Quantification of PFAS Fate in Sludge Handling Systems

by

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Author's Declaration

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Statement of Contributions

Chapter 2 of this thesis is a paper entitled “The analysis of per- and polyfluoroalkyl substances in wastewater sludges and biosolids: which adsorbents should be used for the cleanup of extracts?”, which was published in Environmental Science Water Research & Technology (2023, 9 (3), 794-805). For this paper, I developed the methodology and experimental design, carried out the experiments, analyzed the experimental data, and wrote the paper under the guidance of Dr. Parker and Dr. Pham.

For Chapter 3, I developed the methodology and experimental design, carried out the experiments, analyzed the experimental data, and wrote the chapter under the guidance of Dr. Parker and Dr. Pham.

Abstract

Per- and polyfluoroalkyl substances (PFAS) have been detected in biosolids, raising concerns regarding their potential leaching into soils, groundwater, and plants when biosolids are land applied. In this study, the fate of PFAS in various sludge handling processes was investigated to determine their fate in individual treatment processes and overall treatment sequences. The results are intended to inform decision-making for the development of effective approaches to biosolids management.

An enhanced analytical method was developed to reliably measure the concentrations of 24 PFAS in the solid streams (i.e., sludges and cakes) of sludge handling processes as the current analytical techniques in the literature were not broadly applicable over a diverse range of samples. The enhancement of existing analytical methods focused on the steps employed to clean the methanolic biosolids extracts. In this study, blends of adsorbents (graphitized non-porous carbon (Envi-Carb), primary secondary amine-functionalized silica (PSA), bare silica and, octadecyl-functionalized silica (C18)) with different adsorptive properties were evaluated for use in the clean-up step. Six different biosolid samples were employed to initially screen and then validate the preferred formulation of the adsorbent blend. A blend consisting of 1,000 mg Envi-Carb, 500 mg PSA and 500 mg C18 was found to effectively eliminate background interferences in extracts of the six biosolids. The cleanup method developed in this study has the potential to facilitate the analysis of PFAS in a broad range of sludge and biosolid matrices.

For the analysis of 24 PFAS in recycled liquid streams (i.e., filtrate and centrate) from sludge handling processes, US EPA Method 1633 was modified, and then used. Modifications of US EPA Method 1633 were necessary because this method was intended for the quantification of PFAS in wastewater samples with a total suspended solids (TSS) value lower than 250 mg/L, whereas the TSS concentration in the liquid samples collected in this study ranged between 100 and 7,000 mg/L. A sample preparation process consisting of ultracentrifugation and extraction steps was developed to first separate solids and extract the PFAS adsorbed on them. The modified method in this study could potentially handle liquid samples with TSS up to 7,000 mg/L.

Following the successful development of the analytical methods, two sampling campaigns were conducted to characterize PFAS fate in the sludge handling systems of two full-scale wastewater

treatment plants (WWTPs), namely WWTP-A and WWTP-B. Both systems included gravity drum thickening (GDT), anaerobic digestion (AD), and dewatering (DW). WWTP-B also had dissolved air flotation (DAF), fermentation (FM) and sludge blending (SBT). In both systems, a majority of individual PFAS mass flows did not significantly change through the liquid-solid separation (i.e., GDT, DW and DAF) and the blending processes. It was concluded that these types of physical processes with short sludge retention time (SRT) did not cause major formation or removal of PFAS. In contrast, in both plants, AD resulted in mass flow increases for a majority (13/22) of the detected individual perfluoroalkyl acids (PFAAs), while the mass flows of the detected precursors decreased. More specifically, the mass flows of the precursors decrease ranged between 29-47% and 20-32% in WWTP-A and in WWTP-B, respectively, while the increase in the mass flows of PFAAs ranged between 50% and 70% in WWTP-A, and 30% and 50% in WWTP-B. The degradation of PFAS precursors and formation of PFAAs were attributable to biodegradation. While the FM process at WWTP-B is also a biological process, there was no significant change in a majority (16/24) of mass flows of target PFAS. This lack of precursors degradation and PFAAs formation observed in FM was attributed to the short SRT (4 days) of this process (compared to the longer SRTs of 45 and 20 days of the AD processes in WWTP-A and WWTP-B, respectively). Overall, it was concluded that SRT may be a key factor affecting PFAS fate in sludge handling systems.

With respect to PFAS partition between solid and liquid streams, it was found that PFAS with carbon chain lengths of C3 to C6 were mostly present (between 50% and 86%) in liquid streams, whereas PFAS with chain lengths higher than C6 were mostly present (between 50% and 121%) in solid streams. Furthermore, perfluoro sulfonates (PFSAs) were found to be 1.4 to 4 times more prone to binding to solids as compared to the perfluorocarboxylates (PFCAs) counterparts with the same carbon chain. These results showed that carbon chain length and functional groups are the key factors determining PFAS fate in sludge handling systems. Additionally, of the 24 PFAS measured, perfluorooctane sulfonate (PFOS), which is the compound that has drawn the most attention due to its toxicity and bioaccumulative nature, was found to be the most abundant PFAS. PFOS concentrations were as high as 12.9 µg/kg and 53 ng/L in solid and liquid samples, respectively.

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List of Abbreviations

AD	: anaerobic digestion
Cake	: biosolid/cake
Cent	: centrate
C18	: octadecyl-functionalized silica
DAF	: dissolved air flotation
DS	: digested sludge
DW	: dewatering
Envi-Carb	: graphitized non-porous carbon
EtFOSAA	: N-ethyl perfluorooctane sulfonamido acetate
EW	: elutriation water
Filt	: filtrate
FM	: fermenter
FOSA	: perfluorooctane sulfonamide
FS	: fermented sludge
GDT	: gravity drum thickener
HDPE	: high density polyethylene
HLB	: hydrophilic-lipophilic balance
LHA	: lifetime health advisory
LOQ	: limit of quantification
MAC	: maximum acceptable concentration
MeFOSAA	: N-methyl perfluorooctane sulfonamido acetate
MeOH	: methanol
MS	: mixed sludge
Overf	: overflow
PAP	: perfluoroalkyl phosphate esters
PFAAs	: perfluoroalkyl acids
PFAC-19ES	: isotopically labeled PFAS mixture standard
PFAS	: per- and polyfluoroalkyl substances;
PFBA	: perfluorobutanoate

PFBS : perfluorobutane sulfonate
PFCAs : perfluorocarboxylates
PFDA : perfluorodecanoate
PFDS : perfluorodecane sulfonate
PFDoA : perfluorododecanoate
PFHpA : perfluoroheptanoate
PFHpS : perfluoroheptane sulfonate
PFHxA : perfluorohexanoate
PFHxS : perfluorohexane sulfonate
PFNA : perfluorononanoate
PFNS : perfluorononane sulfonate
PFOA : perfluorooctanoate
PFOS : perfluorooctane sulfonate
PFOSA : perfluorooctane sulfonamide
PFPeA : perfluoropentanoate
PFPeS : perfluoropentane sulfonate
PFSAs : perfluoro sulfonates
PFTeDA : perfluorotetradecanoate
PFTrDA : perfluorotridecanoate
PFUdA : perfluoroundecanoate
PP : polypropylene
PS : primary sludge
PSA : primary secondary amine-functionalized silica
SBT : sludge blending tank
SPE : solid phase extraction
SRT : solids retention time
Sub : subnatant
Super : supernatant
TPS : thickened primary sludge
TS : total solids
TSS : total suspended solids

TWAS : thickened waste activated sludge

US EPA : United States Environmental Protection Agency

VS : volatile solids

VSS : volatile suspended solids

WAS : waste activated sludge

WAX : weak anion exchange

WWTPs : wastewater treatment plants

4:2 FTS : 4:2 fluorotelomer sulfonate

6:2 FTS : 6:2 fluorotelomer sulfonate

8:2 FTS : 8:2 fluorotelomer sulfonate

Chapter 1

Introduction

The objective of this study is to quantify the fate (formation/removal, partitioning betweenaqueous and solid phases, and transformation) of per- and polyfluoroalkyl substances (PFAS) in sludge handling systems. PFAS are a group of fluorinated chemicals, including perfluoroalkyl acids (PFAAs) and precursors. [1-4] PFAAs can be categorized as perfluorocarboxylates (PFCAs) and perfluoro sulfonates (PFSAs), which have different functional heads attached to a fully fluorinated alkyl chain. More specifically, PFCAs have a carboxylate group, whereas PFSAs have a sulfonate one. With respect to the fully fluorinated alkyl chains, PFSAs and PFCAs can have a range of chain lengths. Perfluorobutanoate (PFBA), perfluorohexanoate (PFHxA), perfluorobutane sulfonate (PFBS) and perfluorohexane sulfonate (PFHxS) are some examples of PFAS with varying chain length. Unlike perfluoro compounds, polyfluoroalkyl compounds, also known as precursors, are compounds that have carbon atoms that are not fully fluorinated. Because of these carbon atoms, PFAS precursors could be transformed into PFCAs and PFSAs via biological and/or chemical processes.[1-4] Figure 1-1 presents the chemical structure of perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), and N-methyl perfluorooctane sulfonamido acetate (MeFOSAA). These are among the most commonly known PFCAs, PFSAs and precursors, respectively.

From the examples of chemical structures given in Fig. 1, it can be seen that PFAS possess one of the strongest chemical bonds (C-F), a hydrophobic tail and a hydrophilic head.[1-4] These properties and PFAS resistance to chemical and biological degradation (except for some precursors) have led to the use of PFAS in firefighting foams and fireproofing materials, non-stick coatings for cookware, waterproofing agents, surfactants, cosmetics, inks, insulation for electrical wiring, and pesticides.[5,6] PFAS manufacturing points and usage have led to their detection in surface water, groundwater, sediments, soils, and biota, thereby creating various risks due to their toxic nature for living organisms.[5,7,8] As a result, PFAS are commonly classified as emerging contaminants.[9-11] Thus, the investigation of the accumulation points has been of great interest to establish possible precautions to reduce PFAS concentrations in nature.

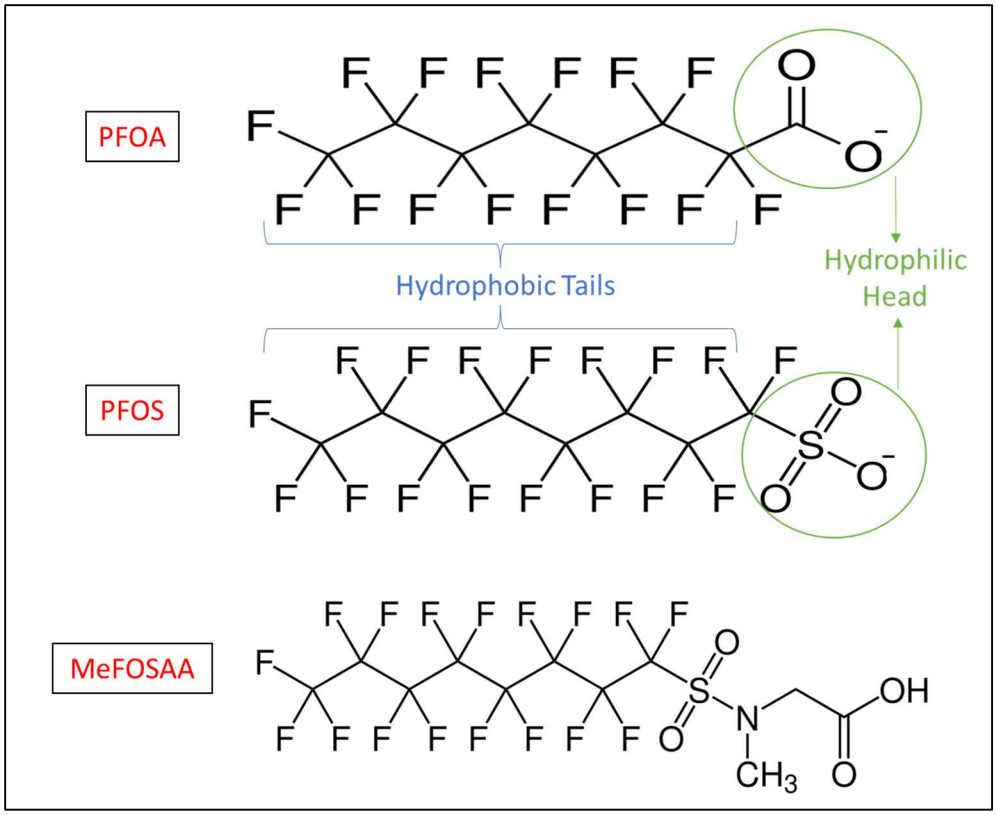


Figure 1-1: Chemical structure of PFOA and PFOS

A number of studies have shown that a major accumulation point of PFAS is wastewater treatment plants (WWTPs). Due to the persistency of PFAS along conventional processes of WWTPs, the discharge of treated wastewater to water bodies and the disposal of biosolids are recognized as common points of PFAS entry into the environment,[6,12-14] and elevated PFAS concentrations in biosolids have been reported in several studies.[15-21] For example, Venkesaten and Halden (2013) demonstrated that biosolid samples collected from 32 US states contained 403 ± 127 µg/kg PFOS, 34 ± 23 µg/kg PFOA and 34 ± 23 µg/kg perfluorodecanoate (PFDA).[19] In the same study, the mean concentrations of other PFAS compounds, such as PFHxA and perfluorooctane sulfonamide (PFOSA), ranged between 2 and 21 µg/kg.[19] In another study, Guerra et. al. (2014) in a survey of 20 wastewater treatment plants in Canada found concentrations of PFOS in treated biosolids ranging from 2.1 to 17,000 µg/kg.[21] Other PFAAs were also found to be present in the study by Guerra et al. Therefore, there has been a growing interest in studying the fate of PFAS through sludge handling systems of WWTPs in which the biosolids are produced.

Biosolids are produced from the treatment of sludges that are generated from primary (primary/raw sludge) and secondary (waste-activated sludge) treatment processes in WWTPs (Fig. 2).[22] Typically, the sludges are treated to reduce sludge weight and volume, and eliminate pathogens and odours. A typical sludge treatment sequence consists of the thickening of primary and waste activated sludge, stabilization of these thickened sludges and subsequent dewatering.[23] While thickening and dewatering processes are mostly employed to reduce the volume of free water, stabilization processes are employed to reduce sludge's odour and putrescence, as well as the level of pathogenic organisms. Stabilization is commonly achieved through biological processes. Aerobic and anaerobic digestion are the two commonly used biological sludge stabilization processes where bacteria break down organic matter to produce stabilized sludge.

To date, only a limited number of studies have investigated the fate of PFAS (e.g., the transformation of polyfluorinated compounds and formation of perfluoro carboxylates and sulfonates, partitioning of PFAS between the aqueous phase and the solid phase) in full scale sludge handling systems.[24,25] Lakshminarasimman et al. (2021) investigated the behavior of PFAS in individual sludge handling processes.[24] Their findings showed that various aerobic and anaerobic digestion systems exhibited both PFAS formation and removal, with some individual PFAS mass flows increasing (278%) and decreasing (71%). In another study, Li et al. (2022) studied aerobic digestion of anaerobically digested sludge and observed a substantial increase in the concentrations of perfluoropentanoate (PFPeA), PFHxA, PFBS and PFOS by 571%, 253%, 340%, and 91%, respectively, while some precursors (6:2 fluorotelomer sulfonate (6:2 FTS), N-ethyl perfluorooctane sulfonamido acetate (EtFOSAA) and MeFOSAA) showed no significant reduction.[25] In both of these studies, it was concluded that the increase in PFAAs concentrations was likely due to the biodegradation of PFAAs precursors. However, these studies did not comprehensively evaluate PFAS fate through an entire train of processes. Therefore, there is a critical need for systematic sampling campaigns to provide data to support the characterization of PFAS in these systems and thereby inform decision-making for future process modifications aimed at mitigating PFAS accumulations in biosolids.

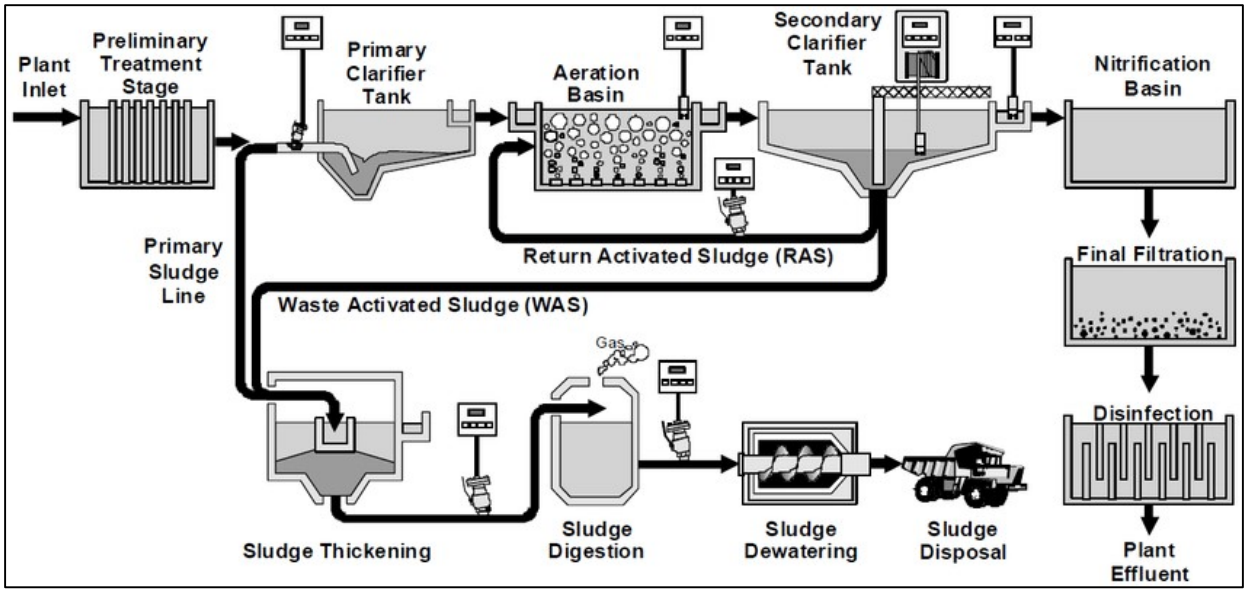


Figure 1-2: Flowchart of a typical WWTP and sludge handling system[22]

To investigate the fate of PFAS in sludge handling systems, PFAS concentrations in the inlet and outlet streams of sludge handling processes should be accurately determined. However, standard methods for the quantification of PFAS in these matrices do not exist. The presence of high levels of salts and organic matter in sludges and biosolids makes PFAS extraction and clean-up of extracts challenging due to the co-extraction of these substances. Furthermore, analytical methods for the liquid streams of sludge handling systems (i.e., filtrate and supernatant) are challenged by the presence of suspended solids that could clog the cartridges used in solid phase extraction (SPE).[26,27] Therefore, there is a need for robust and reliable methods that could be employed for a diverse range of sludges and biosolids and which can address the high levels of solids present in recycle streams.

Given the knowledge gaps mentioned above, the main objective of this research is to study the fate of PFAS in sludge handling processes, focusing specifically on:

- The development of analytical methods for the quantification of PFAS in sludges, biosolids and associated liquid recycle streams
- The investigation of the fate (formation/removal, distribution and transformation) of a variety of PFAS throughout common sludge handling systems

Chapter 2

The Analysis of Per- and Polyfluoroalkyl Substances in Wastewater Sludges and Biosolids: Which Adsorbents Should Be Used for The Cleanup of Extracts?

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Abstract

The analysis of PFAS in wastewater treatment sludges and biosolids is an evolving practice for which a widely recognized standard method does not currently exist. While a number of analytical techniques have been reported in the literature, individual methods were found to not be broadly applicable over a range of sludge/biosolid matrices. This study critically examined the method employed to clean up biosolids extracts prior to LC-MS/MS analysis, which was determined to be crucial to obtaining acceptable chromatogram quality and recoveries of a range of PFAS and associated surrogates. When graphitized non-porous carbon (Envi-Carb) was employed alone or in combination with WAX, as described in US EPA Draft Method 1633, precipitates were formed when methanolic extracts were reconstituted with water, preventing direct analysis by LC-MS/MS. Centrifugation and filtration of reconstituted extracts to remove precipitates did not sufficiently eliminate interferences to allow PFAS quantification. The use of blends of adsorbents (Envi-Carb, primary secondary amine-functionalized silica (PSA), bare silica, octadecyl-functionalized silica (C18)) with different adsorptive properties that could remove a broader range of interfering substances was evaluated. A blend consisting of 1,000 mg Envi-Carb, 500 mg PSA and 500 mg C18 was found to effectively eliminate background interference while providing an optimal trade-off between chromatogram quality and PFAS recovery. When integrated into the overall analytical method, the recoveries of PFAS were between 40 and 160% except for some precursors when six different biosolids were analyzed. The cleanup method developed in this study has the potential to facilitate the analysis of PFAS in a broad range of sludge and biosolid matrices.

2.1. Introduction

Owing to their widespread use in the last several decades, per- and polyfluoroalkyl substances (PFAS) have been detected in diverse environmental compartments, including in surface water, groundwater, sediments, soils, as well as in wildlife and human.[28-31] The two most common PFAS, perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), are common groundwater and surface water contaminants and are posing widespread risks to drinking water resources worldwide.[32-33] Given their bioaccumulative and toxic nature,[9-11] regulatory criteria for PFOS, PFOA and other PFAS are expected to be established in the near future. For example, Health Canada has proposed a Maximum Acceptable Concentration (MAC) of 600 ng/L for PFOS and 200 ng/L for PFOA in drinking water.[34,35] Health Canada has also proposed drinking water screening values between 200 and 30,000 ng/L for other PFAS, including perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluorobutanoate (PFBA), perfluoropentanoate (PFPeA), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), and perfluorononanoate (PFNA).[36] Very recently, the United States Environmental Protection Agency (US EPA) issued final drinking water lifetime health advisory (LHA) levels of 10 ng/L for hexafluoropropylene oxide-dimer acid (also known as GenX) and 2,000 ng/L for PFBS.[37] The US EPA also issued interim updated drinking water LHA levels of 0.02 ng/L for PFOS and 0.004 ng/L for PFOA.[13] These levels are a few orders of magnitude lower than that issued in 2016 (i.e., 70 ng/L for PFOS and PFOA, individual or combined).[38] As the regulatory requirements on these compounds in the environment become increasingly stringent, the need to quantify and control PFAS discharges will grow in importance.

The research conducted over the past decade has shown that municipal biosolids can contain significant amounts of PFAS.[15-21] For example, analyses of the biosolid samples from 32 US states revealed that PFOS and PFOA were the most abundant PFAS (403 ± 127 and 34 ± 23 μg/kg of dry weight (dw), respectively), and that other compounds such as PFHxA, perfluorodecanoate (PFDA), and perfluorooctanesulfonamide (PFOSA) were also present (at a tens of μg/kg level).[19] Similarly, a survey of 19 wastewater treatment plans in Australia found that the total concentration of PFAS in biosolids ranged between 4.2 to 910 μg/kg dw,[20] while a survey of 20 wastewater treatment plants in Canada found that the concentration of PFOS in biosolids ranged between 2.1 to 17,000 μg/kg dw.[21] Other perfluoroalkyl acids (PFAAs) were also present in the Canadian

biosolids.[21] While PFAAs are the PFAS that have been measured the most frequently, two recent studies reported that PFAA precursors, such as di-substituted polyfluorinated phosphate esters, were the dominant PFAS in the studied biosolids.[20,39] Some of these precursors could be transformed into PFAAs. Since it is a common practice around the world to use biosolids as fertilizer, there have been significant concerns that biosolid-amended agricultural fields could be potential PFAS point sources, which could contaminate soil, crops, grazing animals, surface water, and groundwater. Although currently biosolids are not widely regulated with respect to PFAS,[40] this situation may change in the near future, especially in light of the recently reported cases of PFAS contamination following the application of contaminated biosolids to soils.[41-44] In fact, Maine recently became the first state in the US that bans the land application of PFAS-contaminated biosolids.[45] Overall, there is a growing interest in quantifying PFAS in biosolids and assessing associated risks in order to gain information that will help support decision-making with respect to future biosolids management practices.

As with our quickly growing knowledge about PFAS fate and transport, analytical methods for the quantification of PFAS in environmental samples are also rapidly evolving. With respect to biosolids, a standard method for the analysis of PFAS in this matrix does not currently exist. Thus, researchers have been devising their own analytical methods or adopting the methods developed by others. While there are some variations among the methods published in the literature, most methods consist of three main steps, which begin with extracting biosolids with an organic solvent, followed by cleaning up the extract with an adsorbent to remove interfering organic and inorganic compounds that coeluted with PFAS in the extraction step, and finally analyzing PFAS in the cleaned-up extract by liquid chromatography coupled with mass spectrometry. Of these three steps, extract cleanup appears to be the most variable step, with a wide variety of adsorbents having been employed alone or in combination, including graphitized non-porous carbon (which is more commonly known under the trademark name ENVI-Carb), bare silica, octadecyl-functionalized silica (C18), primary secondary amine-functionalized silica (PSA), weak anion exchange (WAX) polymeric resin, and hydrophilic-lipophilic balance (HLB) polymeric resin.[20,46-61] In what appears to have been the first study investigating the occurrence of perfluoro carboxylates and sulfonates in digested sludges, Higgins et al. developed a method that involved extracting sludge samples with an acidic methanol solvent, and cleaning up the extract by a C18 solid-phase extraction (SPE) cartridge.[51] The method developed by Higgins et al. was adopted by several other researchers,[42,58-

[61] including Moodie et al who used C18 together with PSA in the extract cleanup step.[20] In contrast, while Arvaniti et al., Boiteaux et al., and Sinclair and Kannan also used an acidic methanol solvent in the extraction step, they used either a WAX or an HLB SPE cartridge instead of C18 to clean up the extract.[52-54] HLB resin was also used by Yu et al, who further polished the extract with a silica cartridge.[55] Shortly after the publication by Higgins et al., Powley et al. reported an investigation of the occurrence of perfluoroalkyl carboxylates in sediments and activated sludges.[56] In this study, samples were extracted with a basic methanol solution, and the extract was cleaned up with ENVI-Carb. This method, which was employed in over a dozen other studies including several recent studies,[16,18,49,50,57] currently seems to be the most popular method for the analysis of PFAS in sludges and biosolids. Recently, the US EPA published Draft Method 1633 and 2nd Draft Method 1633 for the analysis of PFAS in various environmental matrices including biosolids.[26,27] These methods involve extracting biosolids with a basic methanol solution (0.3% methanolic ammonium hydroxide), cleaning up the extract with ENVI-carb, concentrating the extract by evaporation and subsequently diluting it with water, and finally cleaning up the diluted extract with WAX resin. Draft Method 1633 and 2nd Draft Method 1633 are, in principle, similar to the methods employed in several earlier studies.[19,21,24,46,48,62]

In the aforementioned studies, the rationale for adsorbent selection was not described. However, the role that adsorbents play in the cleanup step has been discussed extensively by researchers who analyze other organic contaminants in complex sample matrices such as fruits, vegetables, milk, eggs, and meats. Specifically, C18, which is a widely used adsorbent in reversed-phase chromatography, is highly effective at removing lipids and other lipophilic compounds from extracts.[63-65] Somewhat similar to C18, ENVI-Carb adsorbs non-polar organic compounds, particularly those with a planar structure such as sterols and natural pigments.[49-51] In contrast, PSA possesses greater affinity towards hydrophilic compounds such as organic acids including fatty acids, as well as sugar and anthocyanin pigments.[63-65] WAX and HLB have been widely used for the analysis of aqueous samples in order to preconcentrate PFAS.[4] Whereas WAX selectively retains anionic organic compounds, HLB is a less selective adsorbent since it can retain both ionic (positive and negative) and neutral compounds.

