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Study on the fate of per- and polyfluoroalkyl substances during thermophilic anaerobic digestion of sewage sludge and the role of granular activated carbon addition

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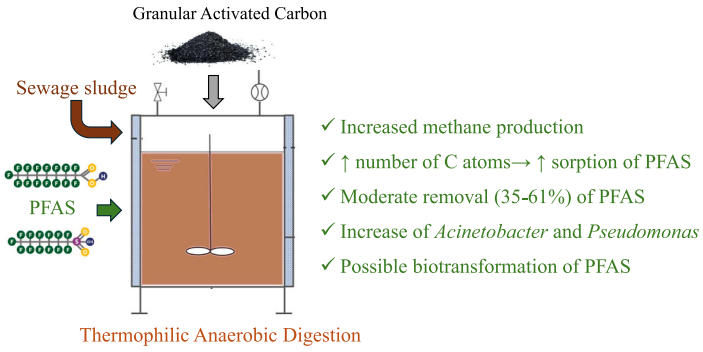
HIGHLIGHTS

- The role of GAC addition and voltage application in thermophilic AD was examined.
- GAC addition enhanced biogas production and decreased dissolved COD and VFAs.
- PFAS sorption amplified by more C atoms and sulfonated headgroup presence.
- Moderate removal (35–61%) for 4 out of 6 PFAS in bioreactors with GAC.
- Increase of *Acinetobacter* suggest its pivotal role in PFAS biotransformation.

GRAPHICAL ABSTRACT



Abbreviations: AD, anaerobic digestion; BMP, biochemical methane potential; CAH, chlorinated aliphatic hydrocarbons; COD, chemical oxygen demand; dCOD, dissolved chemical oxygen demand; DIET, direct interspecies electron transfer; GAC, granular activated carbon; HRT, Hydraulic retention time; K_d, sorption distribution coefficient; OTU, operational taxonomic unit; PMT, persistent, mobile and toxic; PFAA, perfluoroalkyl acids; PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHpA, perfluoroheptanoic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFUdA, perfluoroundecanoic acid; Q, Flowrate; STPs, sewage treatment plants; TEA, terminal electron acceptor; TS, total solids; TSS, total suspended solids; V, Voltage; VFAs, volatile fatty acids; VS, volatile solids.

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ABSTRACT

Limited information is available on the removal of per- and polyfluoroalkyl substances (PFAS) in anaerobic digestion (AD). The fate of six PFAS was studied in thermophilic bioreactors in the presence of granular activated carbon (GAC) and voltage application. Reactors with GAC exhibited lower concentrations of volatile fatty acids and higher methane production compared to those with and without the application of voltage. Analysis of PFAS in dissolved and solid phase showed that their distribution was dependent on perfluorocarbon chain length and functional group. Mass balances showed that PFAS were not removed during conventional AD or after applying voltage; however, significant removal (up to 61 ± 8 %) was observed in bioreactors with GAC for perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorooctane sulfonate (PFOS). Biomass characterization showed that in these bioreactors, the relative abundance of *Acinetobacter* and *Pseudomonas* was higher, indicating their potential role in PFAS biotransformation.

1. Introduction

Anaerobic digestion (AD) is applied to treat and stabilize sewage sludge, as well as produce biogas. However, in recent years, the field of AD technology has faced a new set of challenges related to making it safer and sustainable (Raheem et al., 2018). One major challenge is related to how the quantity of biogas and yield of methane can be increased. Biogas production is a way to create clean and renewable energy as a by-product of the treatment process and to ensure the financial viability of a wastewater treatment unit. The second challenge is related to increasing the removal rate of major pollutants.

To address these challenges, some modifications of conventional AD have been tested, including the addition of conductive materials (Guo et al., 2022) and the application of voltage in the bioreactor (Wang et al., 2022). Conductive materials, such as granular activated carbon (GAC), can improve the efficiency of AD by offering an increased surface area to microorganisms, maintaining stable pH levels, absorbing harmful substances and promoting direct interspecies electron transfer (DIET) (Kalantzis et al., 2023). Applying electrical voltage in the range of 0.3 to 1.0 V can also stimulate methanogenesis by changing the redox potential, making it more favorable for electron transfer to occur (Gatidou et al., 2022). Combining the addition of conductive materials with voltage application has resulted in superior results when compared to utilizing them separately (Mostafa et al., 2021). Additionally, temperature is a crucial variable shaping the formation of existing microbial communities during sludge AD and overcoming the challenge of low biogas production. Wang et al. (2023) reported a 40 % increase in biogas production during thermophilic AD compared to mesophilic AD. The accelerated metabolic activity of microorganisms observed in thermophilic conditions leads to faster degradation of organic matter and complex compounds, which in turn facilitates methanogenesis. To complement these enhanced AD approaches, biological, thermal or physical–chemical pre-treatment steps can also be used prior to entering the AD reactor, to enhance biogas yields (Volschan Junior et al., 2021).

Reaching zero pollution from pollutants including persistent, mobile and toxic (PMT) substances is one of the main objectives of the European Union's Chemical Strategy for Sustainability Towards a Toxic-Free Environment (European Commission, 2021). A substantial amount of these substances find their way into urban sewerage systems eventually reaching sewage treatment plants (STPs) (Arp and Hale, 2022). During conventional wastewater treatment processes, organic micropollutants end up in sewage sludge via sorption to suspended solids (Stasinakis et al., 2013) posing a potential threat for the terrestrial environment during sludge disposal or reuse (Thomaidi et al., 2016). Among the PMT substances detected in sewage sludge, the per- and polyfluoroalkyl substances (PFAS) have attracted significant global attention and concern during recent years due to their wide use and chemical properties (Hale et al., 2022). Their concentrations in sewage sludge have been reported to range between a few ng to several μg per gram of dry weight sludge (Arvaniti and Stasinakis, 2015). There is also limited information available related to their fate during AD.

