS in contrast to 0.7 g_{COD}/L/day with SS). However, micropollutant removal did not increase: on the contrary, it decreased by a factor of 1.6 ± 0.4 (Fig. 2). The addition of readily biodegradable substrates, such as yeast extract, molasses and cellulose, had already been performed in several batch experiments. The results were contradictory: in some cases, the removal rate of PAHs, NP and PCBs increased [14,21,44,45] whereas the removal of PCBs [17] and of brominated micropollutants [46] was not affected. Such discrepancies might be ascribed to different physico-chemical and microbial sludge properties. Depending on the microbial population, overall metabolic stimulation might or might not concern the microorganisms involved in micropollutant degradation.

3.6. Influence of the addition of dissolved and colloidal matter on micropollutant removal

In SupSS, the proportion of dissolved and colloidal matter was multiplied by a 3-fold factor, in comparison to SS (Table 2). As particulate disintegration and hydrolysis are assumed to limit sludge methanisation, higher COD and DM removal was expected for SupSS than for SS. In fact, quite the opposite occurred: COD and DM removal was reduced by approximately 10% (Table 2). This slight decrease in the overall metabolism might be due to the effect of concentration: SupSS contained 19 g_{COD}/L, whereas SS contained 22 g_{COD}/L (Table 2). An inhibitory effect might also have occurred: VFA concentration reached 1.6 g/L in SupSS (Table 2), in which acetate accounted for 44%. Nonetheless, VFAs were totally removed (Table 2): they were not responsible for the inhibition of the methanogenic consortium. The hypothetical inhibition may have been caused by an unidentified constituent of DCM.

Table 3
Comparison of the different feeds and confrontation of micropollutant removal to the relative bioavailability (according to the usual hypotheses) and co-metabolism (assumed to be linked to overall metabolism). The "+" symbol indicates an enhancement, "−" symbol a diminution and "?" indicates that, in the light of the present and literature data, no assumption can be made about the effect.

Feed comparison	Presumable effect on bioavailability	Presumable effect on co-metabolism	Measured effect on micropollutant removal
SS with PS	?	+	+
TTPS with PS	−	+	−
CSS with SS	?	+	−
SupSS with SS	+	−	−

As a result of the decrease in overall metabolism, the potential co-metabolic effect on micropollutant removal would be expected to be negative. Micropollutants were, in fact, less efficiently removed (Fig. 2). The high acetate concentration in feed might contribute to lower their biodegradation, as polyaromatic hydrocarbons biodegradation is supposed to produce acetate and hydrogen [24] The acetate metabolic fluxes modification caused by high acetate load might cause the modification of micropollutant metabolism.

In SupSS, DCM proportion was enhanced without the denaturing phenomenon reported for thermal treatment [29]. According to the bioavailability hypotheses previously mentioned in Section 3.4, micropollutant bioavailability may have been increased. Insofar as micropollutant removal decreased, the decrease in overall metabolism or inhibitory effects might be predominant in comparison to the increase of bioavailability.

3.7. Regression model

The presumable effect on bioavailability level (based on the usual hypotheses presented in Section 3.4), the presumable effect on co-metabolism (in reference to the measured overall metabolism) and the measured micropollutant removal were summarized in Table 3. The anaerobic digestion performance with PS, TTPS, SS, CSS and SupSS tends to show that co-metabolism and bioavailability predominate alternately and limit micropollutant removal. The predominant phenomenon may be determined by the feed characteristics. The feed characteristics differed statistically one from the others in different parameters (Table 2) so that conclusions were difficult to draw about the individual effect of each parameter. To accurately pinpoint the effect of each one, a PLS multivariate linear regression was performed. The regression led to the following model (dimension 6):

$$\begin{aligned}\text{REMOVAL} = {} & 151 + 0.41 \log K_{ow} - 0.077M - 1.94n5C - 3.08n6C \\ & - 1.86n\text{Cl} - 11.8n\text{OH} + 0.08 \log H + 131\text{DCM} \\ & - 24.0\text{Proteins} - 45.7\text{Carbo} - 336\text{Lipids} \\ & - 54.2\text{Cellulose} - 577\text{VFA}\end{aligned}$$

The modelled removal corresponded well with the actual measured removal for each of the 71 individual cases studied (Fig. 3). This indicates that the representation by the selected predictors of removal variability was good. Model accuracy was not improved by including data on COD, DM, protein and carbohydrate removal