To date, only one study has evaluated different extract cleanup strategies for the analysis of PFAS in sludges and biosolids. Specifically, Semerád et al.[66] compared the performance of ENVI-

Carb, HLB, Captiva EMR lipid (i.e., a type of adsorbent that preferentially retains lipids), and ENVI-Florisil (i.e., magnesium silicate, which adsorbs hydrophilic compounds). They also studied the analysis of PFAS in sludges and biosolids by QuEChERS, which is an extraction method widely used for the analysis of trace contaminants in food. QuEChERS consists of a cleanup kit with various adsorbent formulations which, depending on the sample nature, may consist of anhydrous $MgSO_4$, PSA, C18, and ENVI-Carb. It is noted that the exact adsorbent composition employed by Semerád et al. was not described in their paper. Semerád et al. reported that only ENVI-Carb provided an adequate cleanup of extract.[66] As mentioned above, however, HLB and other types of adsorbents were successfully used by other researchers. These conflicting reports could be due to the varying type and quantities of interfering compounds in the extracts among studies. It can be expected that the characteristics of biosolids (e.g., pH, nutrient, fatty acid, lipid, protein, and organic matter content) will be influenced by the sewershed (i.e. residential versus industrial dischargers), wastewater treatment process type, and sludge handling process type (e.g., anaerobic versus aerobic digestion, composting, alkaline treatment).[67] Thus, a "one-size-fits-all" method for the analysis of PFAS in biosolids may not exist, and additional research is needed to compare and contrast extract cleanup strategies.

The objective of this study was to further evaluate the performance of the existing methods of PFAS analysis in sludges and biosolids, focusing specifically on assessing the cleanup of extracts by four commonly used adsorbents, namely ENVI-Carb, PSA, C18 and WAX. As will be presented in the Results and Discussion section, none of the existing methods was capable of adequately cleaning up the extracts generated from the biosolids investigated in this study. This observation motivated us to devise a cleanup method consisting of a combination of ENVI-Carb, PSA and C18. The selection of this adsorbent combination was inspired by the one employed in the study by Yu et al., which investigated the presence of PFAS in dietary milk.[68] The performance of our method was evaluated based on extract physical appearance, chromatogram baseline noise, and analyte recovery. This method was then used to analyze PFAS in six biosolid samples, including three dewatered anaerobically digested sludges, two alkaline-stabilized digested sludges, and a composted dewatered biosolid. The results of this investigation suggest that the method developed in this study may be suited for the analysis of a wide variety of sludge and biosolid samples.

2.2. Materials and Method

2.2.1. Chemicals and materials

Native and isotopically labeled PFAS standards were purchased from Wellington Laboratories (Guelph, Canada). The native PFAS mixture standard (PFAC-24PAR) consists of 11 perfluoroalkyl acids (C_4 – C_{14}), 7 perfluoroalkyl sulfonates (C_4 – C_{10}), 3 fluorotelomer sulfonates (4:2 FTS, 6:2 FTS, 8:2 FTS), 1 sulfonamide (FOSA – perfluorooctane sulfonamide), and 2 sulfonamido acetic acid (MeFOSAA – 2-N-methylperfluorooctanesulfonamido acetic acid, EtFOSAA – N-ethylperfluorooctanesulfonamido acetic acid). These 24 compounds are the analytes of interest in this study. See Table 2-1 for the full list of native PFAS. The isotopically labeled PFAS mixture standard (PFAC-19ES) consists of 19 compounds. The list of these compounds is also presented in Table 1. Graphitized carbon (ENVI-Carb), C18 reversed-phase silica gel, and primary secondary amine (PSA) silica gel were purchased from Sigma Aldrich. All other chemicals, purchased from Fisher Scientific, were either LCMS grade or ACS grade.

2.2.2. Biosolid samples

This study involved six biosolid samples, which are denoted from this point forward as Biosolid 1, 2, 3, 4, 5 and 6. Biosolids 1 – 3 were dewatered anaerobically digested sludges collected from three different wastewater treatment facilities. Biosolids 4 and 5 were generated from the alkaline treatment (at pH 9 and 70 ^{o}C for 20 minutes) of Biosolids 1 and 2, respectively. Biosolid 6 was collected from an aerobic composting facility that processes dewatered municipal biosolids. The samples were collected into high-density polyethylene bottles and shipped on ice. The samples were stored at -20 ^{o}C upon arrival. The frozen samples were then freeze-dried, homogenized using a mortar and pestle, and sieved through a 2 mm stainless steel mesh. The homogenized and sieved samples were stored at 2 – 4 ^{o}C until extraction.

2.2.3. Extraction procedure

The extraction procedure consisted of five steps (Fig. 2-1). In *Step 1*, isotopically labeled PFAS surrogates (2 ng each) were spiked into 0.5 g of the sieved biosolid sample. The spiked sample was then extracted three times with 7 mL 99/1 v/v methanol/30% ammonium hydroxide aqueous solution (i.e., 0.3% methanolic ammonium hydroxide). In each extraction round, the sample and the methanolic ammonium hydroxide solution were mixed on a vortex for 1 minute, sonicated at

35 ºC for 30 min, and the solids were separated by centrifugation at 1,613 RCF for 30 min. The supernatants from the three extraction rounds (20 mL in total) were collected in a 50 mL polypropylene test tube. In *Step 2*, various quantities of ENVI-carb (0 – 1,000 mg), C18 (0 – 500 mg), and PSA (0 – 500 mg) were added to the combined extract. Subsequently, this mixture was vortexed for 1 min, and the adsorbents were separated by centrifugation at 3,100 RCF for 30 min. In *Step 3*, the supernatant was then transferred to a new 50 mL polypropylene test tube, evaporated to dryness, and reconstituted with 0.5 mL 99/1 v/v methanol/glacial acetic acid solution. In *Step 4*, 0.4 mL of this reconstituted sample was transferred to a 2 mL polypropylene microcentrifuge tube and was mixed with 0.4 mL water, resulting in a liquid sample of 50/50 v/v water/methanol. In all cases, it was observed that precipitates were formed in this mixing step. The reason for precipitate formation will be discussed in the Results and Discussion section. To separate these precipitates, in *Step 5* the 2 mL microcentrifuge tube was centrifuged at 24,000 RCF for 10 min, and the supernatant was filtered through a 0.2-μm alumina membrane filter (Whatman Anotop). The filtrate was collected into a 1.5 mL polypropylene vial and subjected to LC/MS/MS analysis.

To confirm that all materials and reagents were PFAS-free, 0.5 g of Ottawa sand was extracted via the procedure described above (i.e., method blank). Also, seven Ottawa sand samples (0.5 g/sample) were spiked with native PFAS (2 ng each) and isotopically labeled PFAS (1 ng each), and the spiked samples were extracted and analyzed. The standard deviation (S) of the analyzed PFAS concentrations was calculated based on these 7 samples, and the limit of quantitation (LOQ) for each PFAS was calculated as LOQ = 10 × S.

To compare the cleanup of extracts by the adsorbents tested in this study and those used in Draft Method 1633, 0.5 g of Biosolid 1 was spiked with isotopically labeled PFAS surrogates (2 ng each) and extracted three times with 7 mL 0.3% methanolic ammonium hydroxide.

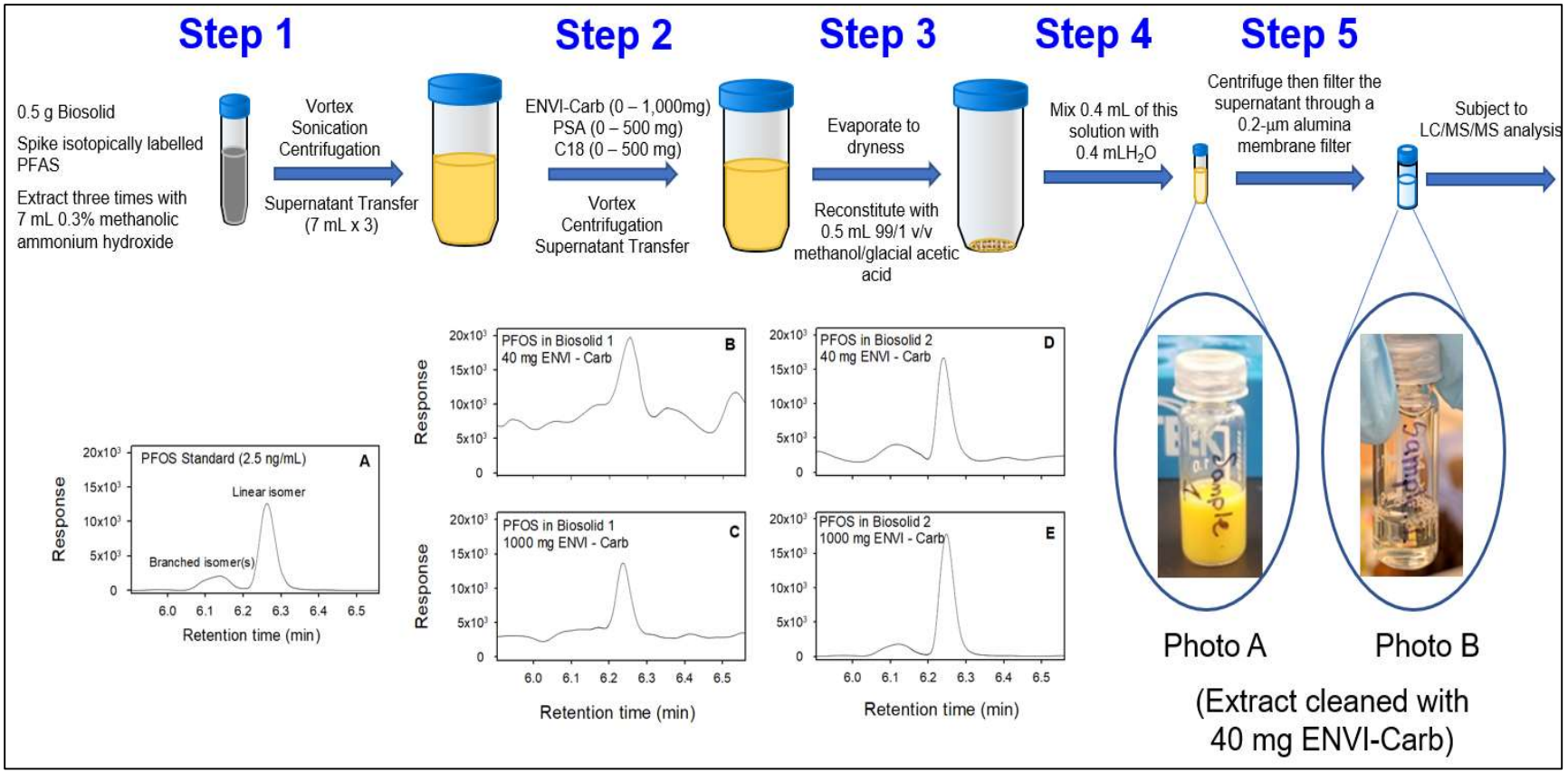


Figure 2-1. The analytical workflow employed in this study for the quantification of PFAS in biosolids. Various adsorbent types and quantities were assessed in Step 2 to clean up the extract generated in Step 1. Photo A presents the physical appearance of the Biosolid 1 extract (50/50 v/v methanol/water) cleaned with ENVI-Carb. The yellow precipitates in this extract could be removed by centrifugation, followed by filtration (Photo B). Panel A presents the chromatogram of a PFOS standard. Panels B & C and D & E present the chromatograms of PFOS in the extracts of Biosolid 1 and Biosolid 2, respectively; these extracts were cleaned with either 40 mg or 1000 mg ENVI-Carb.

Subsequently, the extracts were combined into a 50 mL polypropylene tube that contained 10 mg ENVI-Carb. The mixture was then vortexed, and ENVI-Carb was separated by centrifugation. The supernatant was evaporated down to 1 mL and reconstituted with 49 mL of water, resulting in a solution that contained less than 20% methanol as per the 2nd Draft Method 1633.[27] The resulting solution was subjected to solid-phase extraction with a preconditioned WAX cartridge. The WAX cartridge was then eluted with 5 mL of 1% methanolic ammonium hydroxide. In the final step, the eluent was neutralized with 25 μL glacial acetic acid and mixed with water, resulting in a 50/50 v/v methanol/water sample that was subjected to LC/MS/MS analysis. The workflow described above is presented in Fig. 2-2.

In both methods described above, the combined extract was cleaned by ENVI-Carb prior to evaporation. In several studies, these steps were conducted in reverse — that is the extract was first evaporated to dryness, and the reconstituted methanolic sample was then cleaned with ENVI-Carb.[16,18,49,50,57] To compare these two different approaches, a separate experiment was conducted wherein the extract of Biosolid 1 was evaporated to dryness, reconstituted to 625 μL with 99/1 v/v methanol/glacial acetic acid, and cleaned with 40 mg ENVI-Carb. In the final step, 400 μL of the cleaned extract was mixed with 400 μL of 0.003% ammonium hydroxide in water. This sample was then centrifuged, filtered, and subjected to LC/MS/MS analysis.

The analytes of interest in this study were the 24 PFAS mentioned in the "Chemicals and materials" section. However, only 14 compounds were detected in the five biosolids employed in this study. To investigate how the different cleanup methods described above affected the chromatogram quality of the other 10 compounds, an additional set of experiments was conducted wherein the cleaned-up extracts of Biosolid 1 were spiked with a mixture of 24 native PFAS prior to LC/MS/MS analysis.

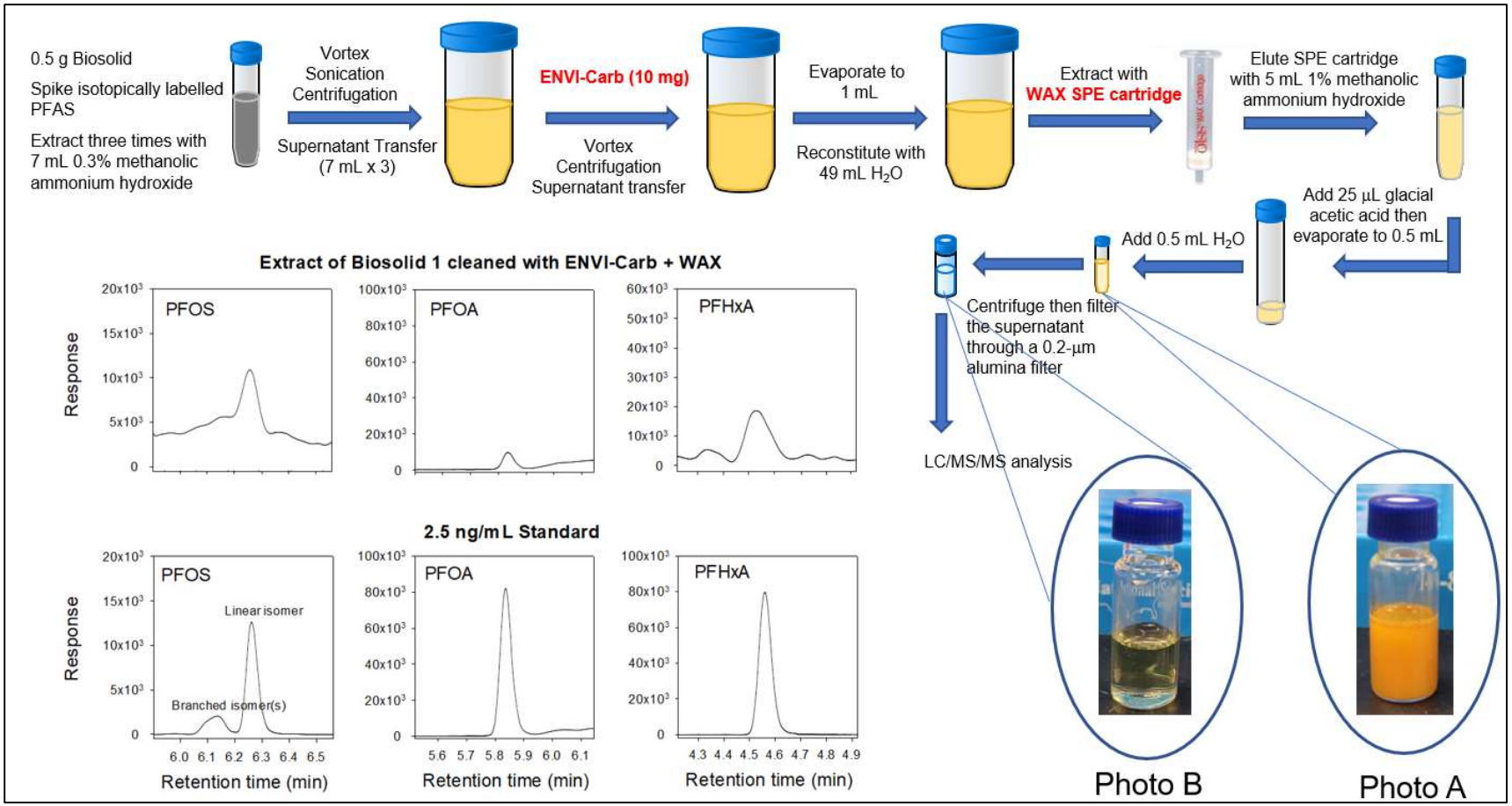


Figure 2-2. The analytical workflow consisting of the ENVI-Carb followed by the WAX cleanup steps, as described in US EPA Method 1633. Photos present the physical appearance of the Biosolid 1 extract after cleanup (Photo A) followed by centrifugation & filtration (Photo B). Panels: Comparison of the chromatograms of various PFAS standards and PFAS in the filtered Biosolid 1 extract.

2.2.4. PFAS analysis and recovery calculations

Samples were analyzed using a Shimadzu 8030 LC/MS/MS. Details about the instrument operating conditions and other relevant information can be found in Table 2-1. The instrument was calibrated using 12 external calibration standards containing 0.05 – 20 mg/L of each native PFAS analyte and 2 mg/L of each isotopically labeled PFAS surrogate. These standards were prepared by spiking various amounts of native and isotopically labeled PFAS into a methanol/water solution to obtain a final sample consisting of 50/50 v/v methanol/water. PFAS concentrations were calculated using the isotopic dilution method.

Table 2-1. LC/MS/MS operating condition and other information.

Instrument	Shimadzu LCMS-8030 Triple Quadrupole Mass Spectrometer		
Ionization	Negative electrospray		
Precolumn	Guard Column Thermo Scientific™ Acclaim 120 Å C18, 4.6 x 10 mm, 5 µm		
Column	Agilent Infinity Lab Poroshell 120 Å EC-C18, 3.0 x 50 mm, 2.7 µm		
Column oven temperature	30˚C		
Injection volume	30 µL		
Mobile phases	A: 10 mM ammonium acetate in LCMS grade water B: 10 mM ammonium acetate in LCMS grade methanol		
Flow rate	0.5 mL/min		
Gradient profile	**Time (min)**	**Eluent A Conc. (%)**	**Eluent B Conc. (%)**
	0	60	40
	0.5	60	40
	4.5	20	80
Gradient profile	10	20	80
	10.5	60	40
	13.5	Stop	

	Analytes	Ion transitions	Internal standards	Ion transitions	Calibration range (µg/L)
Monitored ion transitions	PFBA	213 > 169	[$^{13}C_4$] MPFBA	217 > 172	1 - 20
	PFPeA	263 > 219	[$^{13}C_5$] MPFPeA	268 > 223	1 - 20
	PFHxA	313 > 269	[$^{13}C_5$] MPFHxA	318 > 273	0.05 - 20
	PFHpA	463 > 319	[$^{13}C_4$] MPFHpA	367 > 322	0.05 - 20
	PFOA	413 > 369	[$^{13}C_8$] MPFOA	421 > 376	0.05 - 20
	PFNA	463 > 419	[$^{13}C_9$] MPFNA	472 > 427	0.05 - 20
	PFDA	513 > 469	[$^{13}C_6$] MPFDA	519 > 474	0.05 - 20
	PFUdA	563 > 519	[$^{13}C_7$] MPFUdA	570 > 525	0.05 - 20
	PFDoA	613 > 569	[$^{13}C_2$] MPFDoA	615 > 570	0.05 - 20
	PFTrDA	663 > 619	[$^{13}C_2$] MPFTeDA	715 > 670	0.05 - 20
	PFTeDA	713 > 669	[$^{13}C_2$] MPFTeDA	715 > 670	0.05 - 20
	PFBS	299 > 80	[$^{13}C_3$] MPFBS	302 > 99	0.05 - 20
	PFPeS	349 > 80	[$^{13}C_3$] MPFBS	302 > 99	0.1 - 20
	PFHxS	399 > 80	[$^{13}C_3$] MPHxS	402 > 99	0.05 - 20
	PFHpS	449 > 80	[$^{13}C_3$] MPHxS	402 > 99	0.05 - 20
	PFOS	499 > 80	[$^{13}C_8$] MPFOS	507 > 99	0.05 - 20
	PFNS	549 > 80	[$^{13}C_8$] MPFOS	507 > 99	0.05 - 20
	PFDS	599 > 80	[$^{13}C_8$] MPFOS	507 > 99	0.05 - 20
	4:2FTS	327 > 307	[$^{13}C_2$] M4:2FTS	329 > 81	0.05 - 20
	6:2FTS	427 > 407	[$^{13}C_2$] M6:2FTS	429 > 81	0.05 - 20
	8:2FTS	527 > 507	[$^{13}C_2$] M8:2FTS	529 > 81	0.05 - 20
	FOSA	498 > 78	[$^{13}C_8$] MFOSA	506 > 78	0.05 - 20
	MeFOSAA	570 > 419	[$^{2}d_2$]MMeFOSAA	573 > 419	0.05 - 20
	EtFOSAA	584 > 419	[$^{2}d_2$] MEtFOSAA	589 > 419	0.05 - 20

Recovery values (R) were estimated by comparing the peak area (A_{sample}) of the spiked isotopically labeled PFAS with the peak area ($A_{standard}$) of the respective isotopically labeled PFAS in the calibration standards, i.e., $R = (A_{sample}/A_{standard}) \times 100\%$. A separate set of experiments was conducted to confirm that the recovery values of isotopically labeled PFAS were representative of the recovery values of native PFAS. In this set of experiment, biosolid samples were spiked with both native PFAS (5 ng/compound) and isotopically labeled PFAS (2 ng/compound), and the spiked samples were extracted and analyzed. Biosolid 1 and Biosolid 4 were randomly chosen for this experiment. R for the isotopically labeled PFAS was calculated based on peak areas as described above, whereas R for native PFAS was calculated using the following equation: $R = (m_{spiked} - m_{sample}/m_{spiked}) \times 100\%$, with m_{spiked} and m_{sample} being the PFAS mass spiked into the sample (i.e., 5 ng/compound) and the PFAS mass original present in the sample, respectively.

2.3. Results and Discussion

2.3.1. Method blank and LOQ

The analysis of non-spiked Ottawa sand samples revealed that the reagents and materials used in this study were generally PFAS-free. However, 6:2 FTS was detected in some analyses. Attempts to determine and eliminate the origin of 6:2 FTS cross contamination were unsuccessful. Thus, 6:2 FTS was not included in the analyte reporting list.

The LOQ for each PFAS varied between 0.1 and 1.5 μg/kg (Table 2-2). It is noted that these LOQ values were based upon the extraction of PFAS-spiked Ottawa sand samples and, therefore, should be considered as the "best-case-scenario" LOQ. As will be discussed in detail below, the amount of background organics extracted out of biosolid in *Step 1* of the analytical procedure (Fig. 2-1) varied among the tested biosolids. Since the background organics could affect the chromatograph quality, LOQ would vary among biosolid samples.

Table 2-2. Limit of Quantitation (LOQ) for solid samples. Seven Ottawa sand samples (0.5 g/sample) were spiked with native PFAS (2 ng each) and isotopically labeled PFAS (1 ng each), and the spiked samples were extracted and analyzed. The standard deviation (S) of the analyzed PFAS concentrations was calculated based on these 7 samples, and the limit of quantitation (LOQ) for each PFAS was calculated as LOQ = 10 × S. The values should be considered as the "best-case-scenario" LOQ. Since the background organics eluted in the extraction step could affect chromatograph quality, LOQ would vary among biosolid samples. The LOQ of 6:2 FTS is not reported due to cross contamination in some samples.

	LOQ (µg/kg dry weight)
PFBA	0.5
PFPeA	1.5
PFHxA	0.1
PFHpA	0.1
PFOA	0.1
PFNA	0.1
PFDA	0.1
PFUdA	0.5
PFDoA	0.5
PFTrDA	1.0
PFTeDA	1.0
PFBS	0.5
PFPeS	0.5
PFHxS	0.1
PFHpS	0.1
PFOS	0.1
PFNS	0.5
PFDS	0.5
4:2 FTS	0.5
6:2 FTS	-
8:2 FTS	0.5
FOSA	0.5
MeFOSAA	1.0
EtFOSAA	1.0

2.3.2. Envi-Carb alone

Regardless of whether ENVI-Carb was used prior to or after the extract evaporation step, precipitates were formed in *Step 4*, i.e., when the methanolic extract generated in *Step 3* was reconstituted with water to obtain a sample consisting of 50/50 v/v methanol/water. The formation of precipitates in *Step 4* occurred with all 6 tested biosolids, and was particularly prominent in the case of Biosolid 1 (Photo A in Fig. 2-1). While investigating the identity of the precipitates was beyond the scope of this study, it is hypothesized that the precipitates are organic compounds that were co-extracted from the biosolids. These organic compounds appeared to be more soluble in pure methanol than in 50/50 v/v methanol/water. Thus, once the solvent composition changed, they precipitated out of the solution. Schaefer et al.[22] also observed that biosolid extracts consisting of 50/50 v/v methanol/water emulsified. To address this issue, Schaefer et al added isopropanol to the sample such that the final sample composition was 3/2/2 v/v/v methanol/isopropanol/water.[22] While the use of isopropanol, along with lowering the water fraction in the sample, may prevent precipitation/emulsification, this approach does not remove background organic compounds from the final sample that is then subjected to LC/MS/MS analysis. The presence of background organic compounds in the sample could cause various issues, such as analyte ionization enhancement or suppression, and clogging of the LC column and tubing when the LC eluent composition changes to conditions conducive to the precipitate formation (e.g., when the eluent consists of ≥ 50% water).

While it was found that the precipitates could be effectively separated by centrifugation followed by filtration (Photo B in Fig. 2-1), the LC/MS/MS analysis of the filtrate revealed that certain soluble organic compounds must still be present in the sample, which interfered with PFAS analysis. As an example, Fig. 2-1 presents the chromatograms of a PFOS standard (Fig. 2-1A), and of PFOS in the extracts of Biosolid 1 (Figs. 2-1B and 2-1C) and Biosolid 2 (Figs. 2-1D and 2-1E). When 40 mg ENVI-Carb was used in *Step 3*, the resulting chromatogram baseline was noisy, especially in the case of Biosolid 1. By comparing the baseline of these chromatograms, it can be deduced that there were fewer interfering organic compounds in the Biosolid 2 extract than in the Biosolid 1 extract. This illustrates that the number of organic compounds coeluted with PFAS in the extraction step would vary among biosolid samples. To assess whether interfering organic compounds can be removed with a higher ENVI-Carb dose, the amount of ENVI-Carb used in *Step 3* was increased from 40 mg to 1,000 mg. It is noted that even with this ENVI-Carb dose,

precipitates were formed in the reconstituted extracts in *Step 4*. The LC/MS/MS analysis of the filtered extracts reveals that the baseline noise level was near zero in the case of Biosolid 2 (Fig. 2-1E), suggesting that the soluble interfering organic compounds were effectively removed by the extra ENVI-Carb. For Biosolid 1, the baseline noise level remained high, masking the branched isomer peak (Fig. 2-1C). This result suggests that an even greater dose of ENVI-Carb may be required to clean up Biosolid 1 extract and/or there are organic substances in this sample that could not be effectively removed by ENVI-Carb.