In an earlier study, Liou et al. (2010) investigated the biodegradability of the PFAS perfluorooctanoic acid (PFOA) in sludge under methanogenic conditions, where reductive defluorination could potentially occur (Yu et al., 2022). After an incubation time of 231 days in serum bottles, no removal of PFOA was reported; however, a decrease in concentration was observed in the experiment conducted under cometabolic conditions during the dechlorination of trichloroethene (Liou et al., 2010). Li et al. (2021) demonstrated that AD at 35 °C can form perfluoroalkyl acids (PFAA) from precursor substances, such as fluorotelomer alcohols. Therefore, mass balances of PFAS in AD reactors can be complicated as there can be co-occuring production and loss processes of individual PFAS, such as PFOA. To date, biological mineralization of PFAS to fluoride ions has not been reported, even under AD conditions (Ross et al., 2018). From a thermodynamic point of view, it seems plausible that PFAS might function as the terminal electron acceptor (TEA) in metabolic processes and their biotransformation could resemble the biological reductive dechlorination reported for organochlorine compounds. Yu et al. (2022) previously reported the microbial reductive defluorination of two branched unsaturated per- and polyfluorinated carboxylic acids in anaerobic experiments with dehalorespiring cultures. Cao et al. (2022) reported the biotransformation of PFOA in experiments conducted with anaerobic granular sludge while Choi and Kan (2024) observed a decrease of PFOS concentration in biochemical methane potential (BMP) tests conducted under mesophilic conditions. Despite the wide use of thermophilic anaerobic reactors for sewage sludge treatment, to the best of our knowledge, no information is available about the fate of PFAS during thermophilic AD. Previous studies have, focused on other groups of organic micropollutants such as pharmaceuticals (Samaras et al., 2014) and siloxanes (Gatidou et al., 2016). Additionally, the modifications of the AD process through GAC addition or/and the application of voltage, have not yet been studied with regard to the effect on PFAS removal.

The primary goal of this work was to study the fate of PFAS during thermophilic sludge AD. The hypothesis of the work was that the modification(s) of AD would result in PFAS removal during sludge treatment. The role of GAC addition and voltage application on the performance of the process and the removal of target micropollutants was also examined. For this reason, four lab-scale anaerobic reactors were operated in parallel under thermophilic conditions for a period of 104 days and monitored for the fate and removal of six PFAS (perfluoroheptanoic acid, PFHpA; PFOA; perfluorononanoic acid, PFNA; perfluorodecanoic acid, PFDA, perfluoroundecanoic acid, PFUdA; linear and total PFOS). These substances were selected due to their wide use and often detection in sewage sludge worldwide (Arvaniti and Stasinakis, 2015). In parallel, the performance of the reactors was examined for the removal of volatile solids (VS), biogas, and volatile fatty acids (VFAs) production. In addition, the composition of biomass in each bioreactor was also investigated by applying high-throughput 16S rRNA gene sequencing.

2. Materials and methods

2.1. Analytical standards and reagents

All PFAS standards were supplied from Alfa Aesar (Germany) (≥95 %). Stock solutions were prepared in methanol (1000 mg/L) and stored at −18 °C from which a mixture of target compounds was prepared (320 mg/L) for spiking purposes. Methanol (MeOH) and acetonitrile (ACN) were of LC-MS grade and supplied by Merck (Germany). Ultra-pure HCl (32 %) and acetic acid (99.7 %) were also supplied by Merck (Germany). Syringe RC-filters (0.2 μm) and paper filters (5–13 μm) were obtained by Macherey-Negel (Germany) and LLG (Germany), respectively, while OASIS HLB cartridges (200 mg, 6 mL) were supplied by Waters (Milford, MA, USA). Ultra-pure water was produced in the laboratory by a Milli-Q/Milli-RO Millipore system.

2.2. Operation of thermophilic anaerobic digesters

Four continuous stirred tank reactors operated with a hydraulic retention time (HRT) of 20 days and a working volume of 1 L under strictly anaerobic conditions. To determine the effect of GAC addition and the application of voltage on micropollutants removal, the following four combinations were tested: In AD Reactor 1 (AD-V) voltage was applied at an electrical potential of 0.8 V (V); in AD Reactor 2 (AD-GAC-V) GAC (Ravasol B-830, Inaqua, Germany, effective size: 0.8–1.0 mm) was added and voltage was applied; in AD Reactor 3 (AD-GAC) GAC was applied without the application of voltage, and in AD Reactor 4 (AD) neither voltage nor GAC was applied (Table S1). The reactors were constantly maintained at a temperature suitable for thermophilic microbial activity (55 °C) using a water bath. In reactors AD-V and AD-GAC-V, the voltage was continuously applied using a digital bench DC Power supply (QJ3005C, QJE) through carbon cloth electrodes (4 cm x 5 cm), fully submerged into the sludge.

During start-up of the bioreactors, anaerobic digested sludge from a pilot-scale mesophilic anaerobic digester treating agro-industrial wastewater was used as inoculum (Kalantzis et al., 2023) while in reactors AD-GAC and AD-GAC-V, 10 g of GAC were also added to ensure an initial concentration of 10 g/L. During the experiments the sludge feed consisted of a mixture of 70 % secondary sludge and 30 % primary sludge. The secondary sludge was from Mytilene STP (Lesvos, Greece), while the primary sludge was provided by the STP that serves Antissa village (Lesvos, Greece). The anaerobic reactors were allowed to acclimate to thermophilic conditions for a period of two weeks prior to the introduction of target micropollutants. Following a two-week acclimation phase, a mixture of PFAS was spiked to the sludge feed to achieve an initial concentration of 100 μg/L and the reactors operated for a period of three months with daily sludge feeding of 50 mL to maintain an HRT of 20 d. The daily feed for AD-V-GAC and AD-GAC reactors also included 0.5 g of GAC to maintain a stable GAC concentration of 10 g/L.