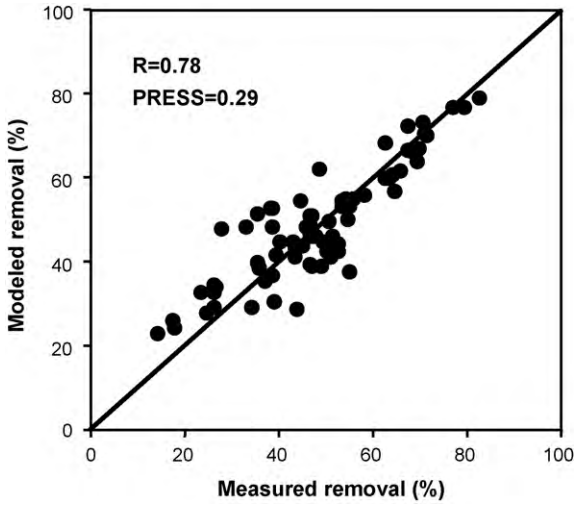

Fig. 3. Modelled vs. measured values of the removal of PAHs, NP and PCB180 during the anaerobic digestion with PS, TTPS, SS, CSS and SupSS.

(data not shown), suggesting that the relevant information is to be found in the feed characteristics.

The relative importance of the feed and micropollutant characteristics could be assessed by comparison of the centered and reduced regression coefficients (Fig. 4). The VFA content was found to be the most influential parameter. This was due to the lesser removal of micropollutants reported with the SupSS feed, rich in VFAs. A possible direct inhibition by VFAs was mentioned in the previous paragraph, but the inhibition may have been caused by an undetermined compound. The effect of such an inhibitor would be linked to the presence of VFAs. Lipid content was the second most important parameter. In the first place, hydrophobic material may not be readily accessible for microorganisms, and thus it lowers the overall co-metabolism. Furthermore, it favours the sorption of micropollutants to particles [29] and may thus lower bioavailability. Both these effects may account for the strong negative impact of lipids. The presence of cellulose, the third most important negative parameter, was shown to increase overall metabolism but to decrease the metabolism of sludge matter, resulting in a drop in micropollutant removal.

The only positive parameter of the feed was the proportion of DCM, probably because it determined the aqueous and bioavailable fraction of micropollutants, and because DCM is a readily bioaccessible substrate.

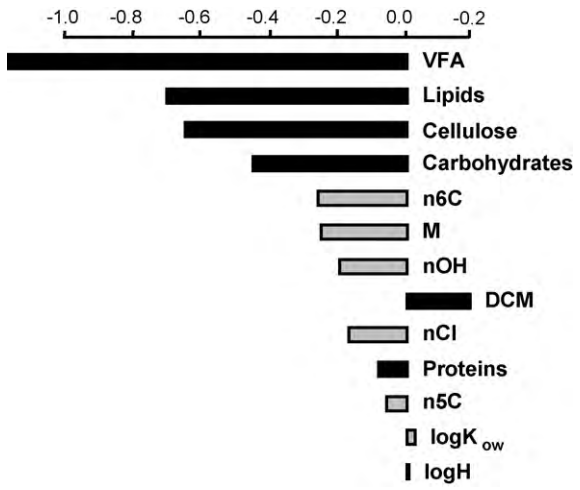

Fig. 4. Centered and reduced regression coefficients. Coefficients related to the characteristics of the sludge feed are black coloured, whereas those related to micropollutants are represented in grey.

The characteristics of micropollutants also account for differences in removal rates. The number of 6 carbon rings had the most negative effect. Indeed, the higher the aromaticity, the more recalcitrant to biodegradation [23,25]. The influence of Henry's law constant ($\log H$) was negligible, which confirms the very low removal through volatilisation.

4. Conclusion

The removal of PAHs, NP and PCBs by anaerobic digestion varied greatly as a function of feed sludge characteristics. The sample of primary sludge was slightly richer in proteins and lipids than the sample of secondary sludge. These differences may account for the slightly lower removal from PS of COD and micropollutants.

The thermal treatment applied to the PS diminished the removal of micropollutants, while at the same time it stimulated the overall metabolism. When cellulose was added, micropollutant removal did not increase despite higher overall metabolism. These results demonstrate that co-metabolic synergy with the metabolism of micropollutants is not determined by the overall metabolism. Such synergy might be linked to a more specific metabolism or co-metabolism did not limit micropollutant removal in these cases.

In contrast, the overall metabolism in the reactor fed with a high proportion of DCM dropped while the bioavailability of micropollutants was hypothetically enhanced, resulting in lower micropollutant removal. In this case, limitation due to co-metabolism was likely to have predominated over limitation caused by bioavailability.

Depending on the reactor, greater or lesser removal might be explained either by variations in co-metabolism or by different levels of bioavailability. This suggests that no one mechanism can be identified as the absolute limiting factor. More research is needed to investigate and accurately quantify both mechanisms and determine their relative significance. Nonetheless, the results suggest that a detailed characterization of the feed may help to predict the removal of micropollutants.

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