2.3.3. Envi-Carb and WAX

As mentioned above, the extracts of biosolids are thought to contain organic compounds that are more soluble in methanol than in 50/50 v/v methanol/water. It is further hypothesized that these organic compounds are hydrophobic enough to be retained by WAX, which adsorbs analytes via both ion exchange and hydrophobic interaction mechanisms.[68] If this hypothesis is correct, it is expected that these organic compounds would also be present in the final sample because they would be eluted out of the WAX cartridge by 1% methanolic ammonium hydroxide. In other words, the combination of ENVI-Carb and WAX is not expected to effectively remove the organic compounds that cause precipitation. Consistent with this hypothesis, it was observed that when the Biosolid 1 extract was cleaned with ENVI-Carb + WAX and subsequently mixed with water, precipitates were formed in the 50/50 v/v methanol/water sample (Photo A in Fig. 2-2). In Draft Method 1633 as well as in several other studies that employed ENVI-carb and WAX to clean biosolid extracts,[19,21,24,26,27,46,48] the 50/50 v/v methanol/water sample was directly subjected to LC/MS/MS analysis without any additional pre-treatment. However, given the physical appearance of the samples in our study, they were not deemed appropriate for LC/MS/MS analysis. Thus, the samples were further subjected to centrifugation followed by filtration. This additional treatment step resulted in a transparent, yellow/brown-colored solution (Photo B in Fig. 2-2). The color of the solution suggests that some soluble organic compounds must still be present in the samples. When these filtered samples were analyzed by LC/MS/MS, it was observed that, as in the case when the extracts were cleaned with only ENVI-Carb, the chromatograms of PFOS and PFHxA contained a noisy baseline, broad analyte peaks, and/or unidentified peaks that overlapped with the analyte peak (Fig. 2-2). Moreover, the chromatogram of other perfluoro sulfonate compounds including PFBS, PFPeS and PFHxS were not of acceptable quality (Fig. 2-3 (top

panels) and Fig. 2-4-A); note that because these compounds were not present in Biosolid 1, they were added (at 2.5 ng/mL) to the Biosolid 1 cleaned-up extract prior to LC/MS/MS analysis in order to assess how the presence of background organics affect the chromatogram of these PFAS). While the chromatograms of other PFCAs and precursors were reasonable (Figs. 2-4-B and 2-4-C), it is concluded that the combination of ENVI-Carb and WAX was not effective at cleaning Biosolid 1 extract to the extent that all 24 PFAS could be analyzed.

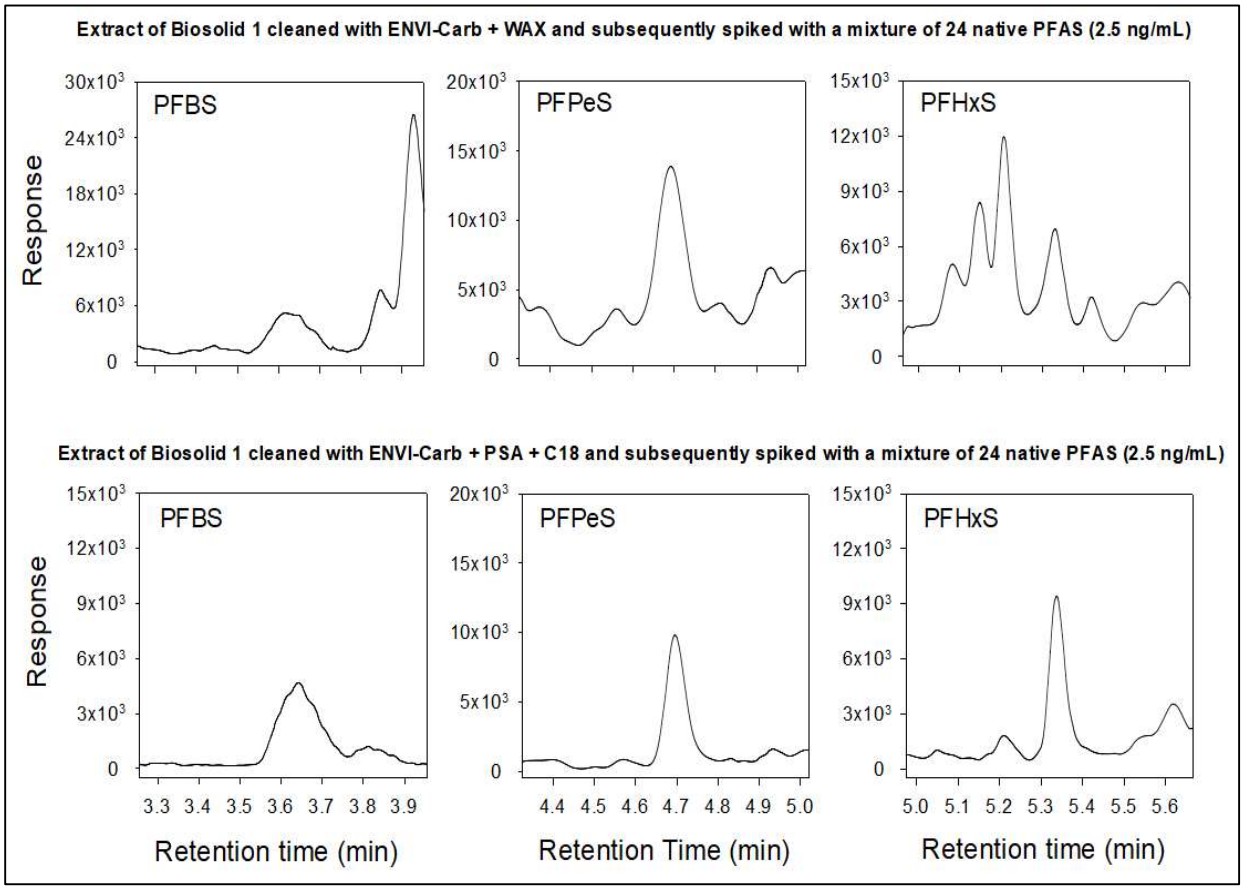


Figure 2-3. Chromatograms of PFBS, PFPeS and PFHxS in the Biosolid 1 extract cleaned with ENVI-Carb and WAX (i.e., the adsorbents used in US EPA Method 1633; top panels) and with a blend of ENVI-Carb, PSA, and C18 (bottom panels). These compounds were not present in Biosolid 1. Thus, the compounds were added to the cleaned-up extract prior to LC/MS/MS analysis.

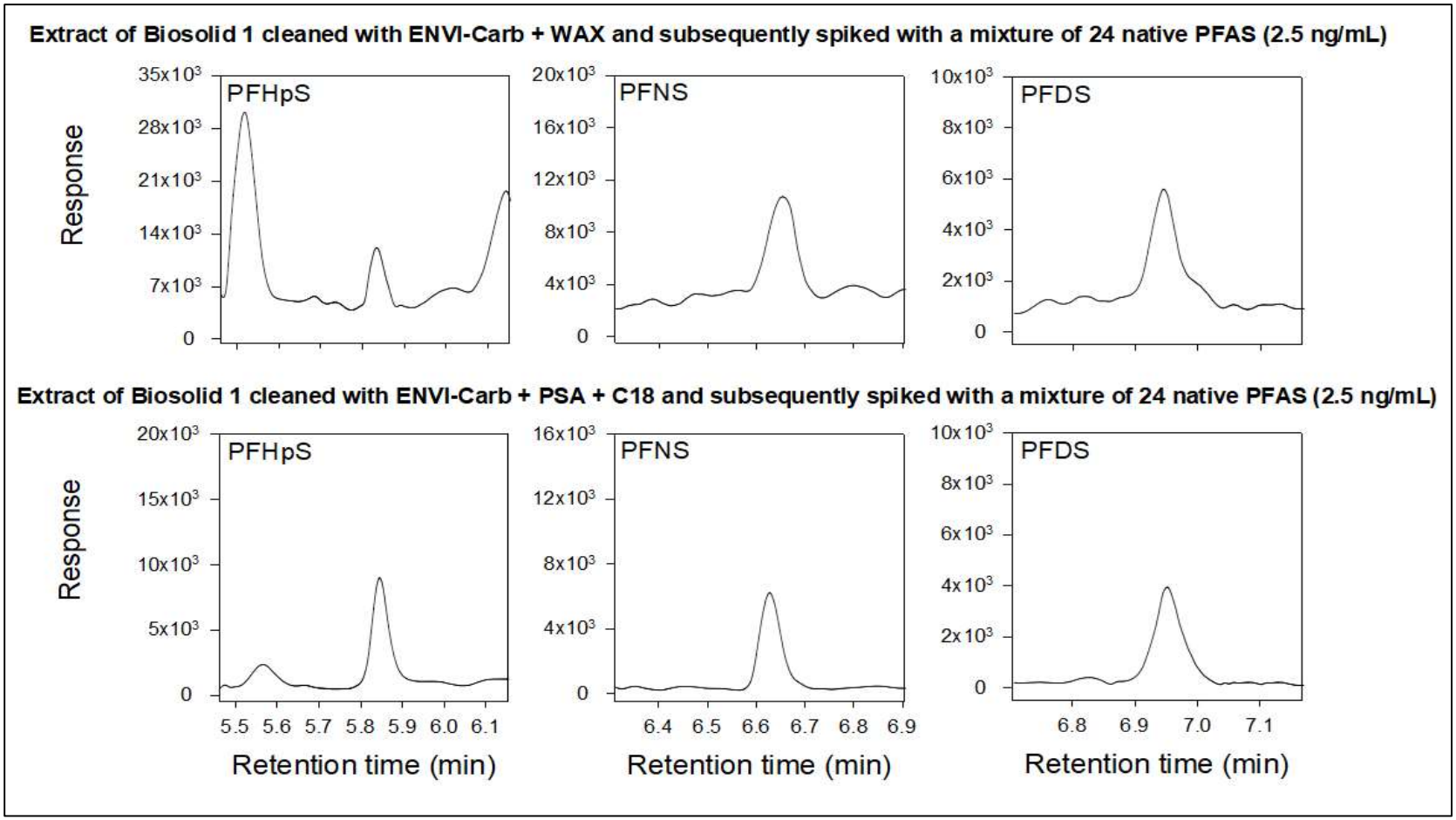


Figure 2-4-A. Chromatograms of perfluoro sulfonates in the Biosolid 1 extract cleaned with ENVI-Carb and WAX (top panels) and with a blend of ENVI-Carb, PSA, and C18 (bottom panels). These compounds were not present in Biosolid 1. Thus, the compounds were added to the cleaned-up extract prior to LC/MS/MS analysis.

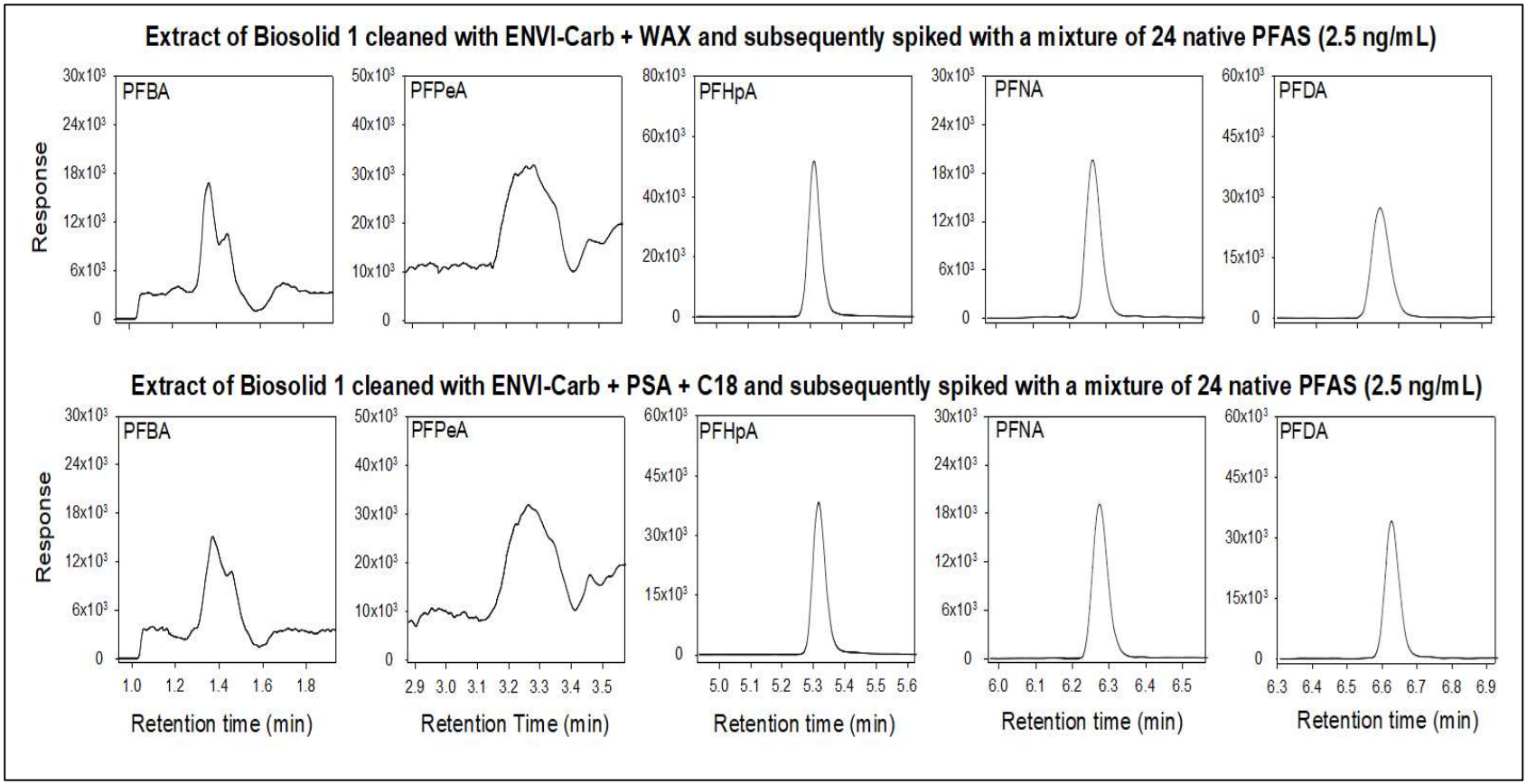


Figure 2-4-B. Chromatograms of other PFCAs in the Biosolid 1 extract cleaned with ENVI-Carb and WAX (top panels) and with a blend of ENVI-Carb, PSA, and C18 (bottom panels). The cleaned-up extracts were spiked prior to LC/MS/MS analysis.

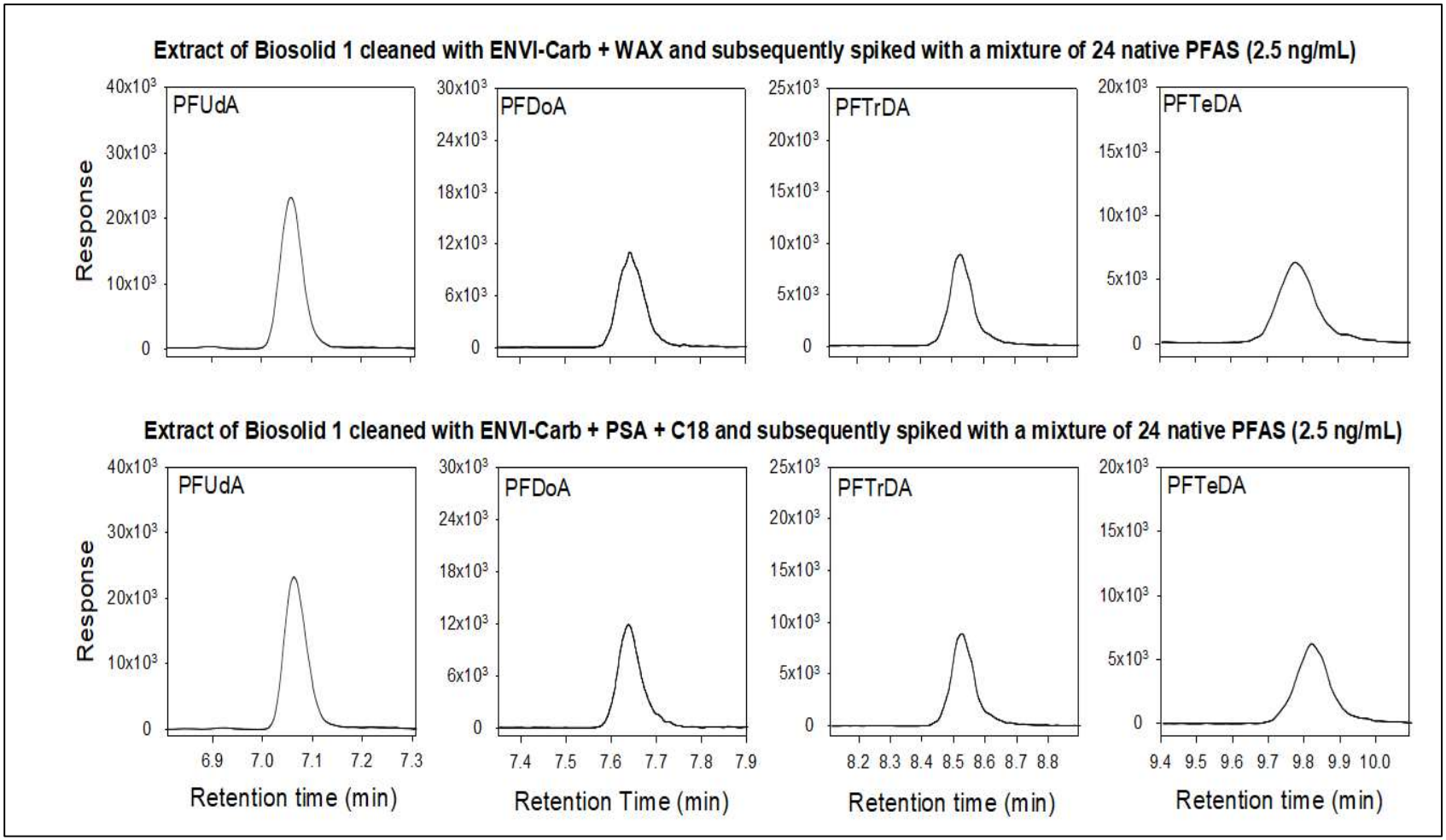


Figure 2-4-B cont'd. Chromatograms of other PFCAs in the Biosolid 1 extract cleaned with ENVI-Carb and WAX (top panels) and with a blend of ENVI-Carb, PSA, and C18 (bottom panels). The cleaned-up extracts were spiked prior to LC/MS/MS analysis.

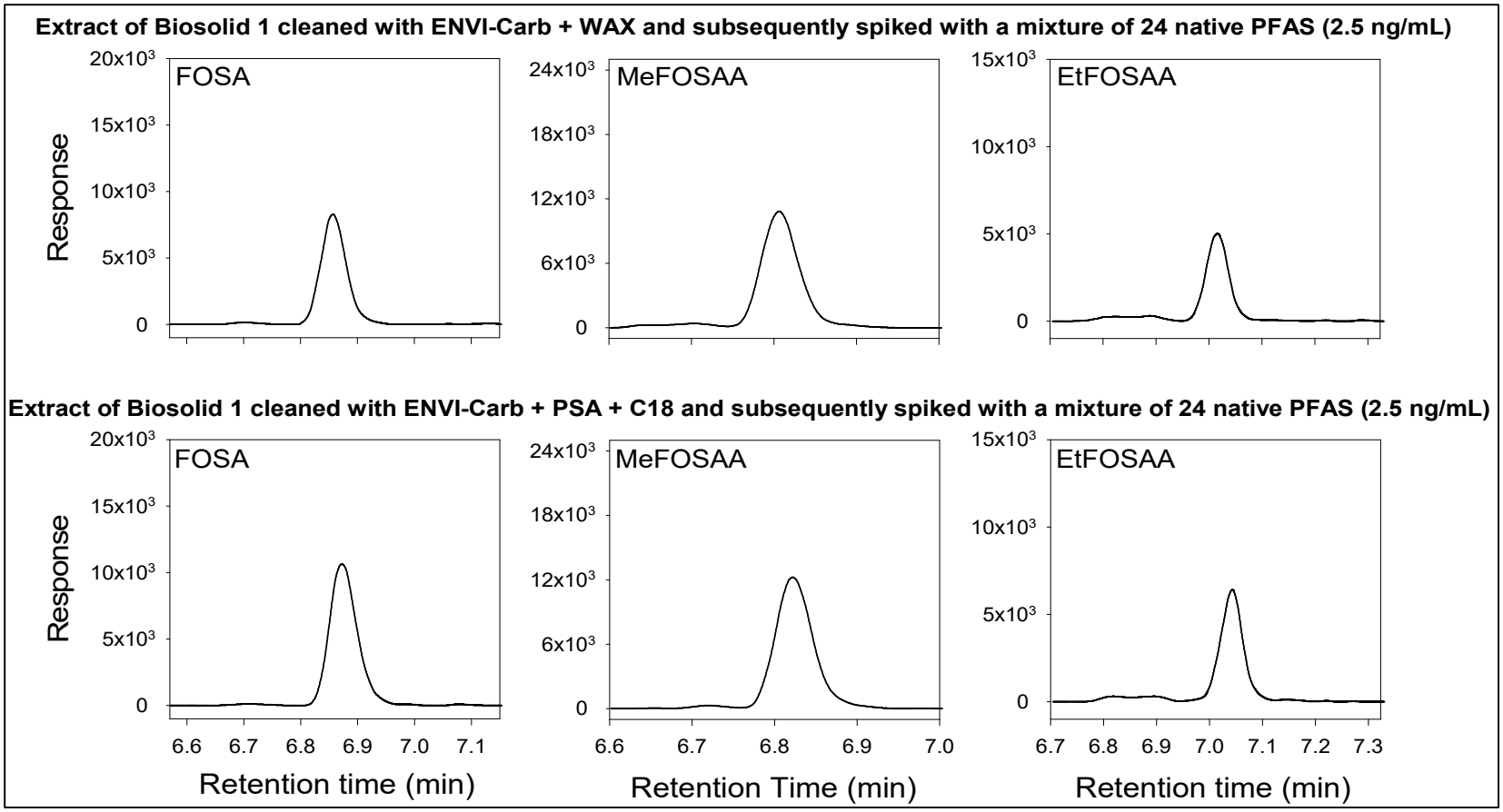


Figure 2-4-C. Chromatograms of precursors in the Biosolid 1 extract cleaned with ENVI-Carb and WAX (top panels) and with a blend of ENVI-Carb, PSA, and C18 (bottom panels). The cleaned-up extracts were spiked prior to LC/MS/MS analysis.

2.3.4. PSA, C18 and Envi-Carb

A few studies have used PSA and/or C18 instead of ENVI-Carb to clean up biosolid extracts.[20,47,51] In our study, the Biosolid 1 extract cleaned by PSA and C18 alone or combined remained highly opaque even after centrifugation and filtration (Photos A, B and D in Fig. 2-5). Considering these samples' physical appearance, they were deemed not suitable for LC/MS/MS analysis and were not studied further.

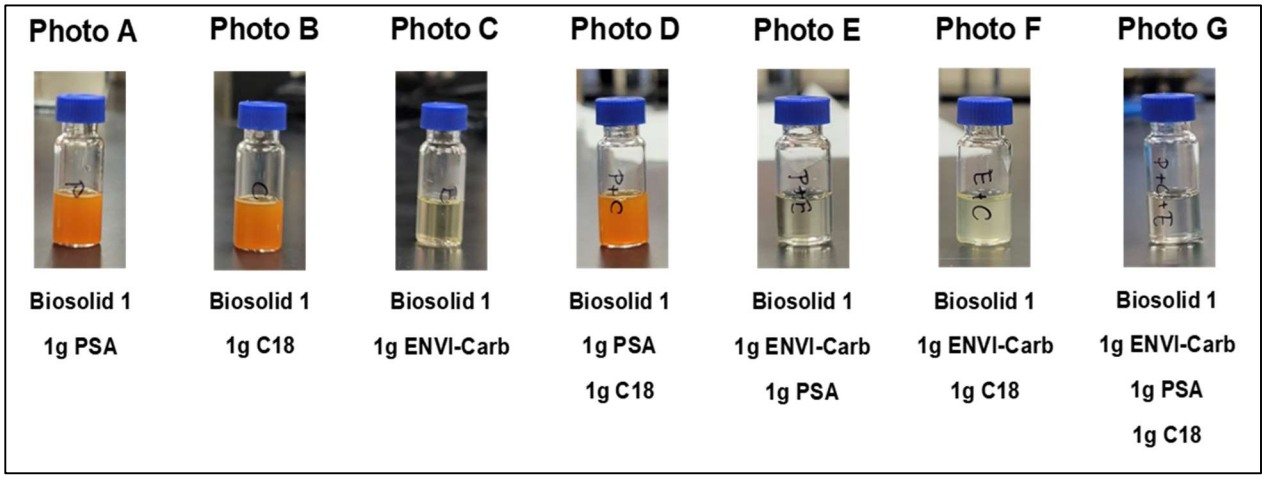


Figure 2-5. Photos A, B, C, D, E, F and G present the physical appearance of the Biosolid 1 extract cleaned with various adsorbents specified below each photo.

The Biosolid 1 extracts that were treated by ENVI-Carb and different combinations PSA and/or C18 were much less opaque (Photos C, E and F in Fig. 2-5), suggesting that ENVI-Carb was essential for the cleanup of this sample. At the highest adsorbent doses (i.e., 1,000 mg of each adsorbent), the final sample was transparent and nearly colorless (Photo G in Fig. 2-5). Further, inspection of the chromatogram of this sample reveals that the baseline noise level was significantly reduced, and the analyte peaks were well resolved (Fig. 2-3 bottom panel and Fig. 2-6). When the amounts of PSA and C18 were reduced to 500 mg, 400 mg, 300 mg, and 200 mg, the baseline noise level in the chromatogram progressively increased (Fig. 2-7). The baseline noise level was also higher when either PSA or C18 was absent (Fig. 2-7).

The results presented above demonstrate that the cleanup of biosolid extracts is affected by both adsorbent type and amount. For Biosolid 1, it was essential that the extract is treated with all three adsorbents in order to obtain clean chromatograms. While higher doses of adsorbents will improve

the removal of background organic compounds, it is recognized that increasing adsorbent dose will reduce recovery efficiency, owing to the adsorption of PFAS to the adsorbents. As can be seen from Fig. 2-8, the recovery values for isotopically labeled compounds were higher when Biosolid 1 extract was treated with less adsorbent. When the extract was treated with 1,000 mg ENVI-carb + 1,000 mg PSA + 1,000 mg C18, the recovery values ranged between 40 – 100%. When the amounts of PSA and C18 decreased by half (i.e., 1,000 mg ENVI-carb + 500 mg PSA + 500 mg C18), the recovery values increased to 60 – 130%. These recovery values are comparable to those reported in several other studies.[16,20,24,30,32-34,45] The recovery values further increased to 80 – 180% when the amounts of PSA and C18 were reduced to as low as 200 mg. As was mentioned earlier, however, the baseline noise level was also appreciably higher with this treatment combination. For the isotopically labelled fluorotelomer sulfonates (M2-6:2 FTS, M2-4:2 FTS, and M2-8:2 FTS), the recovery values were between 140 – 450 % under all cleanup conditions. A recovery value greater than 100% could be attributed to ionization enhancement by the matrix. Previous studies have also observed high recoveries for these same compounds.[41,42,50,51,69,70]

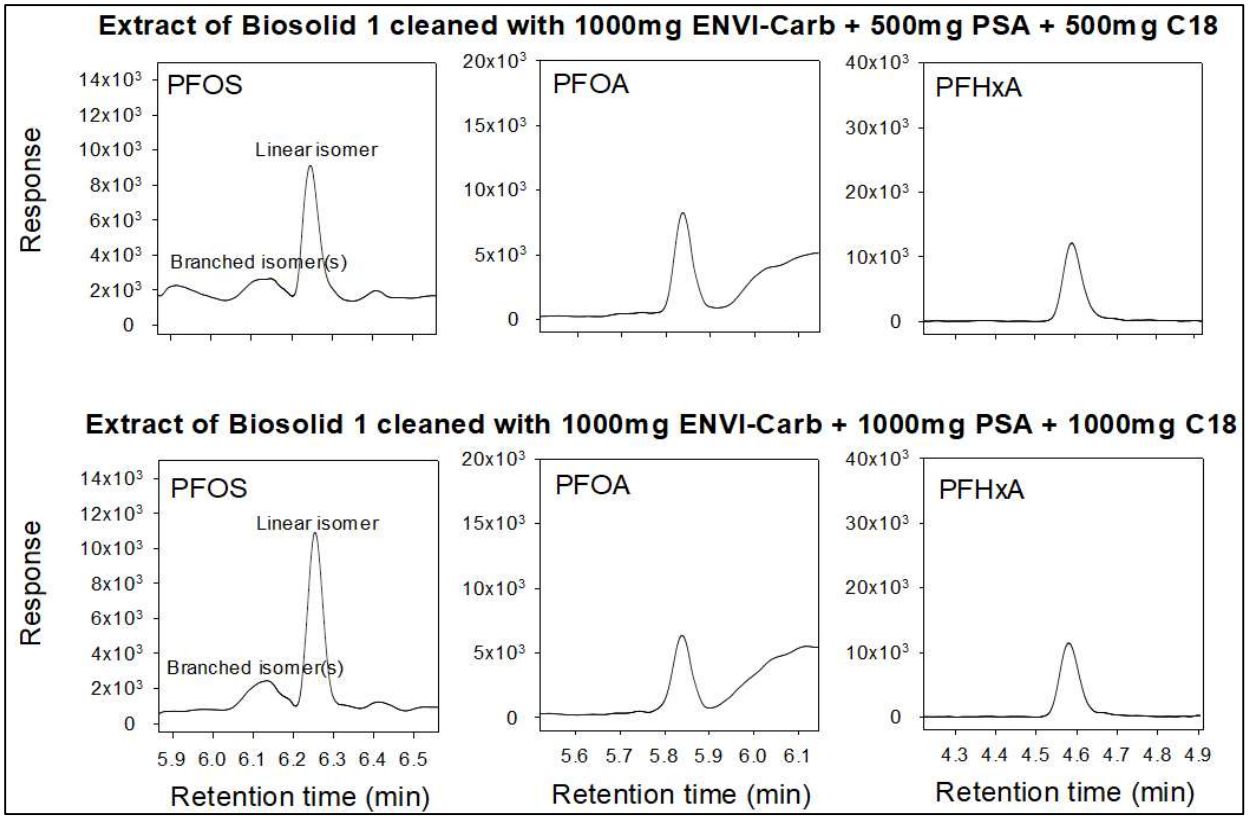


Figure 2-6. Chromatograms of various PFAS in the Biosolid 1 extract cleaned with blends of ENVI-Carb, PSA, and C18.