To monitor the performance of the systems, samples were taken twice a week from the inlet and outlet of the reactors for COD, total solids (TS), VS, and VFAs, and during Day 70, 77, 84 and 91 for the target microcontaminants. Biogas volume and composition were measured twice a week. Samples for DNA analysis were taken at the end of the experimental phase. Collected samples were centrifuged and stored at −20 °C until analysis.

2.3. Analytical methods

TS, VS, total suspended solids (TSS), and dissolved COD (dCOD) were analyzed following the procedures described in Standard Methods (APHA, 2005). For the measurement of COD, the samples were centrifuged, filtered (47 mm LLG-Filter papers, LLG-Labware, Germany), and analyzed using the closed reflux colorimetric method. pH values were determined using a Consort C932 portable pH meter, biogas volume was measured using the water displacement method while its composition was determined using a Geotech GA5000 Portable Gas Analyzer. An Agilent 6890 N GC–MS System with a flame ionization detector was utilized for the determination of VFAs. 1 μL of centrifuged and filtered sample was directly injected into the inlet. The carrier gas used was helium, and the run time for every sample analysis was 15 min while the detector and injector temperature were set at 250 °C (Atasoy et al., 2019). For the calibration of the instrument, a volatile free acid standard purchased from Sigma Aldrich was used.

PFAS were analyzed both in liquid and solid phases according to Arvaniti et al., (2014b). Isolation of target compounds from the liquid samples was performed using Oasis HLB SPE cartridges. Solid samples were extracted by ultra-sonication and cleaned up following the SPE method applied for liquid phase. An Exion UPLC system coupled to a AB Sciex QTRAP mass spectrometer 6500+ (AB Sciex, Canada) was used for the qualitative and quantitative determination of target compounds. A Xterra MS C18 (2.1 x 100 mm, 3.5 um) column was used for chromatographic separation and a XBridge C18 (2.1 x 50 mm, 2.5 um) was employed as delay column between autosampler and mixer for delay of PFAS contamination come from UPLC system and autosampler. The flow rate was 0.3 mL/min and the mobile phase was consisted of methanol (A) and buffer with 5 mM ammonium acetate and 5 mM 1-methyl-piperidine in water. A gradient program was employed with 10 % (A) for 3 min, ramping to 40 % (A) at 3.1 min, ramping to 90 % (A) at 26 min, held isocratically to 28 min and ramping back to 10 % (A) at 29 min and held until 32 min for re-equilibration. Mass spectrometer was operated in negative mode, equipped with Turbo-V ion-spray source, with operating conditions: Ion Spray Voltage, −4500 V; Curtain Gas (CUR), 40 psi; Ion Source Gas 1 and 2, 35 psi; Source temperature 250 °C. Multiple Reaction Monitoring (MRM) was the scan type and fragments are presented in Table S2. Data were collected and processed using AB Sciex Analyst software version 1.7/Sciex OS version 2.1.6.59781.

2.4. High-throughput 16 S rRNA gene sequencing

At the end of the experiment, the microbial profile of the suspended sludge contained in each bioreactor was evaluated. A mass of 250 mg underwent DNA extraction using the DNeasy Power Soil Pro Kit (Qiagen, Germany). The extracted genomic DNA was then sent to DNASense ApS (Denmark) for analysis, following the methodology outlined by Gatidou et al. (2022). For the PCR amplification, universal primers targeting the 16S rRNA gene were used. Specifically, the 515FB primer (GTGYCAGCMGCCGCGGTAA) served as the forward primer and the 1391R primer (GACGGGCGGTGWGTRCA) was used as the reverse primer. These primers are well-suited for amplifying a wide range of microbial DNA, hence providing a comprehensive overview of the microbial communities in the samples. The PCR conditions were meticulously set: an initial denaturation at 98 °C for 3 min, followed by 25 cycles of amplification (each cycle comprising 98 °C for 30 s, 62 °C for 20 s, and 72 °C for 2 min), and a final elongation step at 72 °C for 5 min.

Amplicons were purified using CleanNGS SPRI beads and eluted in nuclease-free water. Sequencing libraries, prepared using the SQKLSK114 kit, were cleaned up similarly and quantified with the Qubit dsDNA HS Assay kit. Library quality and size were confirmed by gel electrophoresis on a Tapestation 2200. Sequencing was performed on a MinION R10.4.1 flowcell using MinKNOW software (v23.04.6) and basecalled with guppy g6.5.7 using the super accurate basecalling configuration. The sequencing reads were filtered (320–2000 bp, phred score > 15) using filtlong v0.2.1 and mapped to the MiDAS database (release 4.8.1) with minimap2. Quality filtering ensured < 5 % deviation in sequence alignment length and mapping quality > 0.79. Reads corresponding to low-abundant operational taxonomic units (<0.01 %) were excluded. Data analysis was conducted using R (v4.3.0) with packages including ampvis2, tidyverse, seqinr, ShortRead, and iNEXT (analysis facilitated by DNASense ApS).

2.5. Equations and statistical analysis

The removal of studied PFAS was calculated using Equation (1):

$$Removal(\%) = \frac{M_{in} - M_{out}}{M_{in}} \times 100 \quad (1)$$

where, M_{in} is the mass of each PFAS in the influents and M_{out} is the mass of each PFAS in the effluents.

The masses of PFAS (expressed as ng/d) were calculated by considering the fraction of the PFAS that were found in the dissolved and solid phase, according to Equation (2):

$$M = (C_{dis} \times Q) + (C_s \times TSS \times Q) \quad (2)$$

where, Q is the flowrate (L/d), C_{dis} is the concentration of target PFAS in the dissolved phase (ng/L), C_s is the concentration of target PFAS on the suspended solids (ng/mg) and TSS is the concentration of the total suspended solids (mg/L).

The values of sorption distribution coefficient, K_d (as L/kg) for PFAS were calculated using Equation (3):

$$K_d = \frac{C_s}{C_{dis}} \quad (3)$$

where, C_s the concentration of the target substance in the solid phase (ng/kg) and C_w the concentration of the target substance in the dissolved phase (ng/L).