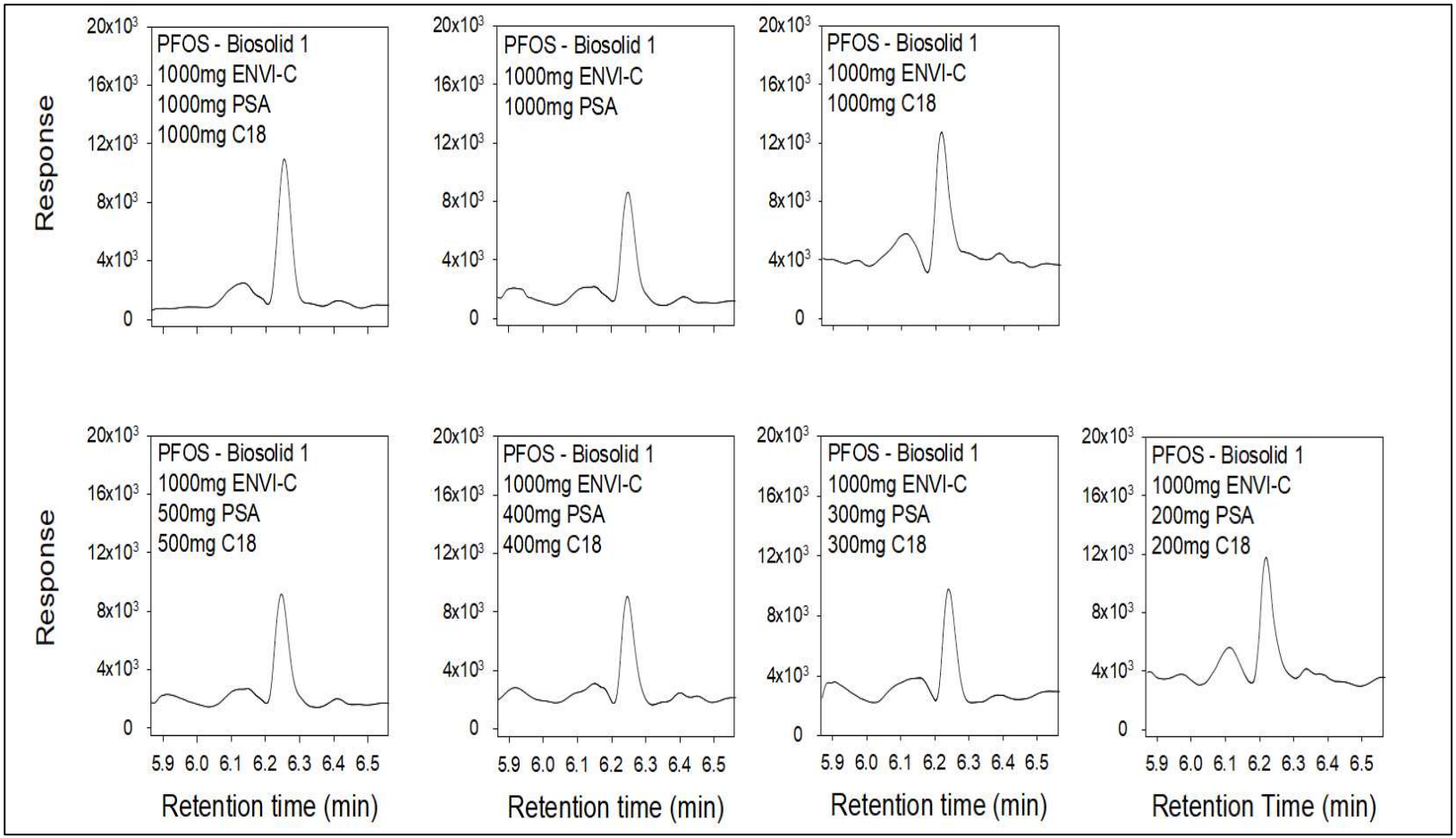


Figure 2-7. Chromatograms of PFOS in the Biosolid 1 extract cleaned with blends of ENVI-Carb, PSA, and C18.

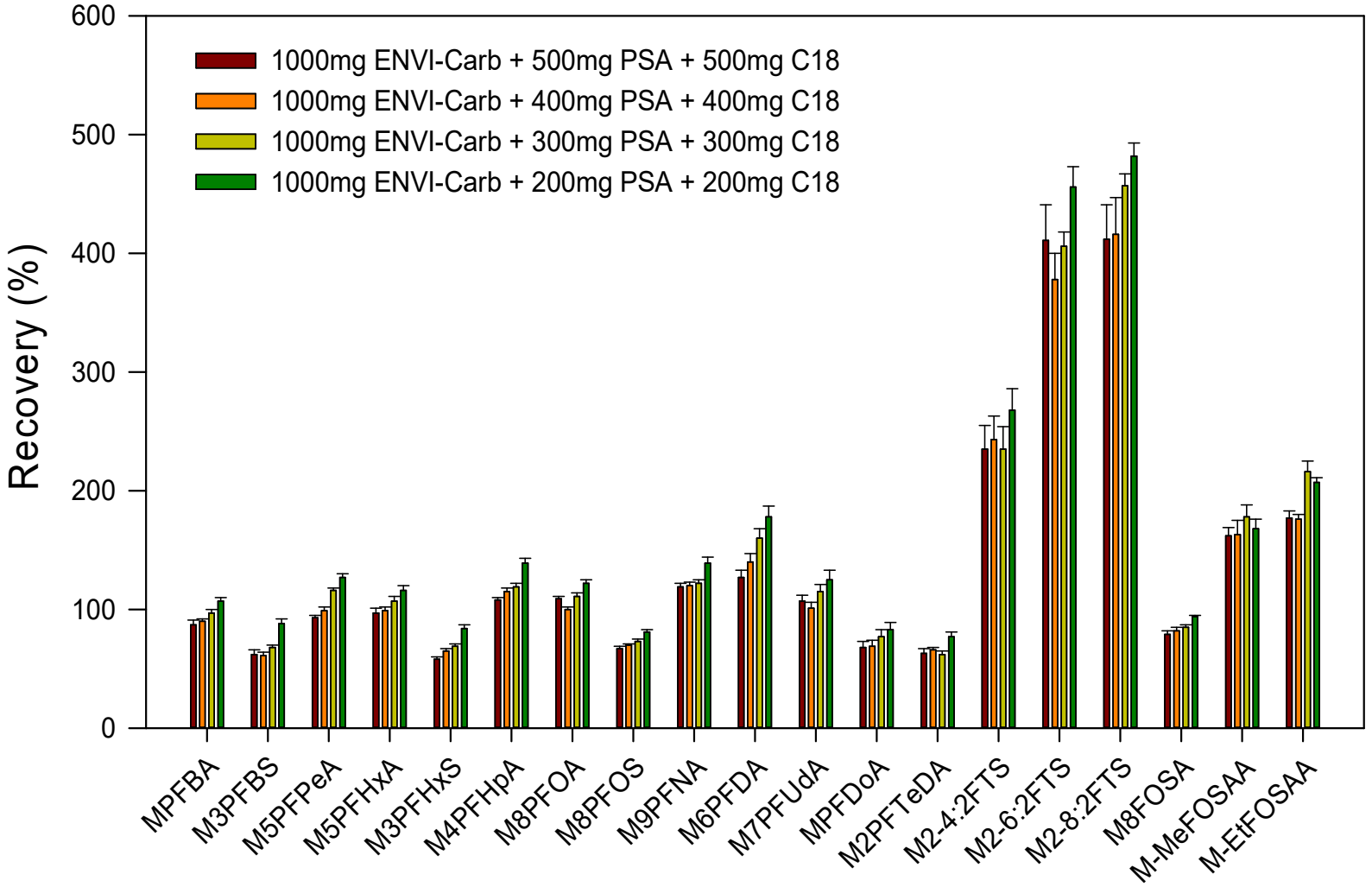


Figure 2-8. Mean Recovery Values (n=3) of mass-labelled PFAS of Biosolid 1 cleaned with 1,000 mg ENVI-Carb and various amounts of PSA and C18.

2.3.5. PFAS in six biosolid samples

Considering the trade-off between analyte recovery and chromatogram quality, the treatment combination consisting of 1,000 mg ENVI-carb + 500 mg PSA + 500 mg C18 was deemed most appropriate for the cleanup of Biosolid 1 extract. This adsorbent formulation was then applied to the analysis of Biosolids 2 – 6. For these biosolid samples, the chromatograms, presented in Fig. 2-9 consisted of well-resolved analyte peaks and low baseline noise levels. While varied across the six studied biosolid samples, the recovery value for each isotopically labeled compound generally fell in the 40 – 160% range (Fig. 2-10). With the biosolid samples (Biosolid 1 and Biosolid 4) that were spiked with both native and isotopically labeled PFAS, the average recovery values (based on triplicate spiked samples) of the native PFAS were comparable ($p > a = 0.05$) to those of the isotopically labeled counterparts (Table 2-3). Thus, the concentration of the native PFAS can be calculated based on the R of the isotopically labeled compounds along with the analyte/surrogate peak area ratio.

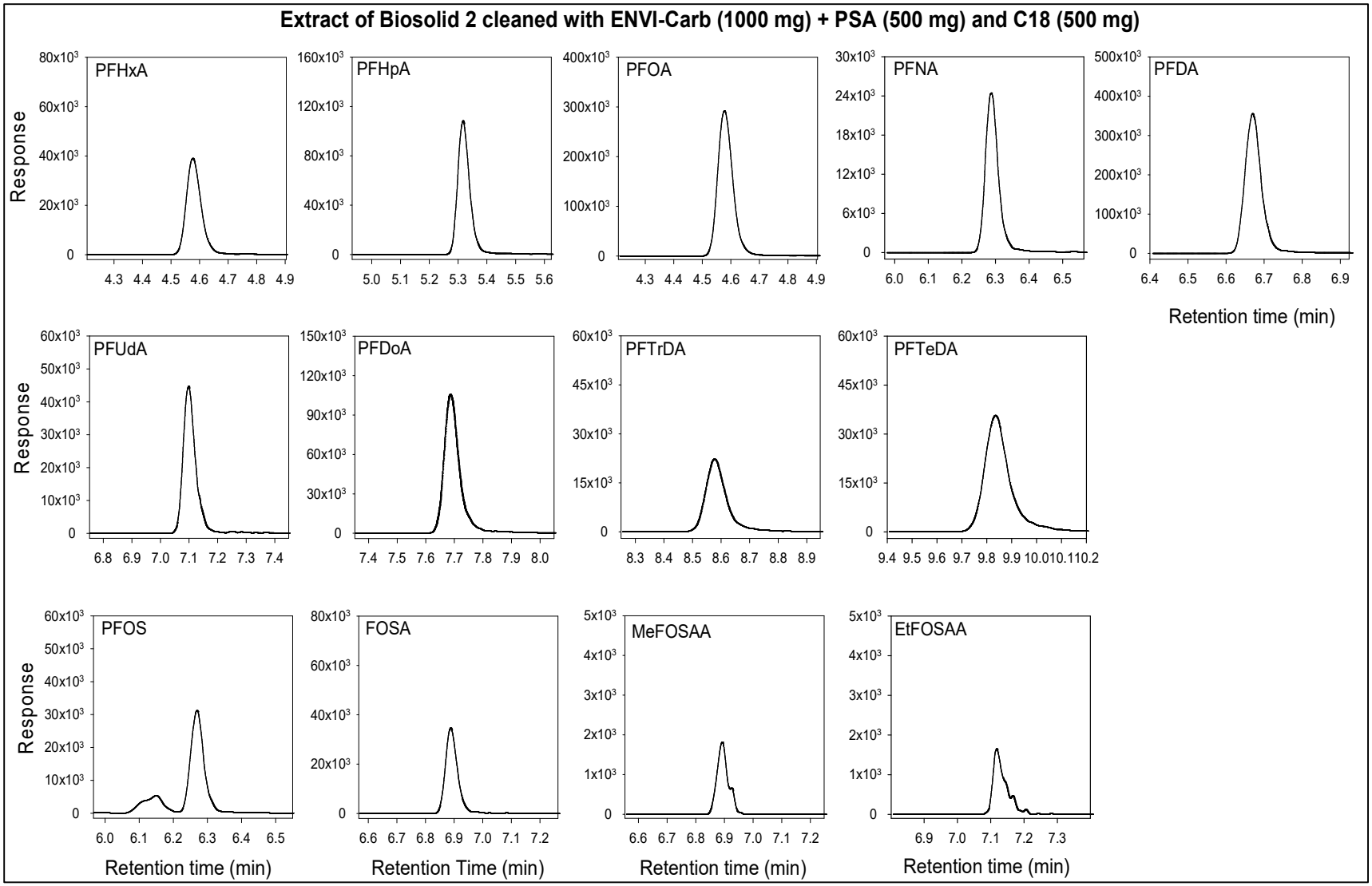


Figure 2-9-A. Chromatograms of all PFASs in the Biosolid 1 extract cleaned with ENVI-Carb (1000 mg), PSA (500 mg) and C18 (500 mg).

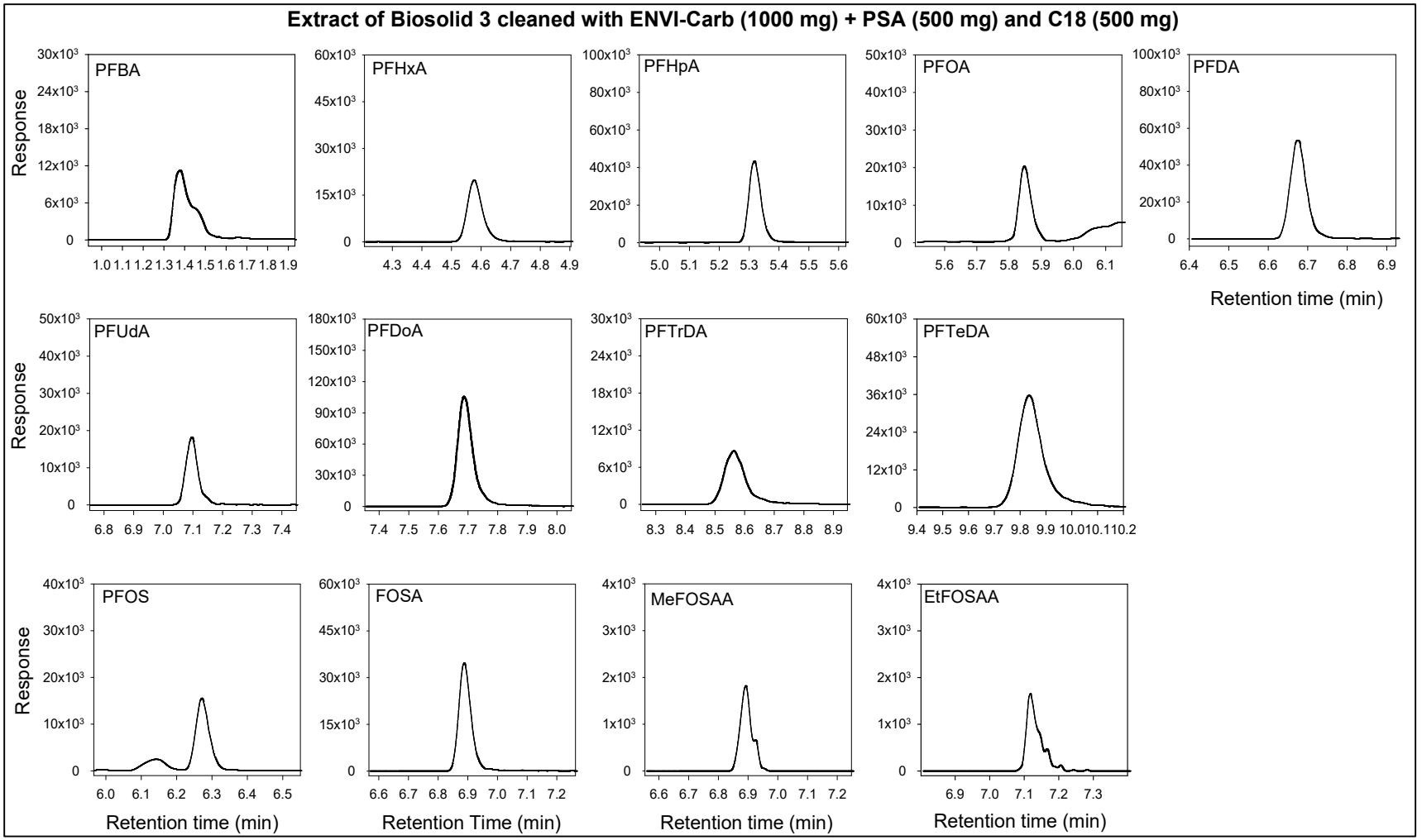


Figure 2-9-B. Chromatograms of all PFASs in the Biosolid 3 extract cleaned with ENVI-Carb (1000 mg), PSA (500 mg) and C18 (500 mg).

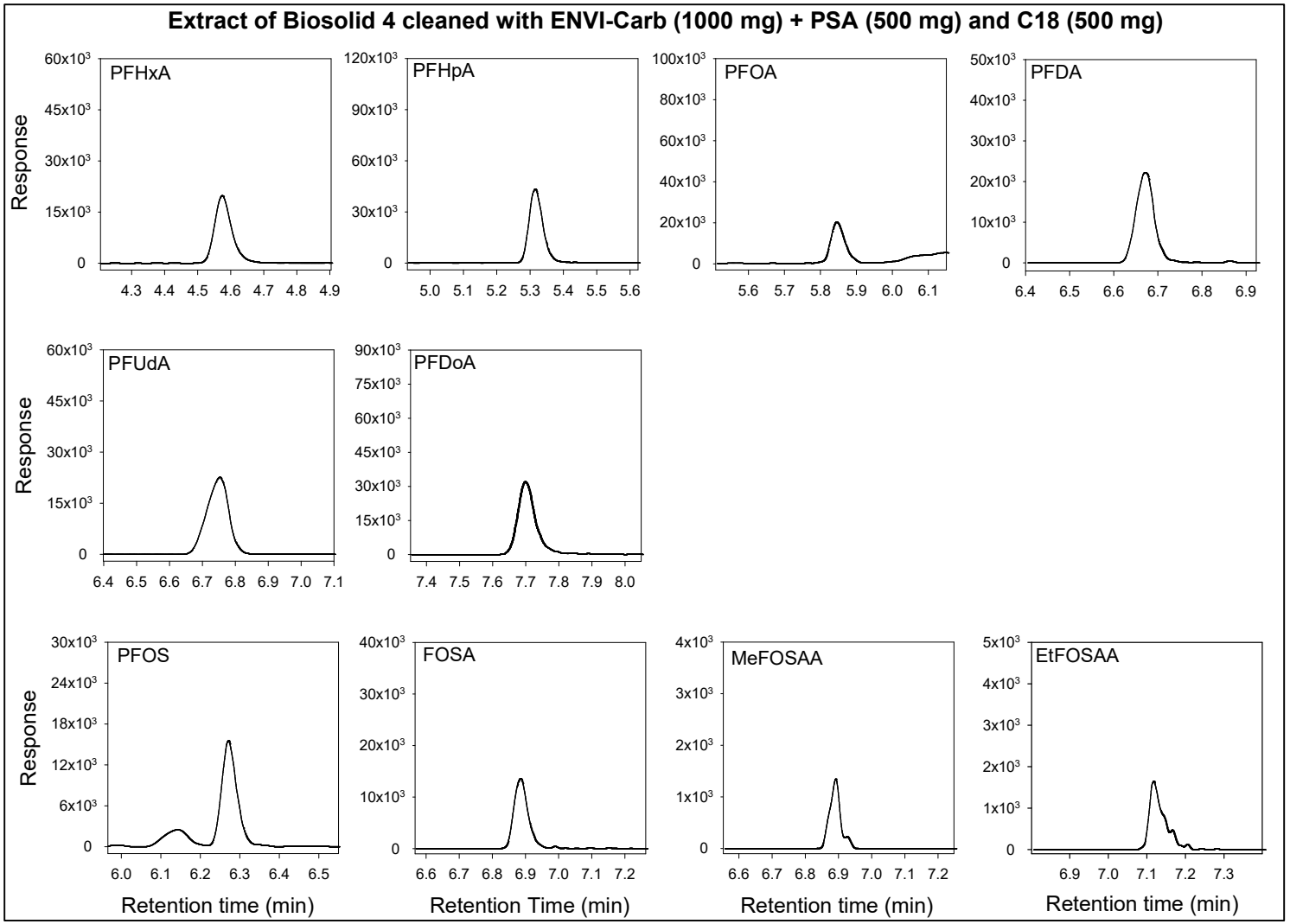


Figure 2-9-C. Chromatograms of all PFASs in the Biosolid 4 extract cleaned with ENVI-Carb (1000 mg), PSA (500 mg) and C18 (500 mg).

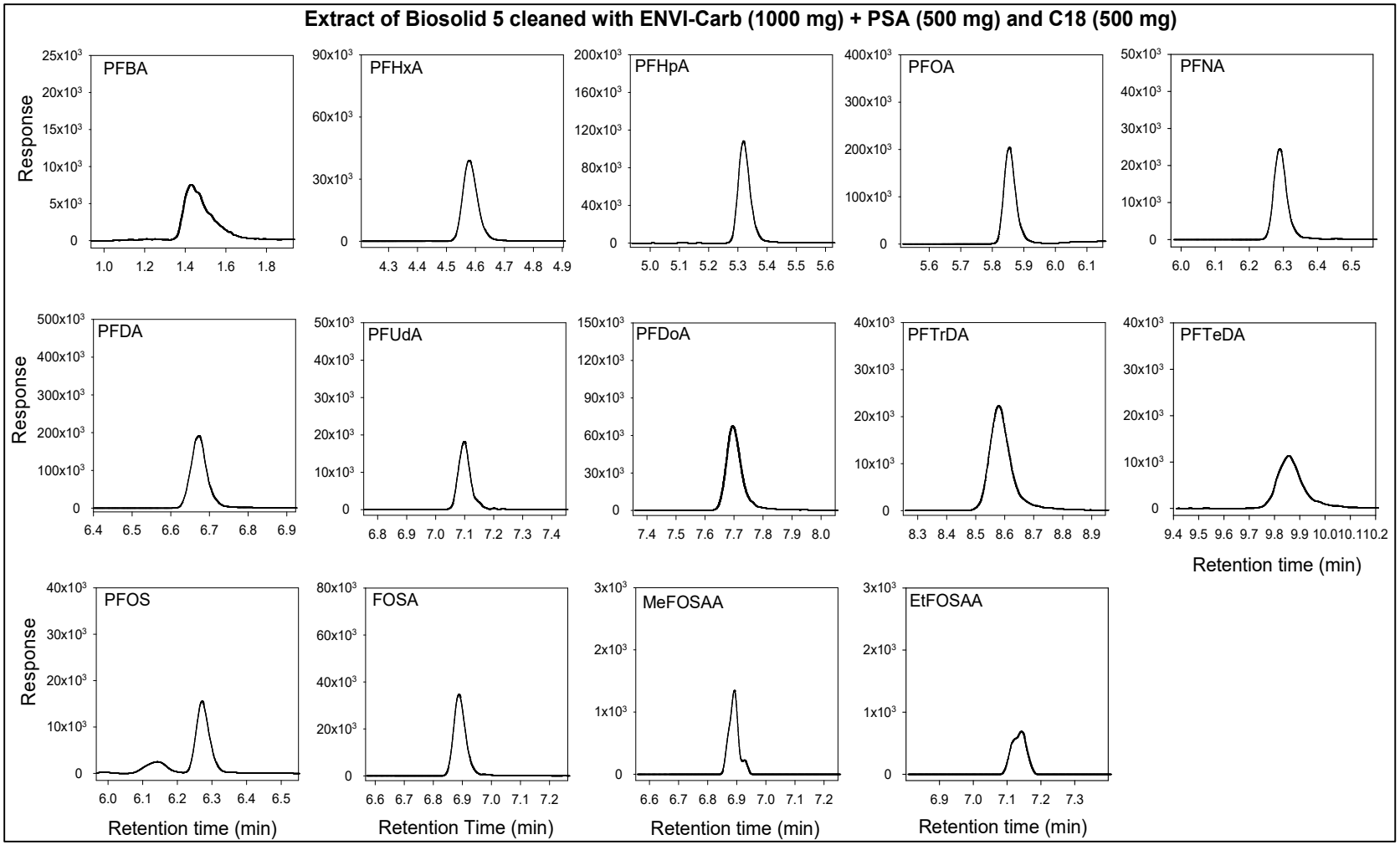


Figure 2-9-D. Chromatograms of all PFASs in the Biosolid 5 extract cleaned with ENVI-Carb (1000 mg), PSA (500 mg) and C18 (500 mg).

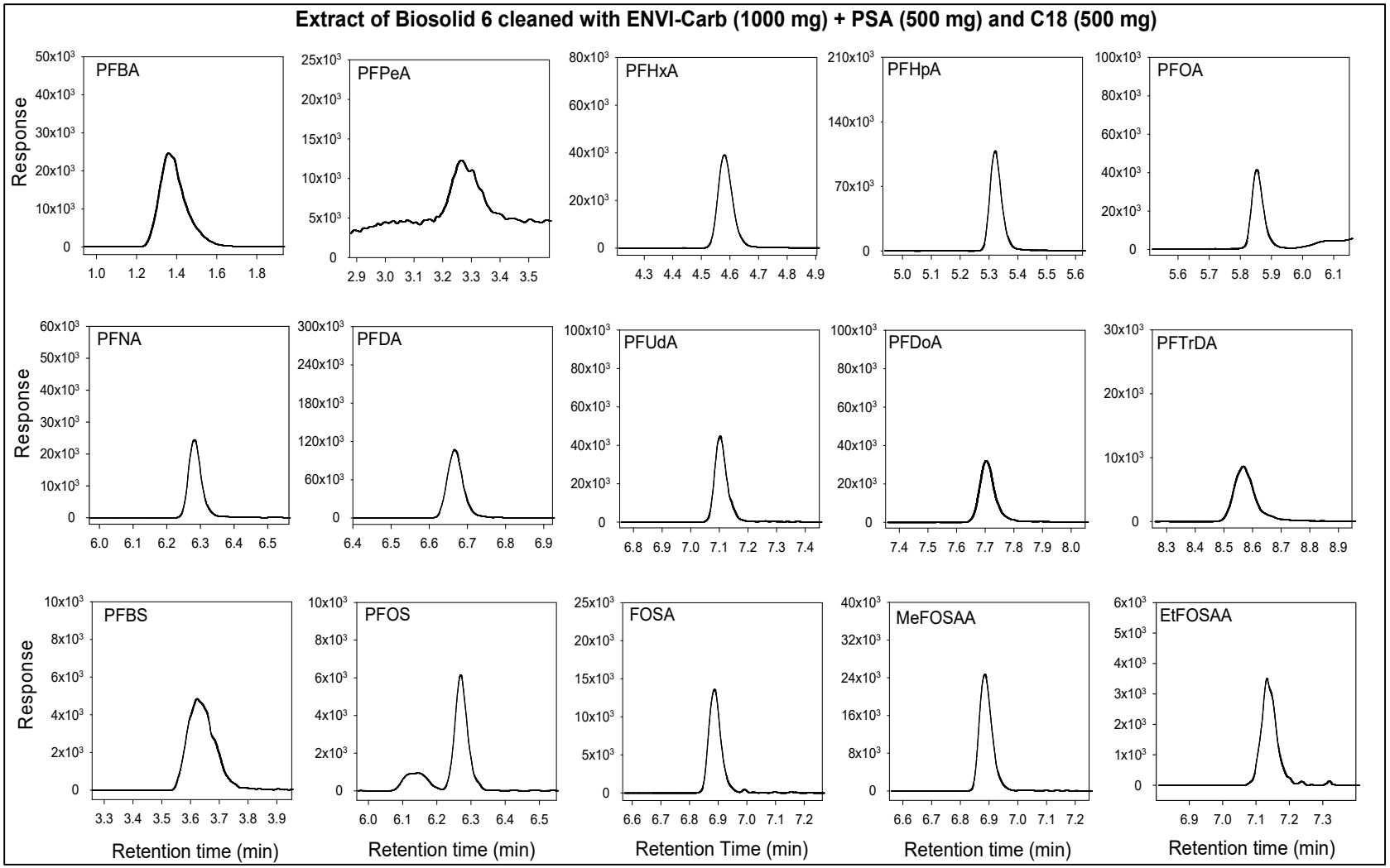


Figure 2-9-E. Chromatograms of all PFASs in the Biosolid 6 extract cleaned with ENVI-Carb (1,000 mg), PSA (500 mg) and C18 (500 mg).

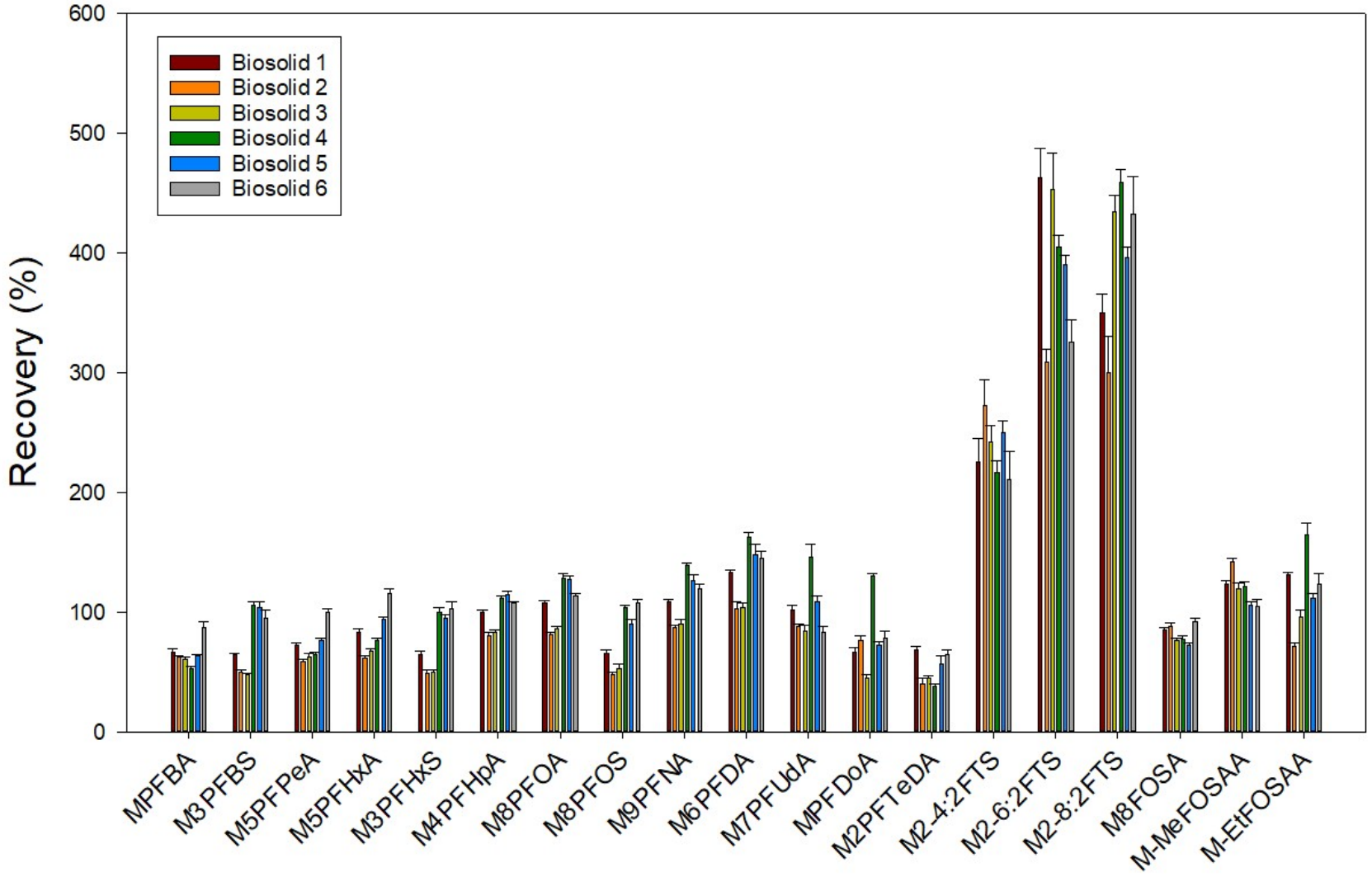


Figure 2-10. Mean Recovery Values (n=3) of mass-labelled PFAS of Biosolid 1, 2, 3, 4 and 5 cleaned with 1,000 mg ENVI-Carb + 500 mg PSA + 500 mg C18.

Table 2-3. Recovery values for native and isotopically labeled PFAS. Biosolid 1 and Biosolid 4 were spiked with both native PFAS (5 ng/compound) and isotopically labeled PFAS (2 ng/compound), and the spiked samples were extracted and analyzed.

Biosolid 1

Analytes	Recovery	Internal standards	Recovery	p
PFBA	95 ± 4	[$^{13}C_4$] MPFBA	87 ± 4	0.07
PFPeA	95 ± 6	[$^{13}C_5$] MPFPeA	92 ± 5	0.50
PFHxA	97 ± 7	[$^{13}C_5$] MPFHxA	101 ± 4	0.29
PFHpA	108 ± 5	[$^{13}C_4$] MPFHpA	112 ± 2	0.30
PFOA	102 ± 3	[$^{13}C_8$] MPFOA	108 ± 3	0.10
PFNA	137 ± 6	[$^{13}C_9$] MPFNA	130 ± 8	0.28
PFDA	160 ± 11	[$^{13}C_6$] MPFDA	156 ± 10	0.62
PFUdA	109 ± 6	[$^{13}C_7$] MPFUdA	117 ± 7	0.09
PFDoA	95 ± 4	[$^{13}C_2$] MPFDoA	85 ± 5	0.08
PFTrDA	85 ± 5	[$^{13}C_2$] MPFTeDA	74 ± 4	0.05
PFTeDA	80 ± 5	[$^{13}C_2$] MPFTeDA	74 ± 4	0.14
PFBS	78 ± 5	[$^{13}C_3$] MPFBS	84 ± 3	0.17
PFPeS	90 ± 4	[$^{13}C_3$] MPFBS	84 ± 3	0.17
PFHxS	96 ± 6	[$^{13}C_3$] MPHxS	91 ± 3	0.21
PFHpS	94 ± 4	[$^{13}C_3$] MPHxS	91 ± 3	0.25
PFOS	93 ± 3	[$^{13}C_8$] MPFOS	93 ± 2	1.00
PFNS	98 ± 5	[$^{13}C_8$] MPFOS	93 ± 2	0.22
PFDS	100 ± 9	[$^{13}C_8$] MPFOS	93 ± 2	0.38
4:2FTS	215 ± 12	[$^{13}C_2$] M4:2FTS	190 ± 10	0.05
6:2FTS		[$^{13}C_2$] M6:2FTS	410 ± 20	
8:2FTS	325 ± 13	[$^{13}C_2$] M8:2FTS	298 ± 13	0.05
FOSA	100 ± 5	[$^{13}C_8$] MFOSA	107 ± 4	0.11
MeFOSAA	137 ± 6	[$^{2}d_2$]MMeFOSAA	134 ± 3	0.58
EtFOSAA	138 ± 7	[$^{2}d_2$] MEtFOSAA	144 ± 3	0.14

Biosolid 4

Analytes	Recovery	Internal standards	Recovery	p
PFBA	86 ± 6	[$^{13}C_4$] MPFBA	81 ± 5	0.28
PFPeA	89 ± 4	[$^{13}C_5$] MPFPeA	93 ± 2	0.25
PFHxA	109 ± 4	[$^{13}C_5$] MPFHxA	111 ± 3	0.41
PFHpA	125 ± 5	[$^{13}C_4$] MPFHpA	128 ± 3	0.49
PFOA	134 ± 4	[$^{13}C_8$] MPFOA	133 ± 2	0.70
PFNA	156 ± 7	[$^{13}C_9$] MPFNA	152 ± 3	0.37
PFDA	181 ± 6	[$^{13}C_6$] MPFDA	175 ± 3	0.23
PFUdA	158 ± 7	[$^{13}C_7$] MPFUdA	164 ± 5	0.25
PFDoA	141 ± 5	[$^{13}C_2$] MPFDoA	142 ± 3	0.85
PFTrDA	66 ± 4	[$^{13}C_2$] MPFTeDA	68 ± 6	0.60
PFTeDA	74 ± 3	[$^{13}C_2$] MPFTeDA	68 ± 6	0.26
PFBS	116 ± 7	[$^{13}C_3$] MPFBS	115 ± 4	0.78
PFPeS	111 ± 7	[$^{13}C_3$] MPFBS	115 ± 4	0.43
PFHxS	109 ± 6	[$^{13}C_3$] MPHxS	114 ± 5	0.27
PFHpS	119 ± 4	[$^{13}C_3$] MPHxS	114 ± 5	0.29
PFOS	105 ± 5	[$^{13}C_8$] MPFOS	110 ± 4	0.21
PFNS	101 ± 6	[$^{13}C_8$] MPFOS	110 ± 4	0.10
PFDS	104 ± 5	[$^{13}C_8$] MPFOS	110 ± 4	0.16
4:2FTS	232 ± 14	[$^{13}C_2$] M4:2FTS	210 ± 10	0.06
6:2FTS		[$^{13}C_2$] M6:2FTS	399 ± 10	
8:2FTS	369 ± 13	[$^{13}C_2$] M8:2FTS	400 ± 20	0.05
FOSA	97 ± 3	[$^{13}C_8$] MFOSA	100 ± 3	0.26
MeFOSAA	121 ± 5	[$^{2}d_2$]MMeFOSAA	129 ± 3	0.11
EtFOSAA	156 ± 6	[$^{2}d_2$] MEtFOSAA	170 ± 10	0.13

The concentrations of each PFAS in Biosolids 1 – 6 are presented in Fig. 2-11. In general, the total PFAAs concentrations in the biosolid samples our study, which ranged between 16 and 96 mg/kg, were comparable to those reported in previous studies. Based on the total concentration as well as the individual concentration of each PFAA in Biosolids 1, 2, 4 and 5, it appears that alkaline treatment resulted in a decrease in the concentration of PFAAs. The total PFAAs (inset of Fig. 2-5) decreased from 96.2 ± 1.7 mg/kg in Biosolid 2 to 62.4 ± 0.9 mg/kg in Biosolid 5, which was generated by treating Biosolid 2 at pH = 9 and 70 ºC for 20 minutes. The total PFAA concentrations in Biosolid 1 and Biosolid 4 (which was generated by treating Biosolid 1 generated at pH = 9 and 70 ºC for 20 minutes) were 16.0 ± 0.3 mg/kg to 15.2 ± 0.4 mg/kg, respectively (inset of Fig. 2-11). The difference between these two values was statistically significant ($p < a = 0.05$). One possible explanation for this trend was that PFAAs leached out of biosolid during alkaline treatment, due to a pH increase. The pH of the as-received biosolid samples is: Biosolid 1, pH = 7.8; Biosolid 4, pH = 9.1; Biosolid 2, pH = 6.3; Biosolid 5, pH = 8.7. It has been established that the affinity of PFAAs to solid surfaces, including biosolids, decreases as pH increases.[57, 71]

Of the three PFAA precursors measured, the concentration of FOSA (i.e., a sulfonamide) in the pre- and post-alkaline treatment samples did not differ appreciably. Both Biosolids 1 and 4 contained 1.6 ± 0.1 mg/kg FOSA, while Biosolid 2 and 5 contained 5.3 ± 0.1 mg/kg and 5.2 ± 0.3 mg/kg FOSA, respectively. In contrast, the concentration of MeFOSAA and EtFOSAA (i.e., the sulfonamido acetic acid compounds) decreased by 20 – 70% after alkaline treatment. To determine if this change is due to partitioning and/or transformation, future research will need to measure the concentration of these compounds in the liquid phase generated from the alkaline treatment in order to examine the mass balance.

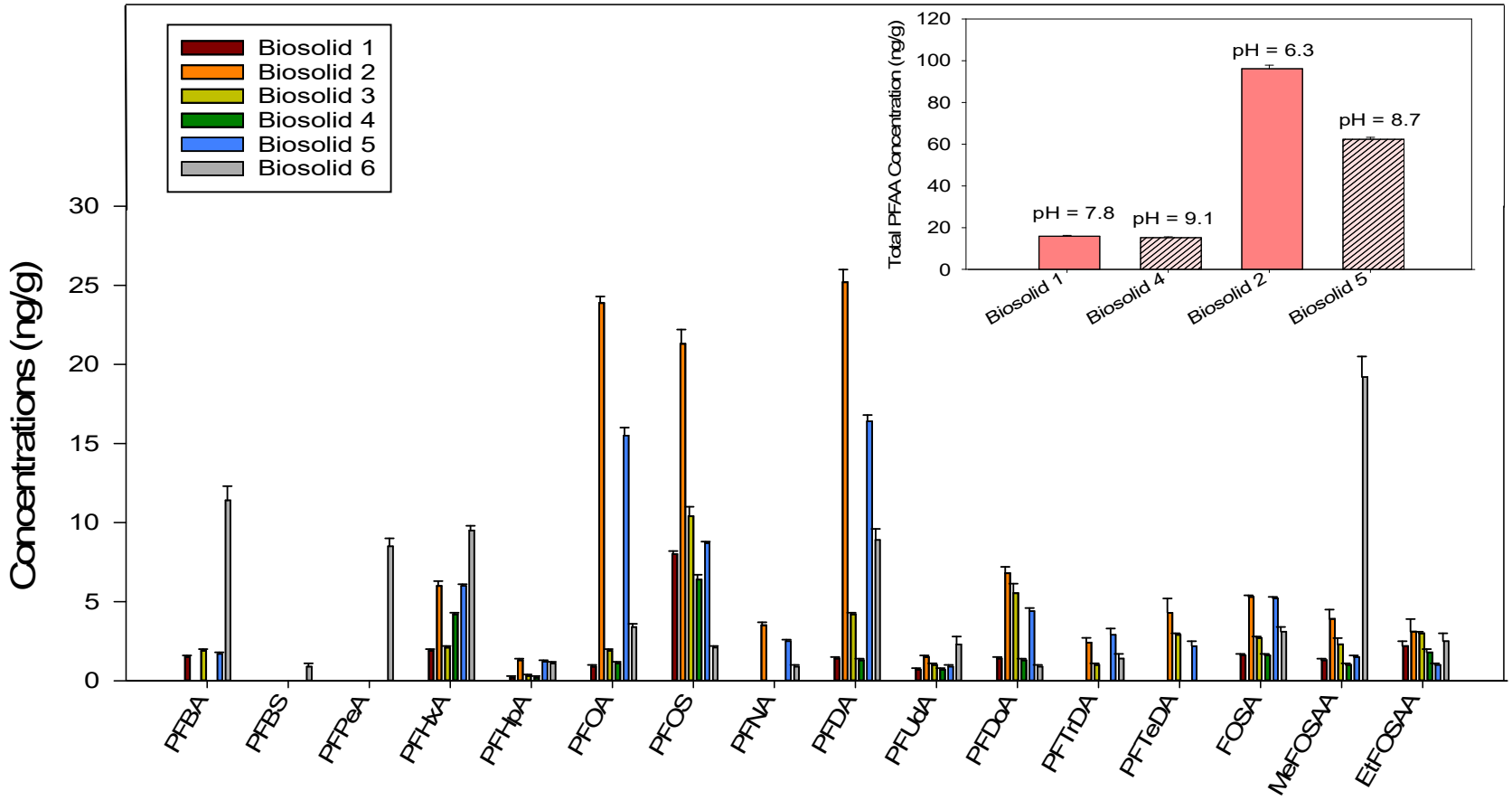


Figure 2-11. The concentrations of various PFAS in the five biosolids investigated in this study. Of the 24 measured PFAS, the following compounds were not present in all biosolids: PFPeS, PFHxS, PFHpS, PFNS, 4:2 FTS, and 8:2 FTS. Due to cross contamination, 6:2 FTS is not reported. Inset: the total PFAA concentration in Biosolids 1, 4, 2 and 5. Biosolids 4 and 5 were produced via thermal alkaline treatment of Biosolids 1 and 2, respectively. The total PFAA concentrations in Biosolid 1 (16.0 ± 0.3 mg/kg) and Biosolid 4 (15.2 ± 0.4 mg/kg) were statistically different ($p < a = 0.05$).

2.4. Conclusion

Several analytical methods for the quantification of PFAS in wastewater sludges and biosolids were proposed over the past two decades, including the recently published US EPA Draft Method 1633. In all methods, the cleanup of extract to eliminate interfering organic and inorganic compounds prior to LC-MS/MS analysis is critical to obtaining acceptable chromatogram quality and recoveries of PFAS from these complex matrices. While a variety of adsorbents have been employed in the cleanup step, we argue that a "one-size-fits-all" method may not exist. Specifically, in this study, we observed that none of the existing methods could prevent precipitate formation when methanolic extracts from the five studied biosolids were reconstituted with water. Centrifugation and filtration of the reconstituted extracts to remove precipitates did not sufficiently eliminate interferences to allow PFAS quantification. To overcome this issue, the use of blends of adsorbents (ENVI-Carb, PSA, C18) with different adsorptive properties that could remove a broader range of interfering substances was evaluated. A blend consisting of 1000 mg ENVI-Carb, 500 mg PSA and 500 mg C18 was used to successfully analyze PFAS in the five studied biosolids. While this adsorbent formulation shows considerable promise, the optimum formulation could be sample-specific, given that sludges and biosolids vary widely in characteristics. Thus, the adsorbent formulation should be modified to obtain an optimal trade-off between chromatogram quality and PFAS recovery. Additional research is needed to assess the robustness of the cleanup method developed in this study against a broader range of sludge and biosolids matrices.

Chapter 3

Quantification of PFAS Fate in Sludge Handling Systems

Abstract

PFAS are highly toxic compounds that have been detected in biosolids and are of concern due to the potential for leaching to soils and groundwater upon biosolids land application. This study evaluated the fate of PFAS in two full-scale Canadian sludge handling systems, namely WWTP-A and WWTP-B. Both systems included thickening, anaerobic digestion and dewatering. WWTP-B also had dissolved air flotation, fermentation and blending. Out of 15 detected PFAS, PFOS was the most abundant, which is among the most toxic and bioaccumulative PFAS. The concentrations of PFOS were as high as 12.9 μg/kg and 53 ng/L in solid and liquid samples, respectively.

Mass balance analyses of liquid-solid separation (i.e., thickening and dewatering) and blending showed that the mass flows of 92% of the 15 detected individual PFAS did not change through these processes, suggesting that these physical processes characterized with short sludge retention time (SRT) did not result in significant PFAS formation or removal. While the fermentation process (SRT of 4 days) also did not cause a significant change in a majority (16/24) of mass flows of target PFAS in WWTP-B, the anaerobic digestion processes in WWTP-A and WWTP-B (SRTs of 45 and 20 days, respectively) resulted in mass flow increases for a majority (13/22) of the detected individual PFAAs and mass flow decreases for all detected precursors. The PFAAs formation and precursors degradation magnitudes were higher in WWTP-A. These results showed that SRT may be a key factor affecting PFAS fate in sludge handling systems.

In the overall sludge handling systems, the highest increases in mass flows were observed for PFOS and PFDA (>100%) in WWTP-A, and PFOS and PFHxS (>30%) in WWTP-B. Furthermore, the mass flows of the precursors decreased (10-40%) in both WWTPs. In the liquid-solid separation processes, the PFAS with the carbon chain lengths of C3 to C6 were mostly present (between 50% and 86%) in the liquid streams, whereas the PFAS with the chain lengths higher than C6 were mostly present (between 50% and 121%) in the solid streams. Moreover, PFSAs were found to be 1.4 to 4 times more prone to binding to solids as compared to PFCAs with the same carbon chain length. These results showed that carbon chain length and functional groups are the key factors determining PFAS fate in sludge handling systems.

Collectively, the results of this study provide in-depth information about the behavior of PFAS through the entire train of sludge handling systems. The results generated in this study will inform decision-making for the development of effective approaches to biosolids management.

3.1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are anthropogenic compounds that possess both hydrophilic and hydrophobic properties. Moreover, PFAS contain multiple C-F bonds, which are one of the strongest chemical bonds.[1-4] Thus, PFAS can largely withstand physical, chemical or biological degradation. These unique properties of PFAS have made them useful in a variety of consumer products, including firefighting foams and fireproofing materials, non-stick coatings for cookware, waterproofing agents, surfactants, cosmetics, inks, insulation for electrical wiring, and pesticides.[5,6] The wide usage of PFAS and their recalcitrance has led to their detection in surface water, groundwater, sediments, soils, and biota.[28-30] Furthermore, evidence of harmful toxicological properties of these compounds adds to the environmental concern.[9-11] As a result, researchers and regulators have taken a keen interest in developing mitigation strategies and regulatory guidelines for PFAS. To date, regulatory guidelines for PFAS have only been proposed for drinking water, but these guidelines have become increasingly stringent as further information about these compounds has come to light. For example, the US Environmental Protection Agency (US EPA) has recently revised PFAS recommended levels in drinking water for perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) to 0.02 ng/L and 0.004 ng/L respectively.[37,38] Likewise, Health Canada has announced its intention to establish an objective of 30 ng/L for the combined total of all PFAS detected in drinking water.[72,73] As the regulations for drinking water become more rigorous, it can be expected that regulations for other environmental matrices may follow suit. Therefore, quantifying PFAS discharges to the environment will become increasingly important.

Biosolids are the products obtained from the sludge handling systems of wastewater treatment plants (WWTPs). These systems consist of a train of processes where the sludges generated in wastewater treatment are processed and stabilized. The product biosolids are typically nutrient-rich and are often applied to agricultural land as a soil amendment. However, some studies have shown that the presence of PFAS in soils can increase upon the application of biosolids.[41-44] This is consistent with reports of amounts of PFAS in biosolids.[15-21,74-76] For instance, the average PFOA

concentration in biosolids collected from WWTPs around the US ranged from 20 µg/kg to 320 µg/kg, whereas the concentration of PFOS varied between 13 µg/kg and 530 µg/kg. In addition to PFOS and PFOA, perfluorodecanoate (PFDA) and perfluorododecanoate (PFDoA) have also been detected, with concentrations of up to 990 µg/kg and 500 µg/kg, respectively.[15,19,41,76,77] In Canada, biosolids have been reported to contain as much as 17,000 µg/kg PFOS and 770 µg/kg PFOA, while perfluorononanoate (PFNA), PFDA, PFDoA, perfluoroundecanoate (PFUdA) and perfluorooctane sulfonamide (PFOSA) concentrations ranged between 10 µg/kg and 623 µg/kg.[21,24,78,79] In addition to these PFAS, other compounds such as perfluorobutanoate (PFBA), perfluoropentanoate (PFPeA), perfluorotetradecanoate (PFTeDA), perfluorobutane sulfonate (PFBS), perfluoropentane sulfonate (PFPeS), perfluorohexane sulfonate (PFHxS), N-methyl perfluorooctane sulfonamido acetate (MeFOSAA) and N-ethyl perfluorooctane sulfonamido acetate (EtFOSAA) have been reported at concentrations of up to 150 µg/kg.

Given the ubiquity of PFAS in biosolids, information on the factors that influence their fate in sludge handling systems is needed to inform decision-making regarding the development of effective approaches to handle and dispose of biosolids in the future. However, most previous studies only reported the levels of PFAS present in the final product (i.e., biosolids), with only a limited number of studies investigating the fate of PFAS in sludge handling processes.[24,25] Lakshminarasimman et al. (2021) investigated the behavior of PFAS in pelletization, alkaline stabilization, anaerobic digestion, and aerobic digestion.[24] Their findings showed that pelletization and alkaline stabilization led to an overall increase in total PFAS mass flows by 19% and 99%, respectively. In addition, aerobic and anaerobic digestion systems exhibited both PFAS formation and removal, with individual PFAS mass flows either increasing (278%) or decreasing (71%). In another study, Li et al. (2022) studied aerobic digestion of anaerobically digested sludge and observed a substantial increase in the concentrations of PFPeA (by 571%), PFHxA (by 253%), PFBS (by 340%) and PFOS (by 91%), while some precursors (6:2 fluorotelomer sulfonate (6:2 FTS), EtFOSAA and MeFOSAA) showed no significant reduction.[25] The increase in PFAA concentrations observed in both studies was attributed to the biotransformation of perfluoroalkyl phosphate esters (PAP) during anaerobic digestion. It is noted that while these two studies have examined PFAS fate for individual units within sludge handling processes, they did not comprehensively evaluate PFAS fate through an entire train of processes. Additional research is needed to generate information on the fate of PFAS through entire sludge handling systems,

because this insight will inform decision-making for potential process modifications aimed at mitigating PFAS accumulations in biosolids.

The objective of this study was to investigate the fate of PFAS through sludge handling systems. To this end, sampling campaigns were carried out at two WWTPs in Canada that employed both distinct and similar sludge handling processes. Thickening, fermentation, anaerobic digestion, and dewatering processes were collectively studied between the two plants. Solids balances were conducted. Subsequently, PFAS mass flows were estimated using measured solid and liquid concentrations. The fate of PFAS was assessed by comparing the mass flows of PFAS in and out of processes.

3.2. Materials and Method

3.2.1. Chemicals and materials

The details of the mass labelled and native PFAS standards, adsorbents and all other chemicals were provided in Section 2.2.1. Solid phase extraction (SPE) cartridges (weak anion exchange (WAX), 6 cc/150 mg) used for liquid sample extractions were purchased from Waters, Canada.

3.2.2. Sample collection

This study involved sampling campaigns at two WWTPs sludge handling facilities in Canada. The sludge handling facilities are denoted from this point forward as WWTP-A and WWTP-B. WWTP-A is located in a city with a population of 312,000 people, while WWTP-B is one of the WWTPs that treat wastewater in a metropolitan region with a population of approximately 890,000 people. At WWTP-A (Fig. 3-1), the waste activated sludge (WAS) is initially handled by gravity drum thickening (GDT), then the primary sludge (PS) and the thickened waste-activated sludge (TWAS) were subjected to anaerobic digestion (AD) and dewatering (DW). The sludge handling system at WWTP-B employs a GDT and two fermenter units to process PS, and a dissolved air flotation (DAF) unit to thicken WAS. Subsequently, the solid streams are blended (SBT) prior to AD and DW. Fig. 3-1 presents sampling points where solid and liquid samples were taken. All other sample acronyms can be found in the glossary. Samples were initially categorized as solid or liquid based on the visual inspection of the suspended solids levels that settled at the bottom of

the sampling bottle. The details and significance of this categorization to the selection of analytical methods for PFAS will be discussed in Section 3.2.5.