OriginPro 8 SR0 (version 8.0724, Northampton, USA) was used for the construction of figures. One-way ANOVA was used to compare the production of biogas and methane, the removal of VS, and the removal of target micropollutants in different bioreactors. When ANOVA was significant at $p < 0.05$, the Tukey's HSD post hoc test was run to identify differences. R (version 4.3.2) was used for the statistical treatment of the results.

3. Results and discussion

3.1. Operation of the CSTR systems and removal of major pollutants

The values of pH, TS, and VS in influent and effluent sludge, as well as the removal of VS and the production of biogas and methane, are presented in Table 1. During the operation of the reactors at steady state (period after day 54), the average value of pH in each reactor was between 7.12 ± 0.13 (AD) and 7.31 ± 0.14 (AD-V). Slightly higher pH values were noticed during the start-up of their operation, though they were gradually decreased to levels between 7.0 and 7.5 at the end of the experiments (Figure S1). The concentrations of VS in the reactors increased during the study (Figure S2) while their average concentrations at the outlet of each reactor at steady state were 14.6 ± 4.2 g/L (AD-V), 12.8 ± 2.7 g/L (AD-GAC-V), 15.7 ± 6.1 g/L (AD-GAC) and 13.7 ± 4.6 g/L (AD). The average VS removal ranged between 33 ± 24 (AD-V) and 49 ± 20 (AD-GAC-V) (Table 1). The reactor with applied voltage and added GAC exhibited higher removal rates for VS compared to other reactors, indicating a possible enhancement of the hydrolysis step (Fig. 1a). However, these differences were not statistically significant ($p > 0.05$) owing to the large standard deviations. It is worth noting that in all bioreactors, the removal rate of dCOD was negative. During mesophilic AD, COD values are expected to be lower in the effluent because organic compounds are broken down by microorganisms during

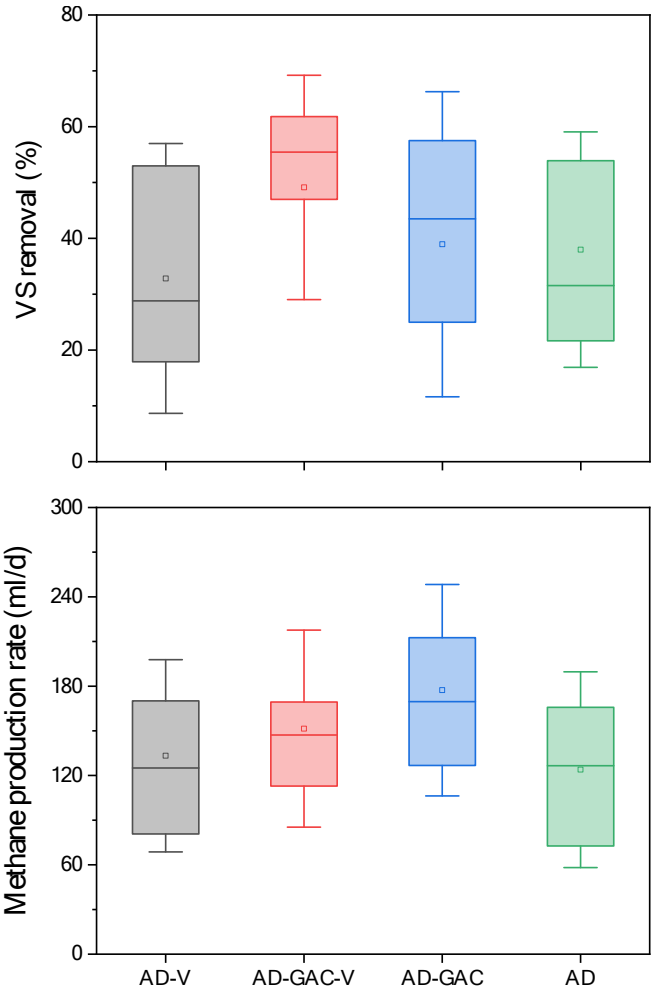

Fig. 1. (a) Volatile solid (VS) removal and (b) daily methane production rate in the lab-scale thermophilic AD systems at steady state (period after Day 54 of the experiment, n = 14). **AD-V**: anaerobic digestion with application of external voltage (0.8 V); **AD-GAC-V**: anaerobic digestion with addition of GAC and application of external voltage (0.8 V); **AD-GAC**: anaerobic digestion with addition of GAC; **AD**: typical anaerobic digestion. Boxes represent median, lower and upper quartiles, while square points inside the box represent the mean values. Standard deviation is indicated by the bottom and the top of the plot, respectively.

Table 1
Influent and effluent characteristics and performance of the anaerobic reactors used in the current study during steady state. Experiments were conducted at thermophilic conditions and the reactors were monitored for a total period of 103 days. As steady state was considered the period after Day 54. Values as expressed as mean ± sd (n = 14).

	pH	TS (g/L)	VS (g/L)	VS removal (%)	disCOD (mg/L)	Biogas production (mL/d)	Methane content (%)
Feed	6.84 ± 0.23^{a}	31.2 ± 4.5^{a}	22.5 ± 4.0^{a}	–	1,099 ± 577^{a}	–	–
Feed (GAC)	6.93 ± 0.21^{a}	35.6 ± 4.7^{a}	26.6 ± 4.5^{a}	–	1,189 ± 684^{a}	–	–
AD-V	7.31 ± 0.14^{b}	22.9 ± 6.1^{b}	14.6 ± 4.2^{b}	33 ± 24^{a}	2,892 ± 736^{b,c}	221 ± 85^{a}	58 ± 7^{a}
AD-GAC-V	7.25 ± 0.14^{b}	20.4 ± 3.8^{b}	12.8 ± 2.7^{b}	49 ± 20^{a}	2,260 ± 1,296^{b}	254 ± 74^{a}	58 ± 8^{a}
AD-GAC	7.26 ± 0.14^{b}	24.4 ± 8.6^{b}	15.7 ± 6.1^{b}	39 ± 27^{a}	2,123 ± 678^{b}	297 ± 101^{a}	59 ± 8^{a}
AD	7.12 ± 0.13^{c}	21.8 ± 7.0^{b}	13.7 ± 4.6^{b}	38 ± 21^{a}	3,856 ± 650^{c}	231 ± 94^{a}	50 ± 14^{a}

* In each column, different letters indicate significant ($p < 0.05$) differences based on one-way ANOVA (Tukey test).

methanogenesis and transformed to biogas. However, in environments with higher temperatures, COD solubilization is increased. Under thermophilic conditions, hydrolysis is more efficient, meaning that complex molecules are broken down into smaller and more soluble organic compounds. The presence of these simple compounds in the dissolved phase leads to an increase in dCOD values (Tiwari et al., 2021). In the current study, the lowest dCOD concentrations in the effluents were observed in reactors containing GAC and they were equal to 2,123 ± 678 mg/L (AD-GAC) and 2,260 ± 1,296 mg/L (AD-GAC-V) (Table 1).

The highest daily biogas yield was observed in reactors AD-GAC and AD-GAC-V (297 ± 101 mL/d and 254 ± 74 mL/d, respectively), and similarly, the highest methane content was recorded in the same reactors (59 ± 8 % and 58 ± 8 %, respectively) (Table 1). The average daily methane production during steady state operation ranged between 124 ± 66 mL/d (AD) and 177 ± 71 mL/d (AD-GAC) (Fig. 1b). The biogas production profile during the transition from mesophilic to thermophilic operation (Figure S3) was consistent with previous findings reported by Tian et al. (2015). Specifically, the one-step startup of a CSTR reactor operated at a HRT of 20 days resulted in a sudden increase in biogas production during the initial days, followed by a subsequent decrease to minimum values before stabilizing after 40 days. Considering the role of GAC, its addition resulted to a slightly higher production of biogas and methane in the studied bioreactors (Table 1, Fig. 1b), however, ANOVA analysis indicated that these differences were not statistically significant ($p > 0.05$). There are few previous studies investigating the effect of GAC or other conductive materials addition on the performance of thermophilic AD digestion. In biochemical methane potential (BMP) tests, Tiwari et al. (2021) reported an enhanced cumulative biogas yield when GAC was added at concentrations of 10 and 20 mg/L while Zhang et al. (2020) observed lowed dCOD concentrations and higher methane yields in thermophilic reactors operating with initial addition of GAC of 15 g/L. Activated carbon could act as a conductive material facilitating DIET. During this process, activated carbon granules act as electric conductors, transferring electrons directly from donor microbes to receiving ones, without the need of reduced molecules (Zhao et al., 2020). These interactions can increase the speed of the chemical reactions necessary for methanogenesis, resulting in higher daily methane yield. Other important factors contributing to higher biogas performance on reactors with added GAC include the high surface area of the granules promoting the formation of biofilm and offering a place to enrich microbes and adsorb dissolved molecules and toxic compounds (Jiang et al., 2021).

Analysis of VFAs in the bioreactors at different times of the experiments and expression of their concentrations as COD-VFAs (mg/L) showed that the maximum sum of VFAs was measured in the AD reactor (1784 ± 508), followed by the AD-V reactor (1421 ± 562) (Table S3). Lower concentrations were observed in the reactors containing GAC at 852 ± 412 mg/L for the AD-GAC and 934 ± 367 mg/L for the AD-GAC-V. A decreasing trend in the concentrations of VFAs produced was observed during the experiment while acetic acid was the dominant VFA in all bioreactors (Figure S4). In the reactors containing GAC, this compound contributed to 57 % (AD-GAC) and 71 % (AD-GAC-V) of the total VFAs production. In the absence of GAC, propionic acid was also measured in substantial concentrations contributing to 28 % (AD) and 25 % (AD-V) of total COD-VFAs concentrations. According to the literature, acetic acid is considered the most favorable compound for methane production and the degradation of propionic acid is considered less advantageous (Khanal, 2008). Two distinct processes influence the concentration of VFAs in anaerobic reactors. On one hand, the hydrolysis of complex substrates and the acidification of their monomers results in the accumulation of VFAs in the reactors. On the other hand, acetogenesis and, to a significant extent, methanogenesis leads to the consumption of VFAs in the reactors. In cases where propionic acid increases in the bioreactor, it can interfere with the activity of methanogens inhibiting methane production (Dai et al., 2022). The adsorption of organic compounds, such as VFAs, onto the surface of GAC could potentially contribute to the decrease in COD and VFAs within reactors that contain GAC. Nonetheless, the observed decrease, along with the increased rate of methane production in these reactors, implies that these organic compounds, even when adsorbed, are eventually converted into methane. Consistent with these findings, Xiao et al. (2022) stated that the presence of GAC could stimulate the growth of acetate-oxidizing bacteria, hydrogenotrophic methanogens, and acetoclastic methanogens. In other studies, Xu et al. (2018) reported that GAC accelerated the consumption of propionate and butyrate while Yang et al. (2017) reported that the transformation of propionate to acetate was enhanced in reactors with GAC.

3.2. Fate of PFAS in thermophilic anaerobic digestion

The distribution of PFAS between dissolved and solid phases was investigated in influent and effluent samples collected during the study. As seen in Fig. 2, the increase of the perfluorocarbon chain length in perfluorocarboxylic acids resulted in a gradual decrease of the percentage that was detected in the dissolved phase. For PFHpA, that contains 7C atoms, the dissolved fraction was higher than 79 % in all types of samples, while the dissolved fraction for PFUdA containing 11C atoms did not exceed 47 %. This is in agreement with Arvaniti et al. (2014) who reported that the sorption affinity of PFAS increases with the increase in the number of carbon atoms in the perfluorocarbon chain. A comparison of PFOS and PFOA that both contain eight carbon atoms but have different functional groups in their molecules, indicated that the presence of sulfonic acid results in a decreased tendency of PFAS to remain in the dissolved phase due to relatively higher sorption. A difference in the distribution between different phases was also observed between linear and total (branched + linear) PFOS. For all types of samples, less linear PFOS was detected in the dissolved phase (Fig. 2), indicating that branched PFOS has a higher solubility.