The sampling campaign in WWTP-A was conducted over a duration of four weeks. During the first week, sampling took place on three consecutive days to characterize daily variations in PFAS concentrations. For the subsequent three weeks, sampling was conducted one day per week to characterize weekly concentration variations. In WWTP-B, sampling was carried out over a period of three weeks, with sampling occurring on a single day each week. Sampling in WWTP-B was shorter due to plant constrictions. On each sampling day and at each sampling point, three subsamples were collected at 3-hour intervals over a period of 6 hours. These subsamples were then blended to create one composite sample. For free-flowing streams, 350 mL samples were collected into a 500 mL HDPE bottle by direct filling from the process control taps by first flushing the taps to get representative samples. Cake samples were scooped due to the high solids content. The details of composite sampling are described in the subsequent section.

Powder-free nitrile gloves were used during sampling, and bottles and scoops were precleaned using LCMS-grade water and methanol to prevent cross-contamination of samples. The bottles were placed into separate Ziploc bags and then stored in coolers for transportation.

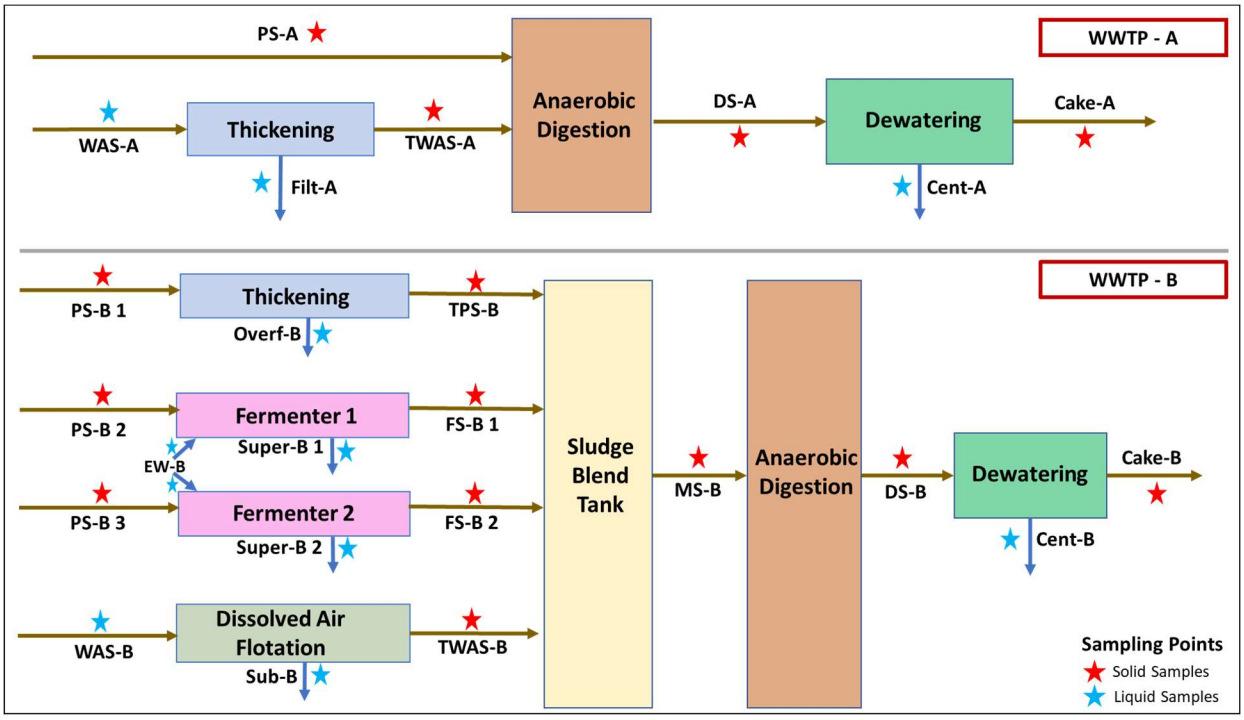


Figure 3-1. The sludge handling systems of WWTP-A and WWTP-B and sampling points.

3.2.3. Sample handling

All samples were transported to the University of Waterloo on ice. The subsamples collected from WWTP-A were transported the same day, while WWTP-B subsamples were shipped by courier with a transit time of 2 days. Upon receipt, solid subsamples were composited by mixing in a clean 1,000 mL PP beakers, and then the composited sample was transferred back to a 500 mL HDPE bottle for storage. Liquid samples were composited and stored in 1,000 mL HDPE bottles. After compositing, approximately 50 mL of sample was used for the characterization of pH, total solids (TS) and volatile solids (VS). The samples categorized as liquid were also tested for total suspended solids (TSS). The remaining portions of liquid samples were either stored at 2 – 4 ºC and extracted the following day or stored at -20 ºC until extraction to minimize biological activity. The remaining portions of solid samples were stored at -20 ºC prior to analysis. Prior to analysis, solid samples were freeze-dried, homogenized using a mortar and pestle, and sieved through a 2 mm stainless steel mesh. The homogenized and sieved samples were stored at 2 – 4 ºC until extraction.

3.2.4. Sample characterization

3.2.4.1.TS and VS

TS and VS measurements were conducted according to Standard Method (SM) 2540B and SD 2540E.[80] Briefly, 1 to 2 g of a sample was placed in an aluminum pan (conditioned for one hour at 550 ºC) with a known weight. The pan containing the sample was then placed in a 105 ºC oven for at least 2 hours or until the last two weight measurements were found to be the same. The increase in weight over the empty dish represented TS. For VS analysis, the same pan was placed into a 550 ºC furnace, and the weight was measured periodically until no change in weight was recorded. The reduction in weight represented the VS. All tests were performed in triplicates to assess analytical variability.

3.2.4.2. TSS and VSS

TSS and VSS of liquid samples were measured according to SM 2540D and SM 2540E.[80] Briefly, TSS was measured by filtering 10 mL of the sample through a 1.6 µm glass microfiber filter (Pall Corporation). The filter had been dried (at least for one hour at 550 ºC) and its weight was recorded prior to filtration. The filter and pan were then placed into a 105 ºC oven and the

weight was measured periodically until no change in weight was recorded. The increase in weight relative to the initial weight represented TSS. For VSS analysis, the same filter was placed into a 550 ^{o}C furnace for at least an hour and reweighed after cooling. The reduction in weight represented VSS. All tests were performed in triplicates to assess analytical variability.

3.2.5. PFAS extraction and analytical procedures

For the categorization of the samples as either liquid or solid, all samples were visually inspected to determine the level of suspended solids that settled at the bottom of the sampling bottles. The cut-off level was determined as 1/5 (the ratio of the length of the settled suspended solids in 500 mL HDPE bottle to the length of the total sample). For the samples collected from the streams marked with a (★) in Fig. 3-1, it was determined that these samples contained more than the cut-off level of suspended solids which were too much to be analyzed by the methods that are used to quantify PFAS in liquid samples, but enough to be dried and analyzed by the methods that used to quantify PFAS in solid samples. Thus, these samples were categorized as solid samples, and the concentration of PFAS in these samples were quantified using the analytical method developed in Chapter 2. For the samples collected from the streams marked with a (★), while visual inspections of the level of settled suspended solids suggested that these samples contained less suspended solids (lower than 1/5 cut-off level), the measured TSS values of these samples were as high as 7,000 mg/L (the TSS concentration of each sample will be presented in Section 3.3 "Results and Discussion"). The high TSS values suggest that PFAS in these samples cannot be analyzed by US EPA Method 1633, because this method is only intended for the quantification of PFAS in wastewater samples having a TSS value lower than 250 mg/L. (According to US EPA Method 1633, if the TSS concentration is higher than 250 mg/L, SPE cartridges used to pre-concentrate PFAS will be clogged). [27] To enable the analysis of PFAS in these samples, US EPA Method 1633 was modified to reduce the number of suspended solids in each sample, following the procedure depicted in Fig. 3-2. Briefly, an unfiltered 200 mL sample was transferred to an ultracentrifuge bottle and spiked with isotopically labelled PFAS surrogates (2 ng each). The spiked sample was then centrifuged for one hour at 40,000 RCF. Subsequently, the supernatant (~190 mL) was decanted into a clean 250 mL HDPE bottle. A volume of 10 ml of 0.1% methanolic ammonium hydroxide solution was added to the ultracentrifuge tube containing the remaining solid-liquid mixture and the suspension was then shaken by hand prior to

transferring to a 15 mL PP tube and vortexing to extract PFAS adsorbed on the solids. The solution was then centrifuged for 15 minutes at 3,100 RCF, and the supernatant was combined with the 190 mL supernatant.

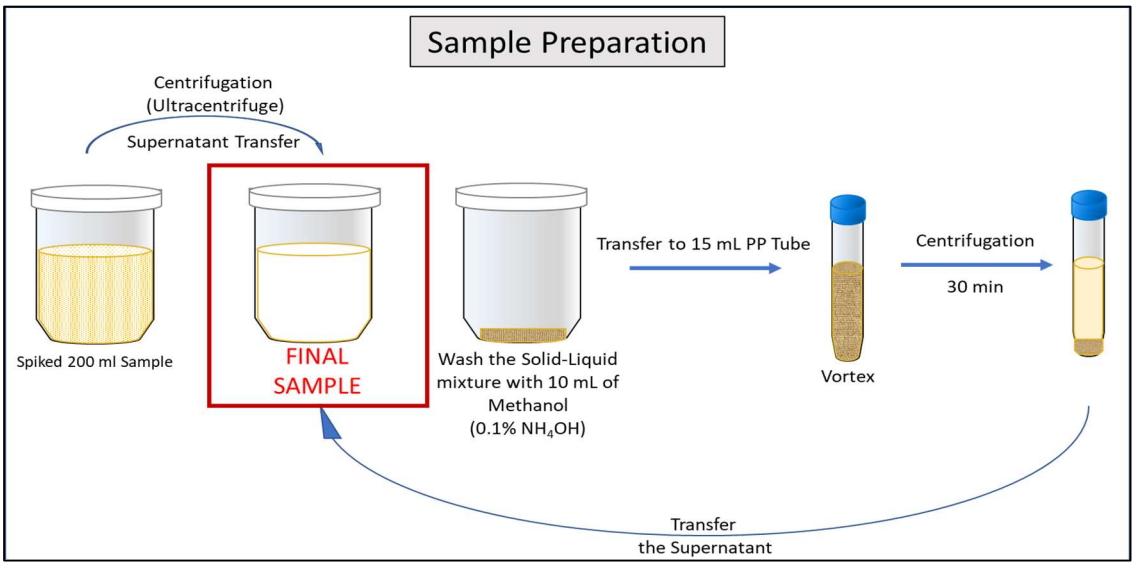


Figure 3-2. Details of sample preparation step in analytical workflow employed liquid samples.

Subsequently, the combined supernatant was subjected to SPE extraction and cleanup (Fig. 3-3). This part of the method consisted of four steps. In *Step 1*, the combined supernatant was passed through an SPE cartridge (WAX, 6 cc/150 mg) that had been conditioned with 1% NH_4OH in methanol and 0.1 M HCOOH in water. In *Step 2*, the loaded cartridge was fully dried under vacuum. In *Step 3*, the analytes retained by the SPE cartridge were eluted with 8 mL of 1% methanolic ammonium hydroxide solution. In *Step 4*, the eluate was evaporated to 0.5 mL and then transferred to a microcentrifuge tube containing 40 mg ENVI-Carb and 25 µL glacial acetic acid for clean-up. The mixture was vortexed for one minute and centrifuged for 15 minutes at 22,000 RCF. Finally, 0.4 mL of supernatant was transferred to a 1.5 mL polypropylene vial and mixed with 0.4 mL water, resulting in a final sample of 50/50 v/v water/methanol that was analyzed a Shimadzu 8030 LC/MS/MS. Details of the instrument operating conditions and recovery calculations were described in Section 2.3.4.

The QA/QC procedures for solid samples were similar to those described in Chapter 2. For liquid samples, QA/QC procedures consisted of the analysis of one LC/MS/MS grade water sample (200 mL) per ten field samples to determine if the reagents and materials were free of target

PFAS analytes. Also, 7 LC/MS/MS grade water samples (200 mL/sample) were spiked with native PFAS (0.16 ng each) and isotopically labelled PFAS (1 ng each), and the spiked samples were extracted and analyzed. The standard deviation (S) of the analyzed PFAS concentrations was calculated based on these 7 samples, and LOQ for each PFAS was calculated as LOQ = 10 × S.

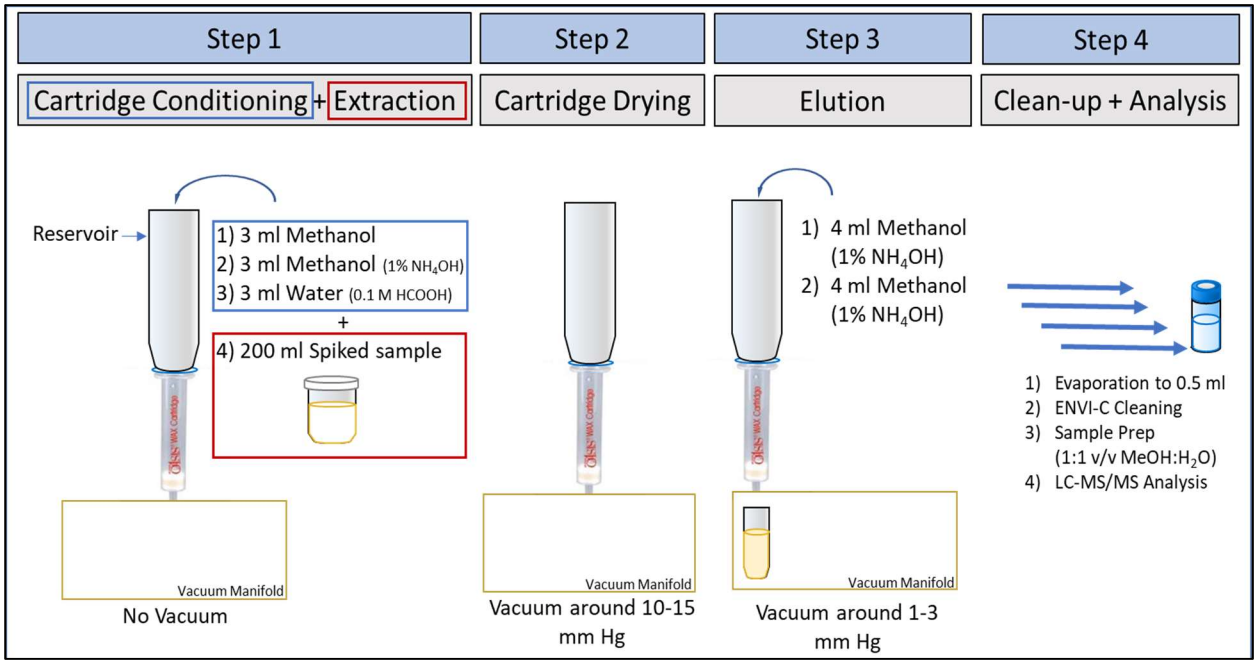


Figure 3-3. Analytical workflow employed liquid samples.

3.2.6. Mass balance calculations

PFAS mass balances were calculated to obtain information that could be employed to understand their fate through the sludge handling systems. The mass balances were based on the flow data collected from the full-scale facilities and the measured PFAS concentrations. The daily recorded flow data (m^3/day) of each stream was provided by the plants over the sampling period (i.e., one month). The flow data of the liquid recycling streams (i.e., filtrate, subnatant, supernatant, centrate) were not available in both plants. For these streams, the flows were calculated as the difference between the measured inlet and outlet flows of each process's unit. Average and standard deviations of the flow data were calculated from this data set. These values were then used in the calculation of solids balances and PFAS mass flows.

Solids mass flows for each stream were calculated using Equation 1.

$$M_{S,avg} = Q_{avg} \times TS_{avg} \times S_g \times 10^3 \quad (1)$$

where $M_{S,\,avg}$ is the average solids mass flow (kg/day), Q_{avg} is the average flow (m^3/day), TS_{avg} is the average total solid (kg/kg) value measured in the lab, S_g is the specific gravity and 10^3 is the density of water (kg/m^3). 1.01, 1.02 and 1.07 were used for S_g of liquid samples, sludges and cake, respectively.[50]

The standard deviations of solids flows were calculated using Equation 2.[81]

$$S_{MS} = \sqrt{\left(\frac{S_Q}{Q_{avg}}\right)^2 + \left(\frac{S_{TS}}{TS_{avg}}\right)^2} \times M_{S,avg} \quad (2)$$

where S_{MS} is the standard deviation of solids mass flow, S_Q is the standard deviation of flow data and S_{TS} is the standard deviation of the total solids test.

The difference between mass flows entering and leaving each process was then calculated and tested using paired t-tests at a 95% confidence level to determine if the solids mass balances could be closed.

The average mass flow of each PFAS compound in liquid and solid streams was calculated using Equations 3-a and 3-b respectively.

$$M_{PFAS-L,avg} = Q_{avg} \times C_{PFAS-\ ,avg} \quad (3\text{-a})$$

$$M_{PFAS-S,avg} = M_{S,avg} \times C_{PFAS-S,avg} \quad (3\text{-b})$$

where $M_{PFAS\text{-}L,\,avg}$ and $M_{PFAS\text{-}S,\,avg}$ are the average PFAS mass flow (mg/day) for liquid and solid samples, respectively, and $C_{PFAS\text{-}L,\,avg}$ and $C_{PFAS\text{-}S,\,avg}$ is the average concentrations of PFAS for liquid (mg/m^3) and solid (mg/kg) samples, respectively.

The standard deviations of the PFAS mass flow in liquid and solid streams were calculated by promulgating the variability of the flow and PFAS concentrations using Equations 4-a and 4-b, respectively.[81]

$$S_{MPFAS-L} = \sqrt{\left(\frac{S_Q}{Q_{avg}}\right)^2 + \left(\frac{S_{CPFAS-L}}{C_{PFAS-L,avg}}\right)^2} \times M_{PFAS-\ ,avg} \quad (4\text{-a})$$

$$S_{MPFAS-S} = \sqrt{(\frac{S_{MS}}{M_{S,avg}})^2 + (\frac{S_{CPFAS-S}}{C_{PFAS-S,avg}})^2} \times M_{PFAS-\ ,avg} \qquad (4\text{-b})$$

where $S_{MPFAS-L}$ and $S_{MPFAS-S}$ are the standard deviation of PFAS mass flows for liquid and solid samples, respectively. $S_{CPFAS-L}$ and $S_{CPFAS-S}$ are the standard deviations of PFAS concentrations for liquid and solid samples, respectively.

Mass balance closure was assessed for each individual PFAS in each process by comparing the mass flows in and out with paired student t-tests at a 95% confidence level.

3.3. Results and Discussion

3.3.1. Flow and solids balances

Solids mass balances were calculated to initially assess the quality of data that was meant to be the basis for the subsequent PFAS mass balance assessments. Volumetric flow data and solids content for the various streams in the sludge handling systems of WWTP-A and WWTP-B are presented in Table 3-1 and Table 3-2. The TS mass flows, calculated based on Eq. 1, are presented in Table 3-3. Based on the results of the paired t-tests ($p > \alpha = 0.05$), it was determined that statistical differences between the influent and effluent TS were only observed for AD in both WWTPs. The apparent destruction of total solids in the anaerobic digesters was approximately 30% and, which was within the range of typical values for such systems.[23] The consistent mass balance closures for TS in the remaining processes provided confidence in the use of the flow and solids content data in the subsequent PFAS mass flow analyses.

Table 3-1. Flows (average of 30 daily flow data recorded in WWTP-A and WWTP-B).

			Flow (Avg ± Std Dev) (m^3/day)	
			In	Out
WWTP - A	Thickening	WAS-A	1,390 ± 50	
		TWAS-A		140 ± 10
		Filt-A		1,250 ± 42
	Anaerobic Digestion	PS-A	230 ± 12	
		TWAS-A	140 ± 10	
		DS-A		360 ± 23
	Dewatering	DS-A	360 ± 23	
		Cake-A		30.1 ± 1
		Cent-A		350 ± 11
WWTP - B	Dissolved Air Flotation	WAS-B	6,946 ± 120	
		Sub-B		5,689 ± 91
		TWAS-B		1,202 ± 52
	Thickening	PS-B 1	3,378 ± 112	
		Overf-B		2,750 ± 127
		TPS-B		733 ± 29
	Fermentation 1	PS-B 2	2,725 ± 99	
		EW-B	447 ± 13	
		FS-B 1		317 ± 15
		Super-B 1		2,436 ± 83
	Fermentation 2	PS-B 3	1,697 ± 77	
		EW-B	1,604 ± 30	
		FS-B 2		455 ± 36
		Super-B 2		2,917 ± 210
	Blending Tank	TWAS-B	1,202 ± 52	
		TPS-B	733 ± 29	
		FS-B 1	317 ± 15	
		FS-B 2	455 ± 36	
		MS-B		2,738 ± 103
	Anaerobic Digestion	MS-B	2,738 ± 103	
		DS-B		3,387 ± 108
	Dewatering	DS-B	2,348 ± 92	
		Cake-B		230 ± 28
		Cent-B		1,600 ± 101

Table 3-2. Solids concentrations (n = 18 for WWTP-A (triplicate tests and 6 different samples collected in 4 weeks) and n = 9 for WWTP-B (triplicate tests and 3 different samples collected in 3 weeks). VS (%) represents the % fraction of TS.

		TS (%)	VS (%)	TSS (mg/L)	VSS (mg/L)	pH
WWTP-A	WAS-A	0.5 ± 0.1	60.2 ± 1.0	3,900 ± 195	3,040 ± 50	7.1 ± 0.1
	TWAS-A	3.0 ± 0.1	75.3 ± 0.3			6.9 ± 0.1
	Filt-A	0.2 ± 0.0	79.9 ± 1.7	1,400 ± 30	1,106 ± 76	7.1 ± 0.1
	PS-A	3.4 ± 0.3	79.4 ± 1.8			6.4 ± 0.2
	DS-A	2.4 ± 0.2	65.1 ± 1.1			7.3 ± 0.1
	Cake-A	25.1 ± 0.4	68.1 ± 0.8			7.5 ± 0.1
	Cent-A	0.1 ± 0.0	67.4 ± 0.4	600 ± 30	456 ± 32	7.6 ± 0.1
WWTP-B	PS-B 1	1.1 ± 0.1	85.2 ± 2.1			6.2 ± 0.1
	Overf-B	0.1 ± 0.0	83.0 ± 2.2	1,100 ± 50	902 ± 54	6.8 ± 0.1
	TPS-B	4.7 ± 0.3	80.9 ± 1.6			5.6 ± 0.1
	PS-B 2	0.6 ± 0.1	84.3 ± 2.8			6.9 ± 0.1
	EW-B					6.6 ± 0.1
	FS-B 1	4.5 ± 0.2	79.8 ± 2.1			5.5 ± 0.1
	Super-B 1	0.1 ± 0.0	79.7 ± 1.9	1,200 ± 50	966 ± 97	6.3 ± 0.1
	PS-B 3	1.0 ± 0.1	85.1 ± 0.9			6.4 ± 0.1
	Super-B 2	0.1 ± 0.0	76.1 ± 1.5	1,200 ± 40	941 ± 83	6.2 ± 0.1
	FS-B 2	3.1 ± 0.1	80.3 ± 1.6			5.3 ± 0.2
	WAS-B	0.8 ± 0.1	76.7 ± 1.6	6,900 ± 70	5,424 ± 131	5.8 ± 0.1
	Sub-B	0.0 ± 0.0	76.6 ± 2.1	100 ± 10	77 ± 6	6.5 ± 0.1
	TWAS-B	4.3 ± 0.3	76.3 ± 1.4			5.5 ± 0.2
	MS-B	4.1 ± 0.2	78.6 ± 0.5			5.6 ± 0.2
	DS-B	2.5 ± 0.1	67.6 ± 1.2			7.5 ± 0.1
	Cake-B	20.4 ± 0.9	69.7 ± 0.9			7.7 ± 0.1
	Cent-B	0.2 ± 0.1	70.0 ± 0.5	2,200 ± 90	1,538 ± 68	7.5 ± 0.1

Table 3-3. Mass flows of total solids.

			Average ± Std Dev. (kg/day)		
			In	Out	p Values*
WWTP - A	**Thickening**	WAS-A	6,255 ± 186		0.26
		TWAS-A		4,214 ± 107	
		Filt-A		1,874 ± 51	
	Anaerobic Digestion	PS-A	7,883 ± 519		0.00
		TWAS-A	4,214 ± 107		
		DS-A		8,743 ± 415	
	Dewatering	DS-A	8,743 ± 415		0.12
		Cake-A		7,540 ± 303	
		Cent-A		1,025 ± 89	
WWTP - B	**Dissolved Air Flotation**	WAS-B	52,330 ± 3,323		0.70
		Sub-B		398 ± 21	
		TWAS-B		51,686 ± 2,617	
	Thickening	PS-B 1	37,158 ± 2,133		0.56
		Overf-B		2,628 ± 199	
		TPS-B		34,167 ± 2,299	
	Fermentation 1	PS-B 2	17,168 ± 1,750		0.81
		EW-B			
		FS-B 1		14,265 ± 733	
		Super-B 1		3,059 ± 195	
	Fermentation 2	PS-B 3	17,312 ± 1,085		0.84
		EW-B			
		FS-B 2		13,919 ± 1,117	
		Super-B 2		3,403 ± 338	
	Blending Tank	TWAS-B	51,686 ± 2,617		0.12
		TPS-B	34,167 ± 2,299		
		FS-B 1	3,059 ± 195		
		FS-B 2	3,403 ± 338		
		MS-B		112,258 ± 3,800	
	Anaerobic Digestion	MS-B	112,258 ± 3,800		0.00
		DS-B		82,982 ± 3,123	
	Dewatering	DS-B	57,526 ± 2,100		0.57
		Cake-B		46,896 ± 1,988	
		Cent-B		11,664 ± 703	

3.3.2. PFAS fate analysis

3.3.2.1. QA/QC

The results of method blanks were initially analyzed to assess the level of background contamination that could be expected in the laboratory. Based on the analysis of the blank extractions, it was determined that the reagents and materials utilized in this study were PFAS-free, except for 6:2 FTS, which was present in several blanks. Attempts to identify and eliminate the 6:2 FTS cross-contamination sources were not successful. As such, 6:2 FTS was excluded from the analyte report list.

LOQ values were estimated for both the liquid and solid analytical methods to establish the minimum concentrations that could be quantified. The LOQ values for the target PFAS in solid extractions were reported in Chapter 2 (Table 2-2). The LOQ for the PFAS compounds in the liquid extractions are presented in Table 3-4 and varied between 0.5 and 3.0 ng/L. The LOQ values were deemed to be sufficiently low to support the subsequent mass balance analyses.

Table 3-4. LOQ of the liquid sample analytical method. The LOQ of 6:2 FTS was not reported due to cross-contamination in blank samples.

	LOQ
	(ng/L)
PFBA	1.0
PFPeA	3.0
PFHxA	0.5
PFHpA	0.5
PFOA	0.5
PFNA	0.5
PFDA	0.5
PFUdA	0.5
PFDoA	0.5
PFTrDA	1.0
PFTeDA	1.0
PFBS	0.5
PFPeS	0.5
PFHxS	0.5
PFHpS	0.5
PFOS	0.5
PFNS	1.0
PFDS	1.0
4:2 FTS	1.5
6:2 FTS	-
8:2 FTS	1.5
FOSA	0.5
MeFOSAA	0.5
EtFOSAA	0.5

The recovery of isotopically labelled PFAS in samples was determined as an additional measure of data quality. Recovery values obtained from liquid and solid sample extractions are presented in Fig. 3-4. The vast majority of the recovery values fell within the range of 50% to 150%, which were similar to the values reported in other studies.[16,20,24,30,32-34,45] The recovery values for M2-4:2FTS, M2-6:2FTS and M2-8:2FTS were found to be up to 250% for liquid samples and 500% for solid samples. These high recovery values were attributed to the matrix facilitating ionization enhancement. Previous studies have also observed high recovery values for the same compounds.[41,42,50,51,69,70] However, the high recovery values have not impacted the target PFAS concentration calculations as native to mass-labelled PFAS ratios were used in the calibration curve and calculations.