The K_d values of the target PFAS in different types of samples are shown in Table 2. As expected, the increase of perfluorocarbon chain length resulted in higher sorption distribution coefficient values while higher K_d values were also observed for the compound containing sulfonated headgroup (PFOS) comparing to that contained carboxylic headgroup (PFOA). A similar trend has also been reported in previous studies with other matrices such as soil (Hubert et al., 2023). A comparison of the calculated K_d values with those previously published in the literature for anaerobically digested sludge showed that differences are often observed between different studies. In a recent study, Li et al. (2022) calculated the K_d values of different PFAS in anaerobically

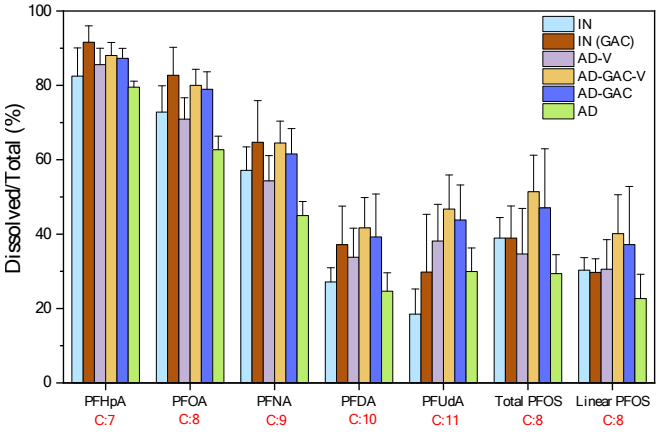

Fig. 2. Percentage of PFAS detected in the dissolved phase of influents and effluents (mean + sd). **IN**: Influent; **IN (GAC)**: influent with addition of GAC; **AD-V**: anaerobic digestion with application of external voltage (0.8 V); **AD-GAC-V**: anaerobic digestion with addition of GAC and application of external voltage (0.8 V); **AD-GAC**: anaerobic digestion with addition of GAC; **AD**: typical anaerobic digestion.

Table 2
Solid-water distribution coefficient (K_d) values for target PFAS in sludge samples taken from the influents and effluents of the thermophilic AD systems (expressed as L/kg).

	PFHpA	PFOA	PFNA	PFDA	PFUdA	Total PFOS	Linear PFOS
IN	7 ± 4	13 ± 5	26 ± 7	91 ± 21	164 ± 63	53 ± 9	77 ± 13
IN (GAC)	3 ± 1	6 ± 3	16 ± 7	53 ± 22	104 ± 101	48 ± 17	70 ± 15
AD-V	7 ± 3	17 ± 5	35 ± 10	86 ± 38	73 ± 36	89 ± 52	101 ± 48
AD-V-GAC	6 ± 2	11 ± 3	23 ± 6	60 ± 18	48 ± 13	41 ± 14	65 ± 23
AD-GAC	5 ± 1	9 ± 23	21 ± 5	56 ± 21	45 ± 14	42 ± 20	65 ± 32
AD	9 ± 23	22 ± 6	45 ± 14	119 ± 57	92 ± 46	93 ± 44	140 ± 81

treated sludge collected from a full-scale AD and reported values of 8 ± 13 L/kg for PFHpA, 28 ± 19 L/kg for PFOA, 130 ± 16 L/kg for PFNA and 592 ± 94 L/Kg for PFOS. On the other hand, in a previous study by Arvaniti et al., (2014a) where sorption experiments had been conducted in the lab, higher K_d values of 162 ± 42 L/kg for PFOA and 1693 ± 357 L/kg for PFOS were reported. The differences observed between reported sorption distribution coefficients are due to various factors such as the different experimental protocols applied in each study for K_d calculation and the chemical characteristics of the matrix (pH, organic content, concentrations of proteins and lipids, particles distribution, ionic strength) (Ebrahimi et al., 2021; Lewis et al., 2023).

There was no removal of target PFAS during thermophilic AD in the conventional AD reactor as well as in the AD reactor where voltage was applied (AD-V). However, the addition of GAC led to a statistically significant (p-value < 0.05) increase in the removal of PFHpA, PFOA, PFNA, total PFOS, and linear PFOS in the reactors AD-GAC-V and AD-GAC (Fig. 3). In these two reactors, removal ranged between 35 ± 7 % for total PFOS to 61 ± 8 % for PFOA, while no difference was observed by the application of voltage in AD-GAC-V. As shown in Fig. 3, the removal decreased with increasing chain length of the perfluorocarboxylic acids; PFDA and PFUdA, which contain 10 and 11C atoms, respectively, were not removed significantly or even found at higher concentrations in the effluents (perhaps due to formation from precursors). Similar removal efficiencies were observed in bioreactors with GAC for total and linear PFOS, while the comparison of PFOS and PFOA showed that the existence of carboxylic acid enhanced PFAS removal. Considering the distribution of the target compounds shown in Fig. 2, it seems that PFAS which are found to a higher extent to the dissolved phase, where they are likely more bioavailable, have a higher potential for removal during AD with GAC.

Biotransformation of PFAS in the bioreactors with GAC is the most likely mechanism for the removal of PFAS in reactors with GAC, considering that analysis was conducted both in the dissolved and particulate phases and given that PFAS are not hydrolyzed or volatized at 55 °C (ECHA, 2023). As outlined in the Introduction there are some recent studies that reported the microbial reductive defluorination of two branched unsaturated per- and polyfluorinated carboxylic acids in anaerobic experiments with dehalorespiring cultures (Yu et al., 2022), the identification of PFOA biotransformation products in experiments with anaerobic granular sludge (Cao et al., 2022) and the 40 % decrease of PFOS with parallel production of fluorine in experiments with biomass originating from anaerobic lagoon (Choi et al., 2024). Studies using full-scale sludge anaerobic digesters have shown contradictory results related to PFAS removal, possibly due to variables associated with either the precursor compounds and/or the composition of the microbial community (Lakshminarasimman et al., 2021). On the other hand, it is well-known that the addition of GAC during AD offers increased surface area to microorganisms and promotes DIET (Dang et al., 2022) while no information is available regarding the effect of DIET on the removal of organic micropollutants. In a recent study, Dai et al. (2022) evaluated the treatment of pharmaceutical industries wastewater in different AD reactors and reported that comparing to the control reactor, the concentrations of some pharmaceutical intermediates such as long-chain alkanes, ester, dehydroepiandrosterone, 3,5-di-*tert*-butylphenon, and 2,2′-methylenebis(6-*tert*-butyl-4-methylphenol) were decreased by 18 %, 29 %, 10 %, 27 %, and 42 %, respectively in the reactor containing 20 g/L GAC. The mechanism for the removal of these substances was not discussed. Further experiments are required to understand the mechanisms of PFAS removal in reactors with GAC, the role of adsorption and whether there are specific pathways induced by GAC or any functional groups in GAC that contribute to PFAS removal.

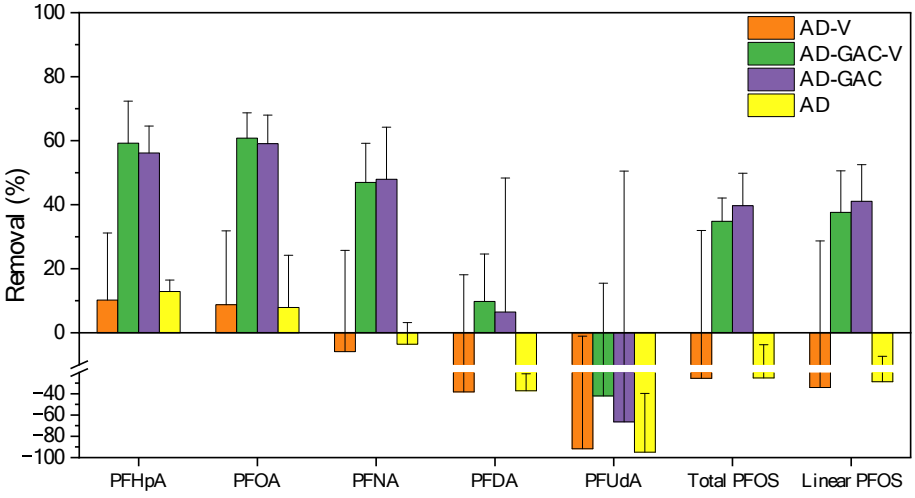

Fig. 3. PFAS removal efficiency observed in different thermophilic AD systems (mean + sd). A negative value represents formation (e.g. from precursors) and a positive value a decrease in the mass of target PFAS at the effluent compared to the influent (see equation (1). **AD-V**: anaerobic digestion with application of external voltage (0.8 V); **AD-GAC-V**: anaerobic digestion with addition of GAC and application of external voltage (0.8 V); **AD-GAC**: anaerobic digestion with addition of GAC; **AD**: typical anaerobic digestion.

3.3. Microbial community analyses

Coprothermobacterota was the most dominant phylum in the AD, AD-GAC, and AD-V bioreactors, and was the second most abundant in the AD-GAC-V system at the end of the experiment (Fig. 4a). This phylum thrives under anaerobic thermophilic conditions. Remarkably, although the initial seed inoculum was cultivated under mesophilic conditions, Coprothermobacterota was dominant across all four bioreactors. In line with these observations, Zhang et al. (2024) inoculated a mesophilic sludge into a thermophilic AD-microbial electrolysis cell system and noted that the presence of Coprothermobacterota gradually increased over time under thermophilic conditions. Proteobacteria dominated in the AD-GAC-V system, and along with Firmicutes, were found in high relative abundance across all systems (Fig. 4a).

Fig. 4b illustrates that at the genus level, *Coprothermobacter* was the most dominant across all systems. *Coprothermobacter* are strictly anaerobic, thermophilic, proteolytic, and hydrogen-producing organisms known for their capability to generate hydrogen through acetate oxidation and for forming growth-promoting syntrophic interactions with methanogens in anaerobic digesters (Dessì et al., 2019). Furthermore, *Coprothermobacter*'s activity is enhanced when a syntrophic relationship is established with hydrogenotrophic methanogens, such as *Methanothermobacter thermautotrophicus* (Gagliano et al., 2015).

Interestingly, in the bioreactor where GAC was added, the presence of *Acinetobacter* was significantly higher (6.7 % for AD-GAC and 12.6 % for AD-GAC-V) compared to the other two, which were below 0.6 %. Details on the dominant *Acinetobacter* species identified within the bioreactors, their relative abundance, and their OTU numbers are available in Table S4. A similar trend was found for *Pseudomonas*, accounting for 4.7 % and 6.4 % for AD-GAC and AD-GAC-V, respectively, and less than 0.8 % for the other bioreactors. Recent studies indicate that *Acinetobacter* play a role in PFAS biotransformation. These bacteria could contribute to PFAS biotransformation and this may explain the differences observed in the systems where GAC was present. Tang et al. (2022) examined the impact of co-contamination with PFAS and chlorinated aliphatic hydrocarbons (CAH) in groundwater at a fluorochemical plant and found *Acinetobacter* to be a dominant genus in this challenging environment. This bacterium, along with *Pseudomonas* and *Arthrobacter*, demonstrated robust adaptation to high concentrations of mixed contaminants, suggesting their potential role in biodegradation processes. The presence of PFAS and CAH led to a significant decrease in microbial diversity but notably increased the abundance of metabolisms related to these pollutants' biodegradation. These findings highlight *Acinetobacter's* potential importance when developing bioremediation strategies for PFAS and CAHs contaminated environments. In another study, Huang et al. (2024) explored the impact of PFAS on SBR systems and found that *Acinetobacter* adapted to contaminants like PFOA and PFBA, emphasizing its significance in PFAS bioremediation. Chen et al. (2022) found that even trace amounts of PFOA affected microbial communities, with *Acinetobacter* gaining a significant advantage under PFOA stress. Yin et al. (2023) observed that the presence of phthalate esters and PFAS promoted the growth of *Acinetobacter calcoaceticus*.