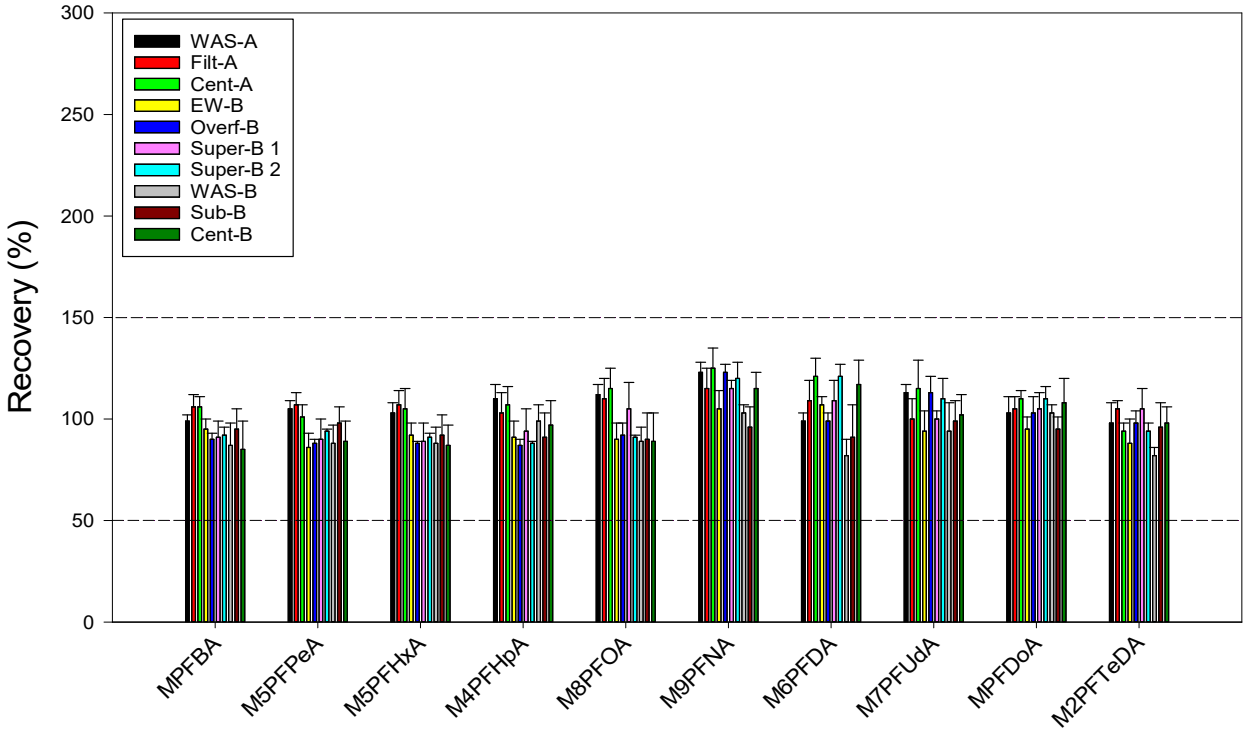


Figure 3-4-A. Mean recovery values (n=18 for WWTP-A (triplicate extractions and 6 different samples collected in 4 weeks) and n=9 for WWTP-B (triplicate tests and 3 different samples collected in 3 weeks) of mass-labelled PFCA of liquid samples.

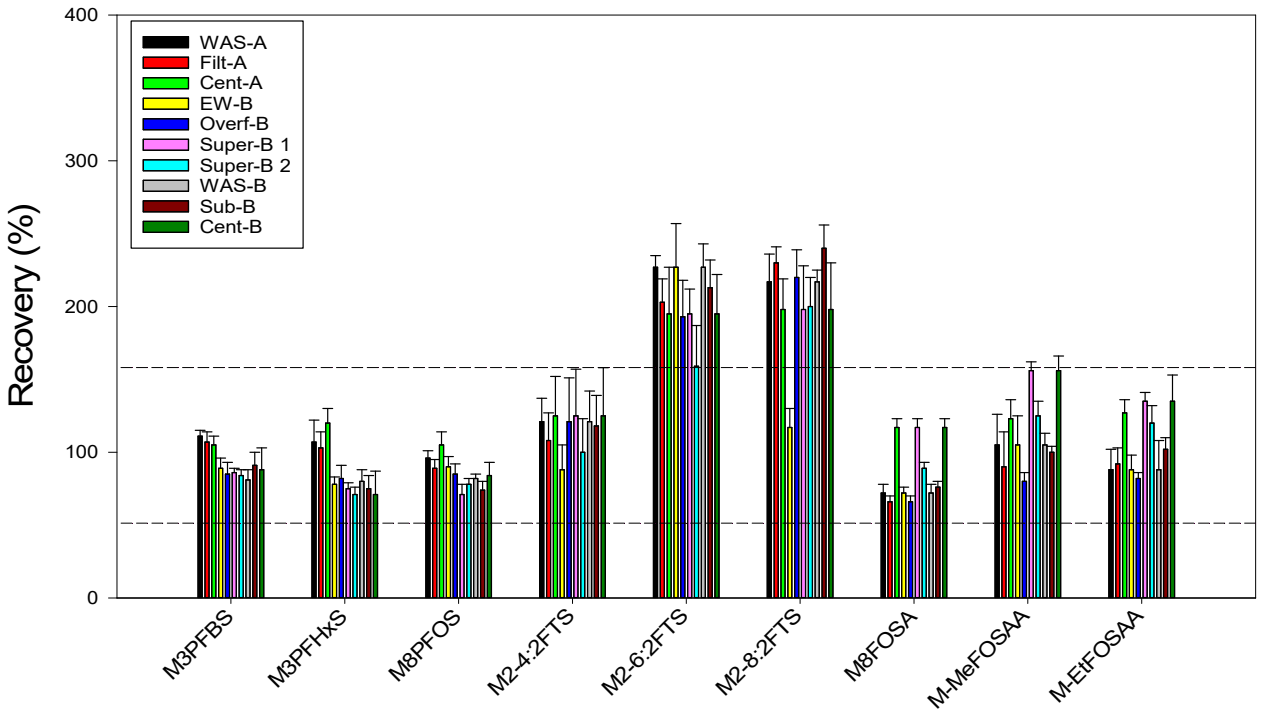


Figure 3-4-B. Mean recovery values (n=18 for WWTP-A (triplicate extractions and 6 different samples collected in 4 weeks) and n=9 for WWTP-B (triplicate tests and 3 different samples collected in 3 weeks) of mass-labelled PFSA and precursors of liquid samples.

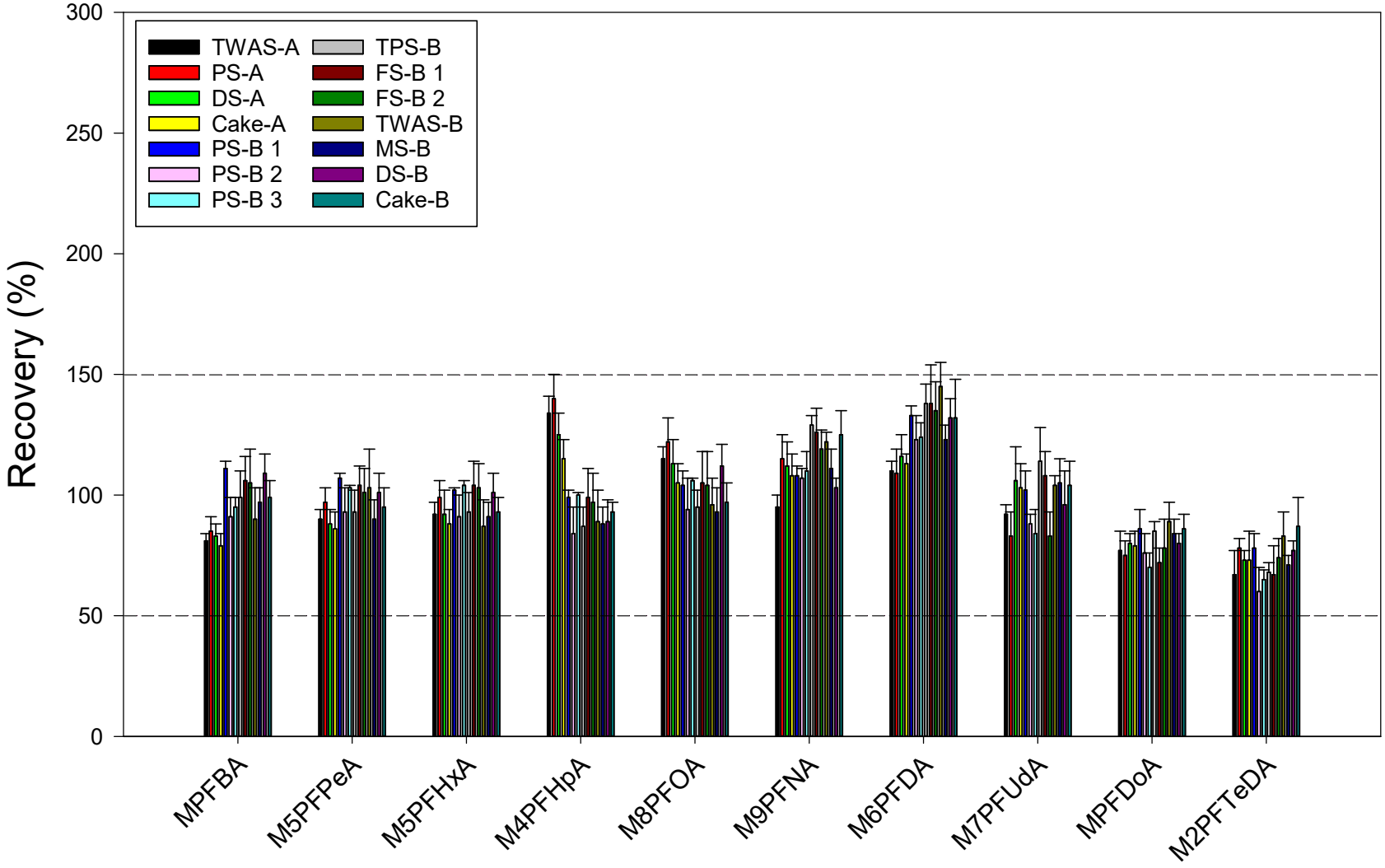


Figure 3-4-C. Mean recovery values (n=18 for WWTP-A (triplicate extractions and 6 different samples collected in 4 weeks) and n=9 for WWTP-B (triplicate tests and 3 different samples collected in 3 weeks) of mass-labelled PFCA of solid samples.

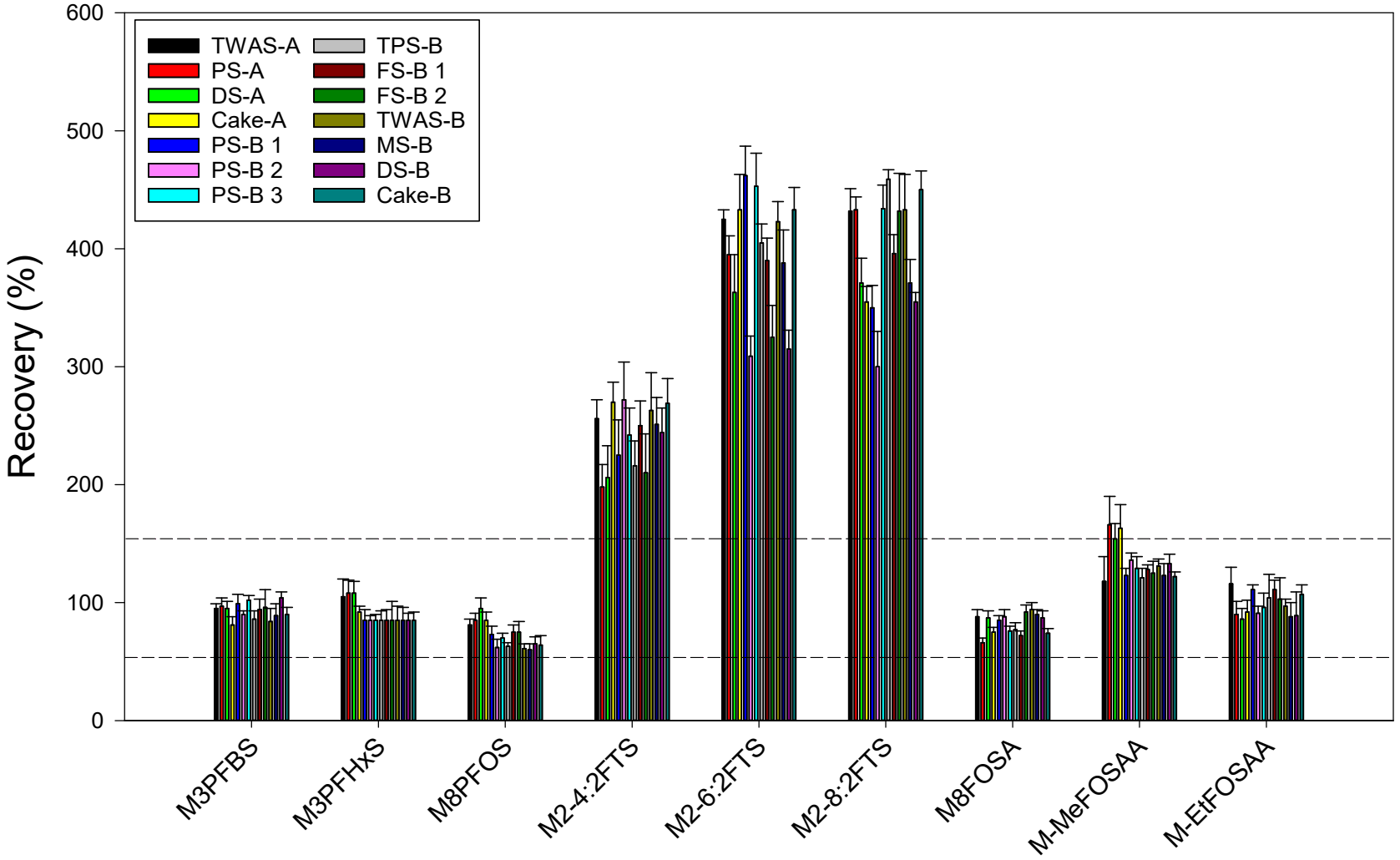


Figure 3-4-D. Mean recovery values (n=18 for WWTP-A (triplicate extractions and 6 different samples collected in 4 weeks) and n=9 for WWTP-B (triplicate tests and 3 different samples collected in 3 weeks) of mass-labelled PFSA and precursors of solid samples.

3.3.2.2. PFAS concentrations

Of the 24 PFAS analytes, 15 were detected in most samples. PFTrDA, PFTeDA, PFPeS, PFHpS, PFNS, PFDS, 4:2 FTS and 8:2 FTS were not detected in any samples and hence are not subsequently discussed. The concentrations of the target PFAS in the liquid and solid samples (Table 3-5 and 3-6) were initially examined to characterize their relative presence in the samples. Seven of the 15 detected PFAS analytes (PFBA, PFHxA, PFOA, PFDA, PFBS, PFOS and MeFOSAA) were selected for detailed presentation and discussion. These compounds represent different functional groups, chain lengths, and degrees of fluorination. The concentrations of these compounds were also compared with data that was available in the literature.

The average individual PFAS concentrations in all solid samples (0.2 and 12.9 µg/kg) (Table 3-5) were examined to establish the level of contamination that was present. Among the analytes, PFOS and PFDA were present at the highest concentrations and reaching up to 12.9 and 8.2 µg/kg, respectively in the DS, TWAS, and Cake samples. Other compounds that were also present at an appreciable level (between 4 and 7 µg/kg) included PFOA, PFHxA, PFDoA and MeFOSAA in the majority same sample types. Other compounds (PFBA, PFPeA, PFHpA, PFNA, PFUdA, PFBS, PFHxS, FOSA and EtFOSAA) were present at lower concentrations (<4 µg/kg) in all solid samples.

The solid sample concentrations were compared between the two WWTPs for the 7 PFAS to assess whether there were differences in the levels of contamination between the two facilities (Fig. 3-5). The concentrations in the inlet streams (PS) of two WWTPs were first compared as they were considered to be indicative of the levels of PFAS entering the WWTPs. Between the two plants, the concentrations of 5 out of the 7 PFAS (PFBA, PFHxA, PFOA, PFDA and PFBS) in the PS of WWTP-B were 2 - 4 times higher than WWTP-A. This difference was attributed to differences in the concentrations of PFAS in the raw wastewater that are impacted by the sewershed characteristics. A comparison of the sewersheds of the two plants was beyond the scope of this work.

The concentrations of PFAS in cake (i.e., biosolid) samples were also examined as they are the product of the sludge handling process train and reflect the levels that could be destined for land application. The concentrations in the cake of WWTP-B were 2 - 3 times higher for four out of the 7 PFAS (PFHxA, PFOA, PFDA, and PFBS). The higher concentrations observed in WWTP-B's

cake samples were consistent with the higher PFAS concentrations in the inlet stream to this WWTP.

The concentrations in the cake samples determined in this study were compared against those reported in previous studies to benchmark the data from this study. Letcher et al. (2020) reported PFOS concentrations between 0.5 and 50 µg/kg, while other individual PFAS concentrations ranged between 1 and 23 µg/kg in various biosolid samples collected from over 20 WWTPs in Canada.[79] Venkatesan and Halden (2013) surveyed composite biosolid samples representing 32 US states and found that PFOS (403 ± 127 µg/kg) was the most abundant while the mean concentrations of other individual PFAS ranged between 2 and 21 µg/kg.[19] It was concluded that the concentrations in the biosolids of this current study were comparable to those reported in the literature.

Table 3-5. PFAS concentrations in solid samples (n = 18 for WWTP-A (triplicate extractions and 6 different samples collected in 4 weeks) and n = 9 for WWTP-B (triplicate tests and 3 different samples collected in 3 weeks).

	Concentration (µg/kg)													
	PS-A	**PS-B 1**	**PS-B 2**	**PS-B 3**	**TWAS -A**	**TWAS -B**	**TPS-B**	**FS-B 1**	**FS-B 2**	**MS-B**	**DS-A**	**DS-B**	**Cake-A**	**Cake-B**
PFBA	0.9 ± 0.1	2.1 ± 0.4	2.6 ± 0.5	1.8 ± 0.3	0.4 ± 0.0	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	1.1 ± 0.2	0.7 ± 0.1	1.9 ± 0.3	1.2 ± 0.2	0.6 ± 0.2	0.5 ± 0.0
PFPeA	2.0 ± 0.1	1.7 ± 0.3	1.7 ± 0.3	2.0 ± 0.4	2.0 ± 0.2	2.6 ± 0.2	1.9 ± 0.3	2.3 ± 0.3	2.0 ± 0.2	2.2 ± 0.4	3.5 ± 0.3	3.5 ± 0.2	2.2 ± 0.2	2.8 ± 0.2
PFHxA	0.6 ± 0.2	4.1 ± 0.7	3.6 ± 0.7	3.9 ± 0.7	2.4 ± 0.4	4.8 ± 0.4	2.7 ± 0.4	2.0 ± 0.3	2.4 ± 0.4	4.7 ± 0.8	3.7 ± 0.4	6.6 ± 0.9	1.9 ± 0.4	6.5 ± 0.5
PFHpA	0.3 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.0	0.5 ± 0.0	0.4 ± 0.1	0.4 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.6 ± 0.1	0.3 ± 0.1	0.7 ± 0.1
PFOA	0.3 ± 0.0	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	3.2 ± 0.5	4.2 ± 0.3	0.9 ± 0.1	0.9 ± 0.1	1.5 ± 0.3	2.4 ± 0.4	2.4 ± 0.2	4.2 ± 0.5	2.0 ± 0.4	5.1 ± 0.4
PFNA	0.2 ± 0.0	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	1.0 ± 0.2	0.5 ± 0.0	0.5 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.0
PFDA	1.1 ± 0.2	1.8 ± 0.3	1.3 ± 0.2	2.1 ± 0.4	8.1 ± 1.2	4.0 ± 0.4	1.8 ± 0.3	1.9 ± 0.2	2.7 ± 0.5	3.6 ± 0.6	8.2 ± 0.2	4.8 ± 0.6	4.6 ± 1.0	6.4 ± 0.5
PFUdA	ND	1.2 ± 0.2	1.4 ± 0.3	2.1 ± 0.4	ND	2.1 ± 0.2	1.4 ± 0.2	2.1 ± 0.3	2.9 ± 0.5	2.0 ± 0.3	ND	2.5 ± 0.3	ND	3.3 ± 0.3
PFDoA	ND	4.0 ± 0.7	2.7 ± 0.5	2.6 ± 0.5	ND	1.6 ± 0.1	4.8 ± 0.7	3.1 ± 0.4	3.0 ± 0.5	3.0 ± 0.5	ND	4.7 ± 0.6	ND	6.0 ± 0.5
PFBS	0.8 ± 0.1	4.3 ± 0.7	3.8 ± 0.7	2.2 ± 0.4	0.4 ± 0.0	0.6 ± 0.1	2.0 ± 0.3	2.2 ± 0.3	2.8 ± 0.5	1.4 ± 0.2	1.4 ± 0.2	2.6 ± 0.3	0.5 ± 0.1	1.9 ± 0.2
PFHxS	0.6 ± 0.1	ND	ND	ND	0.7 ± 0.1	0.6 ± 0.0	ND	ND	ND	0.4 ± 0.1	1.5 ± 0.2	0.7 ± 0.1	1.4 ± 0.3	0.8 ± 0.1
PFOS	2.4 ± 0.4	2.5 ± 0.4	1.5 ± 0.3	1.7 ± 0.3	12.9 ± 0.9	6.4 ± 0.5	2.1 ± 0.3	1.6 ± 0.2	1.8 ± 0.3	4.2 ± 0.5	9.5 ± 0.4	9.4 ± 1.2	10.9 ± 2.0	10.1 ± 0.8
FOSA	1.3 ± 0.2	ND	ND	ND	2.5 ± 0.3	1.2 ± 0.1	ND	ND	ND	0.8 ± 0.1	2.2 ± 0.3	0.5 ± 0.1	2.3 ± 0.3	0.8 ± 0.1
MeFOSAA	1.6 ± 0.4	0.8 ± 0.1	1.7 ± 0.3	1.4 ± 0.3	7.0 ± 1.0	2.4 ± 0.2	1.0 ± 0.1	2.4 ± 0.3	2.5 ± 0.4	2.0 ± 0.3	3.5 ± 0.4	2.3 ± 0.2	3.9 ± 0.5	2.6 ± 0.2
EtFOSAA	3.5 ± 0.7	ND		ND	3.2 ± 0.5	1.9 ± 0.3	ND	ND	ND	1.0 ± 0.2	2.5 ± 0.5	1.0 ± 0.1	3.6 ± 0.4	1.4 ± 0.1

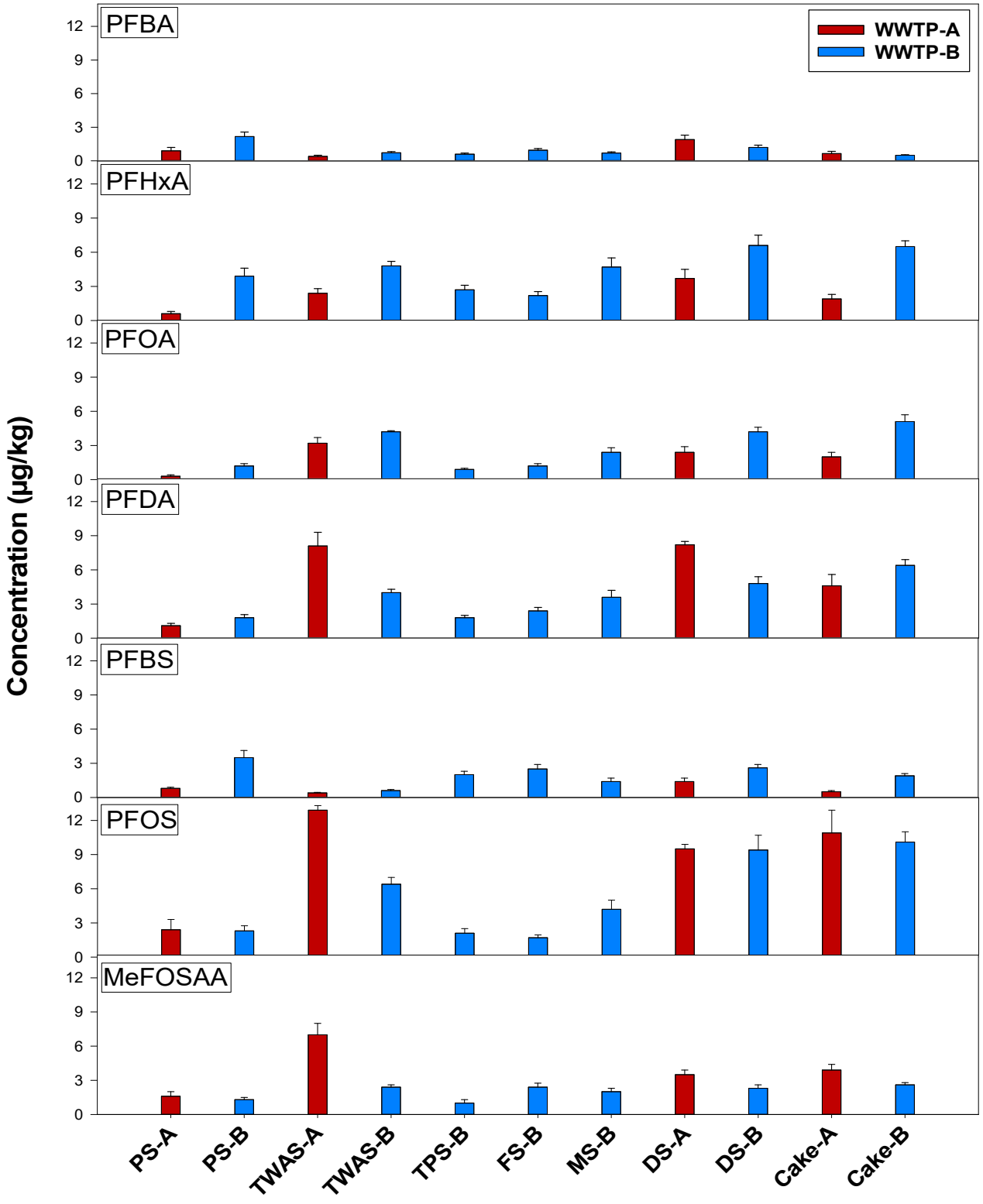


Figure 3-5. Average concentrations (n = 18 for WWTP-A (triplicate extractions and 6 different samples collected in 4 weeks) and n = 9 for WWTP-B (triplicate tests and 3 different samples collected in 3 weeks) of selected PFAS in solid samples.

The individual PFAS concentrations in liquid samples were examined as the sampled streams either represented loading to the sludge handling system (WAS) or are typically recycled to wastewater treatment and hence can contribute to the loading onto wastewater treatment. These concentrations (Table 3-6) ranged between 0.5 and 53 ng/L. PFHxA and PFOS were the most prevalent compounds, with concentrations as high as 42 and 53 ng/L in WAS and Cent samples, respectively. The concentration of PFPeA, PFBS, PFDA, PFBA, PFOA and MeFOSAA ranged between 20 and 30 ng/L in these streams. PFHpA, PFNA, PFUdA, PFDoA, PFHxS, FOSA and EtFOSAA were observed at lower concentrations (<10 ng/L).

The liquid sample concentrations of the selected 7 PFAS were compared between the two WWTPs (Fig. 3-6). The WAS stream concentrations were compared between the plants to assess whether there were differences in this input to the sludge handling systems. The concentrations in the WAS of WWTP-B were, in general, 2 to 5 times higher than those of WWTP-A for 6 out of 7 selected PFAS. This difference in concentrations in WAS streams was attributed to differences in the raw wastewater entering the plants and was consistent with the PS data.