In addition to *Acinetobacter* playing a role in PFAS removal, in the AD-GAC bioreactor, *Bacillus* accounted for 6.7 % compared to 1.4 % in the AD-GAC-V system and less than 0.5 % in the other two systems. This bacterium could also influence the removal of PFOS. Recently, Dai et al. (2022) found that *Bacillus subtilis* exhibited strong adsorption of PFOS, highlighting its significant role in the retention and transport of PFOS in porous media. It should be mentioned that while the increase in *Acinetobacter*, *Pseudomonas* and *Bacillus* populations is notable in the reactors with GAC, further studies and additional types of analyses should be conducted to clarify their potential role in PFAS biotransformation.

The genera *Acetomicrobium* and *Fervidobacterium* were present in all bioreactors at between approximately 2 to 6 % (Fig. 4b). These genera are reported to utilize fats and proteins and are capable of degrading glucose into acetate, CO_2, and H_2 (Ottoni et al., 2022). In the AD reactors, *Methanosarcina* represented a small fraction of the total microbial community but was one of the predominant genus among the archaea. Notably, in the AD-GAC system, its abundance increased to 2.67 %, which was significantly higher than in the other bioreactor configurations. *Methanothermobacter*, a thermophilic hydrogenotrophic methanogen was found in all systems in the range of 0.4–1.14 % (Fig. 4b). These dominant methanogens, *Methanothermobacter* and *Methanosarcina*, are commonly found in thermophilic reactors (Zhang et al., 2020). The prevalence of *Methanothermobacter* is often linked to propionate accumulation and hydrogen inhibition (Zhang et al., 2020). *Methanosarcina* is known for its versatile substrate utilization and faster growth rates relative to other methanogens (Charalambous et al., 2023).

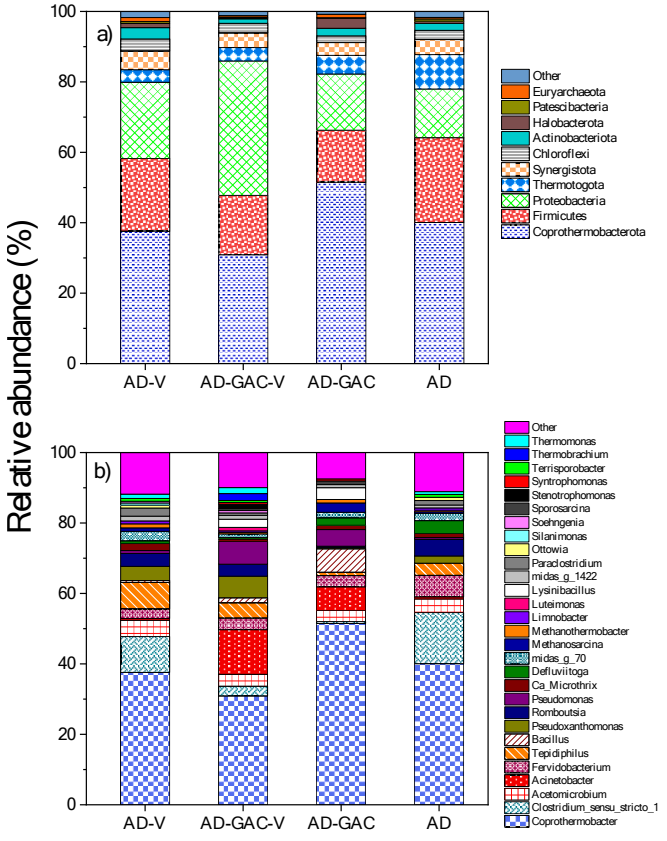

Fig. 4. Relative abundance at the phylum level (a) and at the genus level (b) in the four anaerobic bioreactors used in the current study. AD-V: anaerobic digestion with application of external voltage (0.8 V); AD-GAC-V: anaerobic digestion with addition of GAC and application of external voltage (0.8 V); AD-GAC: anaerobic digestion with addition of GAC; AD: typical anaerobic digestion.

4. Conclusions

The addition of GAC in the AD reactor improved the performance of the system in terms of biogas yield and resulted in a moderate removal of four out of the six studied PFAS. The significant increase in *Acinetobacter* and *Pseudomonas* in the AD-GAC and AD-GAC-V setups indicates their potential role in the biotransformation of PFAS. The sorption of PFAS to sewage sludge increased with increasing number of carbon atoms and the presence of sulfonic acid in their molecules. Overall, this study provides useful scientific data for a potential strategy to remove PFAS from anaerobic digesters treating sewage sludge.

CRediT authorship contribution statement

Michalis Deligiannis: Writing – review & editing, Visualization,

Conceptualization. **Evdokia Gkalipidou:** Investigation. **Georgia Gatidou:** Validation, Methodology. **Marios G. Kostakis:** Validation, Data curation. **Dimitrios Triantafyllos Gerokonstantis:** Software, Investigation. **Olga S. Arvaniti:** Writing – review & editing, Methodology. **Nikolaos S. Thomaidis:** Resources. **Ioannis Vyrides:** Writing – original draft, Methodology. **Sarah E. Hale:** Writing – review & editing, Funding acquisition. **Hans Peter Arp:** Writing – review & editing. **Michail S. Fountoulakis:** Writing – review & editing, Visualization, Conceptualization. **Athanasios S. Stasinakis:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2024.131013.

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