The PFAS concentrations in other liquid streams (Filt, Overf, Sub, Super, Cent) were compared between two WWTPs to obtain insights into compounds that are being recycled back into the wastewater treatment processes (Fig. 3-6). There was no apparent trend in the concentrations of the comparable liquid streams between the WWTPs. It was noted that a majority of PFAAs in the final liquid stream (Cent) exhibited 2 – 4 times higher concentrations as compared to internal liquid streams (Filt, Overf, Sub, Super) in both WWTPs. The subsequently described mass balance analysis was conducted to gain better insight into the fate of PFAS through the individual and overall sludge handling processes.

Table 3-6. PFAS concentrations in liquid samples (n = 18 for WWTP-A (triplicate extractions and 6 different samples collected in 4 weeks) and n = 9 for WWTP-B (triplicate tests and 3 different samples collected in 3 weeks).

	Concentration (ng/L)									
	WAS-A	**WAS-B**	**Filt-A**	**Overf-B**	**Sub-B**	**EW-B**	**Super-B 1**	**Super-B 2**	**Cent-A**	**Cent-B**
PFBA	3.7 ± 0.6	12.5 ± 2.3	3.1 ± 0.2	20.1 ± 3.4	9.1 ± 1.3	2.4 ± 0.3	15.0 ± 2.6	10.5 ± 2.0	29.0 ± 2.0	27.9 ± 2.8
PFPeA	12.0 ± 1.1	16.5 ± 3.0	7.5 ± 0.6	4.3 ± 0.7	4.3 ± 0.6	3.3 ± 0.4	3.1 ± 0.5	2.6 ± 0.5	32.1 ± 2.2	29.1 ± 2.9
PFHxA	22.6 ± 3.1	38.0 ± 3.8	18.7 ± 1.5	29.9 ± 3.0	10.0 ± 0.9	9.1 ± 1.2	11.0 ± 1.9	14.3 ± 2.7	42.1 ± 2.9	27.0 ± 1.4
PFHpA	2.0 ± 0.4	4.5 ± 0.8	1.3 ± 0.1	1.0 ± 0.2	1.0 ± 0.1	0.7 ± 0.1	0.9 ± 0.2	1.2 ± 0.2	2.1 ± 0.1	3.8 ± 0.6
PFOA	20.6 ± 3.2	31.8 ± 5.7	12.6 ± 1.0	5.1 ± 0.9	3.6 ± 0.5	3.1 ± 0.2	5.1 ± 0.9	4.2 ± 0.8	20.1 ± 1.4	19.2 ± 3.3
PFNA	3.4 ± 0.8	3.9 ± 0.7	1.0 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	1.1 ± 0.2	3.9 ± 0.3	3.1 ± 0.5
PFDA	6.6 ± 0.9	33.0 ± 3.6	5.0 ± 0.4	1.7 ± 0.3	1.5 ± 0.2	1.2 ± 0.1	1.2 ± 0.2	1.3 ± 0.2	3.0 ± 0.2	8.9 ± 1.5
PFUdA	ND	15.0 ± 2.7	ND	1.9 ± 0.3	1.1 ± 0.2	0.2 ± 0.0	0.3 ± 0.1	0.6 ± 0.1	ND	2.6 ± 0.4
PFDoA	ND	14.5 ± 2.6	ND	2.1 ± 0.4	1.0 ± 0.1	0.4 ± 0.1	2.3 ± 0.4	1.7 ± 0.3	ND	2.2 ± 0.4
PFBS	7.0 ± 1.1	10.3 ± 1.8	6.7 ± 0.5	32.0 ± 3.2	7.0 ± 1.0	5.5 ± 0.7	7.9 ± 1.3	8.9 ± 1.1	26.2 ± 1.8	29.5 ± 2.9
PFHxS	4.8 ± 0.4	4.4 ± 0.8	3.6 ± 0.3	ND	1.7 ± 0.2	ND	ND	ND	4.0 ± 0.3	5.9 ± 1.0
PFOS	11.9 ± 1.3	53.1 ± 5.8	8.5 ± 0.7	6.2 ± 1.1	3.6 ± 0.5	0.7 ± 0.1	1.9 ± 0.3	2.0 ± 0.4	3.9 ± 0.3	23.5 ± 2.6
FOSA	10.7 ± 1.7	9.3 ± 1.7	1.7 ± 0.1	ND	0.7 ± 0.1	ND	ND	ND	0.9 ± 0.1	1.2 ± 0.2
MeFOSAA	22.1 ± 2.1	17.9 ± 3.2	3.3 ± 0.3	0.2 ± 0.0	0.9 ± 0.1	0.2 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	1.5 ± 0.1	2.4 ± 0.4
EtFOSAA	11.8 ± 0.8	15.8 ± 2.8	2.7 ± 0.2	ND	1.0 ± 0.1	ND	ND	ND	0.5 ± 0.0	2.9 ± 0.5

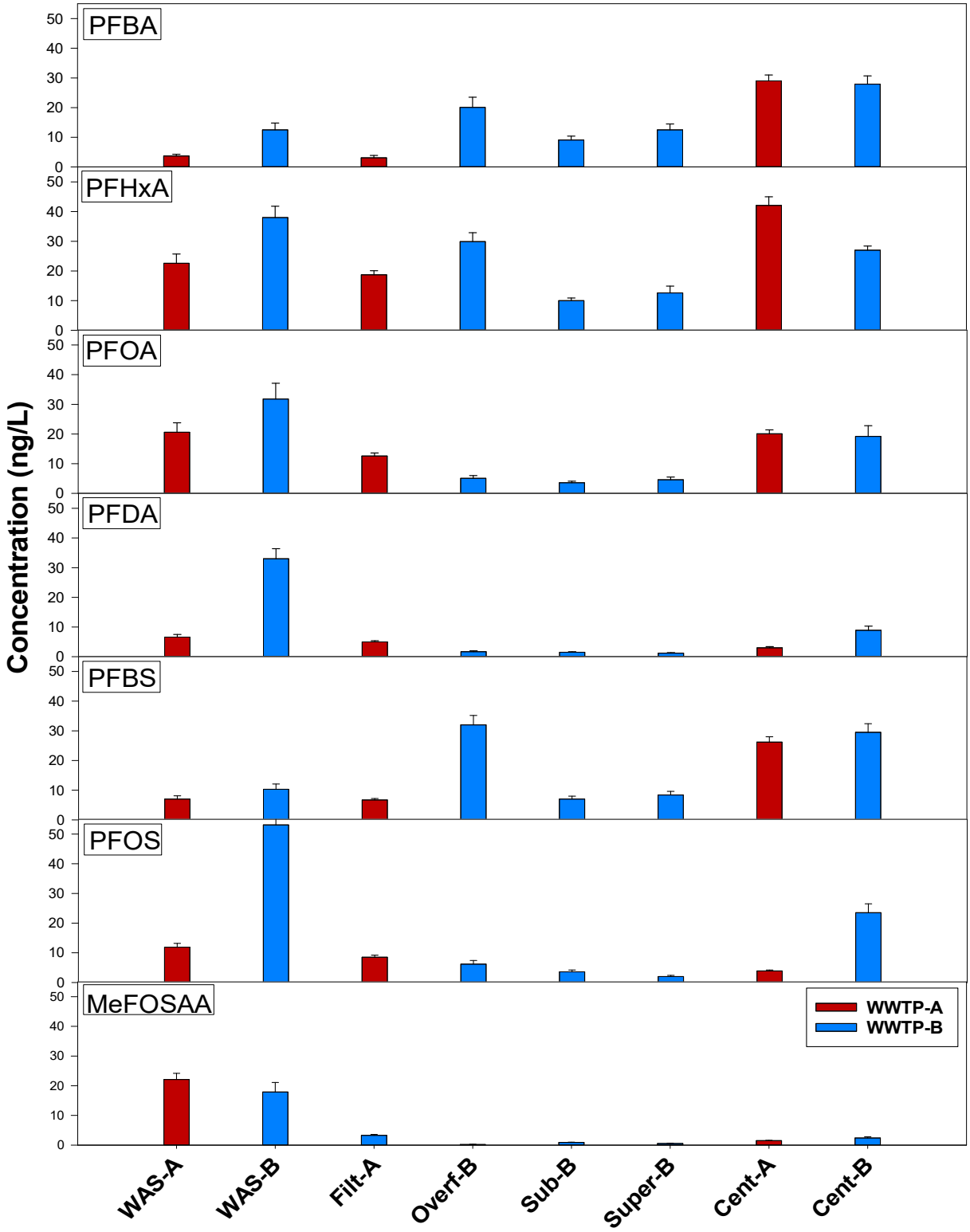


Figure 3-6. Average concentrations (n = 18 for WWTP-A (triplicate extractions and 6 different samples collected in 4 weeks) and n = 9 for WWTP-B (triplicate tests and 3 different samples collected in 3 weeks) of selected PFAS in liquid samples.

3.3.2.3. PFAS mass balances

To assess the mass balance results, the sludge handling processes were divided into four groups based on their underlying operational principles. The first group consisted of liquid-solid separation processes and included the thickening of PS and WAS by GDT, the thickening of WAS by DAF, and the dewatering of DS by DW. The second group involved sludge blending and included the mixing of TWAS, TPS and FS in the SBT. The third group addressed fermentation and included the fermentation and thickening of PS. The fourth group included anaerobic digestion and involved the digestion of blended sludges. The mass flows and balances of the 15 detected PFAS are presented for each group in Figures 3-7, 3-8, 3-9, and 3-10. To assess whether treatment processes statistically impacted the mass flows of PFAS entering and leaving a unit, the paired t-test ($p > \alpha = 0.05$) was performed. The p values are presented in the figures.

3.3.2.3.1. Liquid-solid separation processes

Liquid-solid separation processes (GDT, DAF and DW) are employed to increase the solids concentration and hence reduce the volume of sludges by reducing the volume of free water. GDT uses gravity to settle solids, while DAF encourages solids to float to the surface. DW, on the other hand, uses the force from the rapid rotation of a cylindrical bowl to separate solid phases. GDT and DAF processes are mostly used for the thickening of sludges upstream of digestion, while DW is employed after digestion. Considering the relatively short solids retention times (SRT) of these processes, it was expected that there would be no PFAS transformation or removal. Hence, it was anticipated that similar PFAS mass flows would enter and leave these processes.

The mass flows of the 15 detected PFAS in the solid-liquid separation processes are presented in Fig. 3-7. From this figure, it can be seen that a majority of PFAS mass flows did not significantly change through these processes. The exceptions were PFDA and PFOS in the GDT of WWTP-A, PFPeA, PFHxA and PFHxS in the DAF of WWTP-B, and FOSA in the DW of WWTP-B with p values ranging between 0.00 and 0.04. The incidents of lack of mass balance closure were somewhat random and their causes could not be identified. These represented only 8% of all of the individual PFAS mass balances conducted on the liquid separations processes. As such, it was concluded that the liquid-solid separation processes, which are the physical processes with short SRTs, did not cause major formation or removal of PFAS as hypothesized. The distribution of PFAS into product streams in these processes will be discussed subsequently.

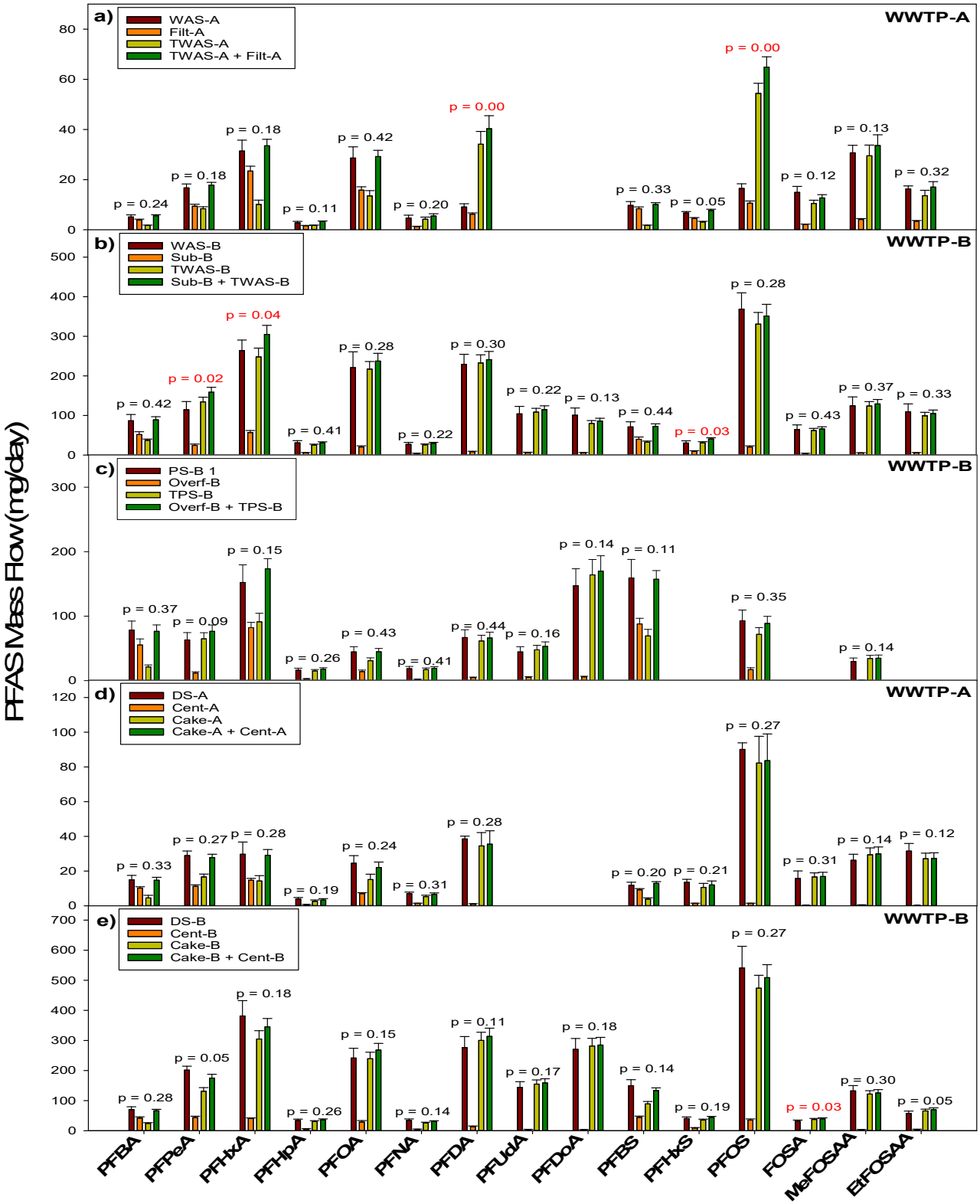


Figure 3-7. PFAS mass flows in liquid-solid separation processes (a) thickening of WAS-A by GDT (b) thickening of WAS-B by DAF (c) thickening of PS-B by GDT (d) dewatering of DS-A by DW and (e) dewatering of DS-B by DW.

3.3.2.3.2. Blending process

Similar to the solid-liquid separation processes, the blending process in WWTP-B has a short SRT and it was expected that there would be a good mass balance closure around this process. Consistent with this expectation, the PFAS mass flows and p values summarized in Fig. 3-8 demonstrated that a large majority (13/15) of the PFAS demonstrated mass balance closure (exceptions were PFHxS (p = 0.04) and FOSA (p = 0.04)). The good mass balance closure for a majority of the compounds was considered indicative of good quality data and that the mass flows entering the AD process were accurately characterized.

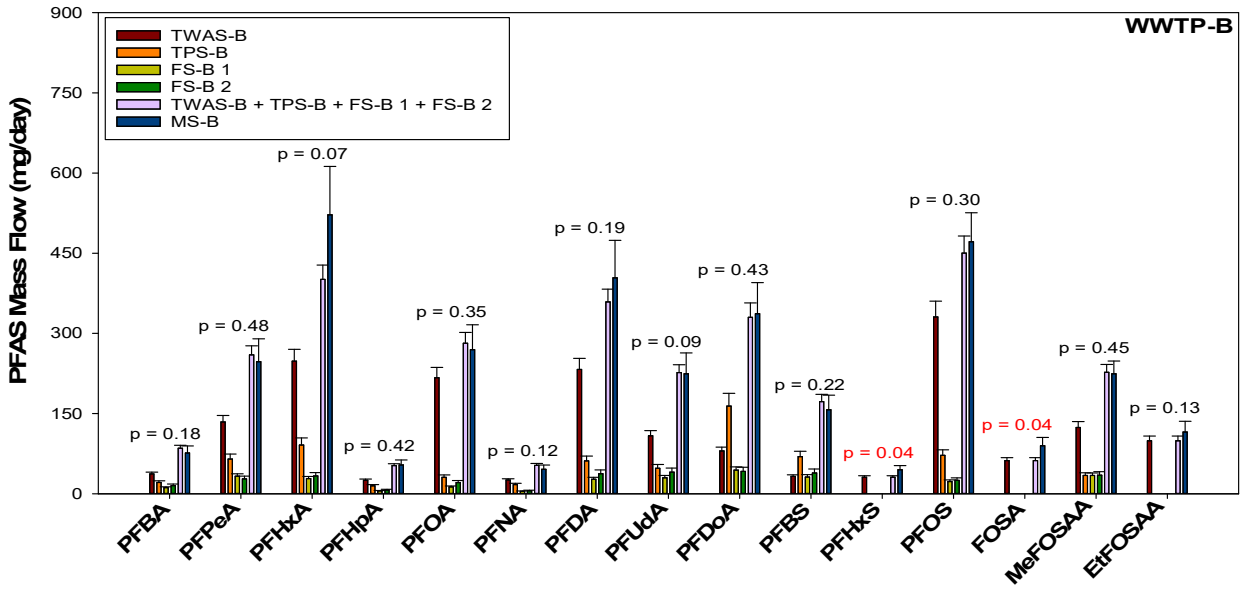


Figure 3-8. PFAS mass balances in the blending of sludges (TWAS-B, FS-B 1, FS-B 2 and TPSB).

3.3.2.3.3. Fermentation processes

Sludge fermentation produces readily biodegradable organic matter by breaking down organic material with the help of anaerobic bacteria. The fermentation process in WWTP-B ferments PS in a continuous-flow mixed reactor that is coupled to a GDT. The SRT of the combined process was approximately 4 days. The two FM units in WWTP-B generate FS with TS values of 3% and 4.5%, respectively. As PFAS precursors have been found to be degraded in anaerobic and aerobic digestion in previous studies,[24,25,82-86] it was considered important to assess whether PFAS degradation would be observed in the FM.

The PFAS mass flows that were calculated for the two FM units are presented in Fig. 3-9. From this figure, it can be seen that most of the PFAS (16/24) demonstrated no significant difference in

the mass flows through FM. Modest increases in mass flows were observed for PFBA (p: 0.04) in FM 2, PFPeA (p: 0.04) in FM 1, PFHpA (p: 0.02) in FM 2, PFNA (p: 0.01) in FM 2, PFDA (p: 0.04) in FM 1 and PFBS (p: 0.01) in both FM units, while a decrease was observed for PFBS (p: 0.03) in FM 1. Among the precursors, MeFOSAA demonstrated no significant difference in the mass flows (p: 0.10) in FM 1, while there was a slight mass flow increase (p: 0.03) in FM 2. The lack of significant PFAA formation and precursor degradation in these processes was attributed to the relatively short SRT of the fermenters compared to typical digestion processes that range between 20 and 45 days. [24,25,82-86]

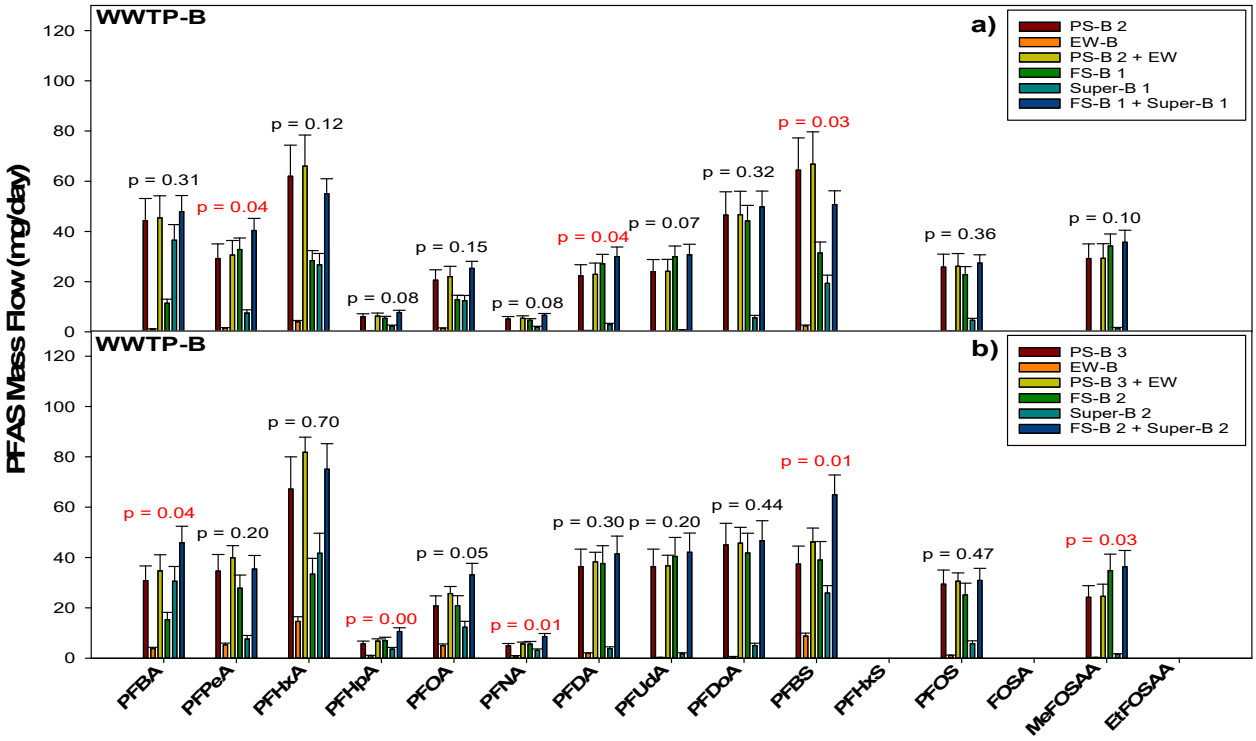


Figure 3-9. PFAS mass balances in fermentation (a) FM-1 (b) FM-2.

3.3.2.3.4. Anaerobic digestion processes

AD is commonly used to stabilize sludges in the absence of oxygen. At WWTP-A the AD process digested a mixture of TWAS and PS and at WWTP-B a combination of TWAS, TPS and FS was treated. Both plants generated DS with TS concentrations around 2.4%. The SRT differed somewhat between WWTP-A and WWTP-B (45 days versus 20 days, respectively). On the basis of literature reports, [24,25,82-85] the degradation of PFAS precursors through this process was anticipated.

The PFAS mass flows for the AD processes at the two WWTPs are presented in Fig. 3-10. From this figure, it can be seen that mass flow increases were observed for a majority (13/22) of the PFAA in both plants. The highest increases in mass flows were observed for PFBA, PFHxA, PFOA, PFBS and PFHxS in WWTP-A (between 50% and 70%), while mass flow increases in the range of 30% and 50% were observed for PFBA, PFOA, PFBS, PFHxS and PFOS in WWTP-B. The elevated levels in WWTP-A may be due to the higher SRT of WWTP-A, which allows for an extended period for the degradation process to occur. The results suggest that the SRT in AD may be a key factor affecting PFAA formation in sludge handling systems.

Among the 6 precursors, only three (FOSA, MeFOSAA and EtFOSAA) were detected in the samples collected from the inlet and outlet streams of the AD process in both plants. The mass flows of the precursors decreased through AD by 29 - 47% in WWTP-A and 20 - 32% in WWTP-B (Fig. 3-9). The difference in the magnitude of mass flow decreases was consistent with the longer SRT of WWTP-A. The trends in precursor transformation agreed with those observed for PFAS formation.

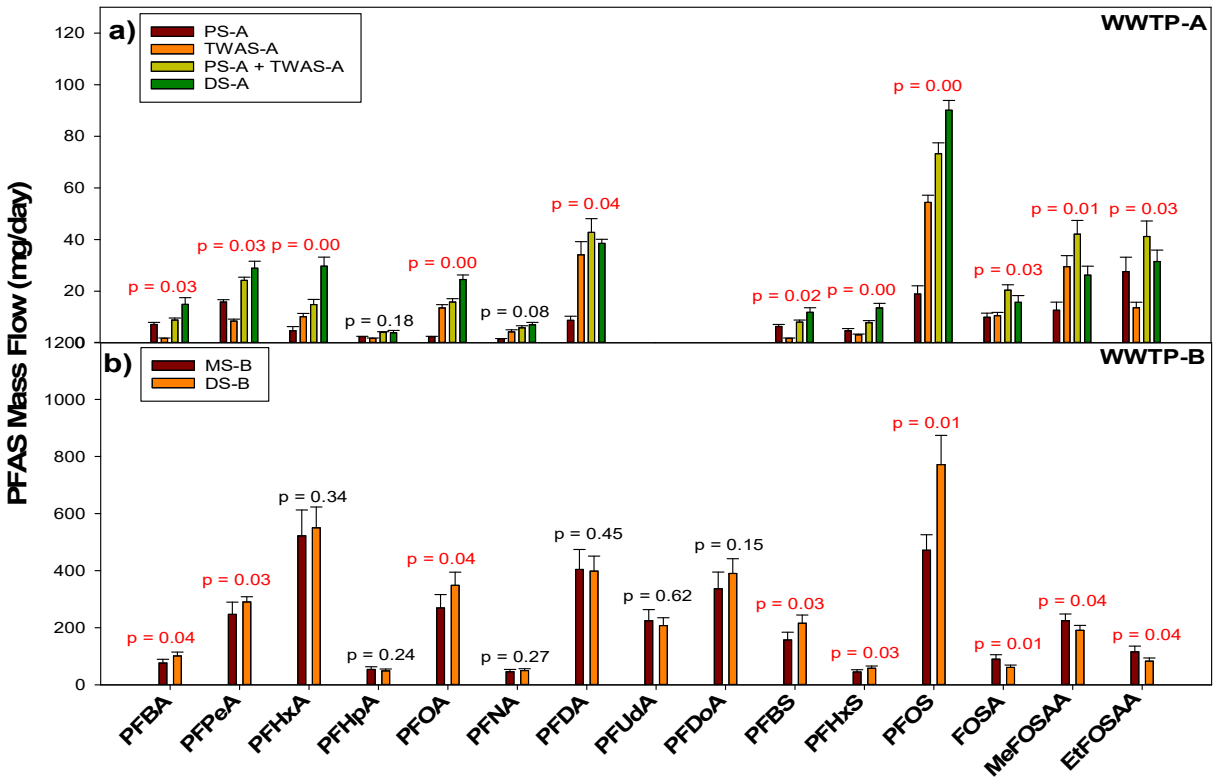


Figure 3-10. PFAS mass balances in AD (a) WWTP-A (b) WWTP-B.

3.3.2.4. PFAS destinations

The distribution of PFAS mass flows between the outlet streams of the GDT, DAF, DW and FM processes was examined to obtain insight into their destination through sludge handling systems, since the liquid streams in these processes are recycled back to the upstream processes within WWTP-A and WWTP-B. In this analysis, the PFAS mass flow in the inlet streams of each unit was set to 100% and the percentage of outlet streams was calculated as the ratio of the PFAS mass flow in the outlet stream to the total PFAS mass flow of the inlet streams. In the subsequent discussion, the impact of functional groups and chain lengths on PFAS destinations is examined.

3.3.2.4.1. Liquid-solid separation processes

The distribution of PFAS mass flows between the solid and liquid streams exiting GDT, DAF and DW are presented in Table 3-7. The distribution of PFAS between liquid and solids streams ranged widely. The underlying reasons for the varying behavior of the PFAS in the different processes are subsequently discussed in detail.

It was anticipated that the long-chain, and hence more hydrophobic, PFAS (≥C7) distribution to solid streams will increase with increasing solids capture in these streams. In WWTP-A, 67% of the solids mass flow was captured in the TWAS stream of the GDT and this ratio was 86% in the Cake stream of the DW. In the same plant, the long-chain PFAS (≥C7) distribution to TWAS ranged between 47% and 89%, while this distribution range was 62% and 115% in the Cake stream of DW, agreeing with the anticipated trend. In WWTP-B, the solids capture in the TPS and TWAS streams of GDT and DAF processes were around 93% and this ratio was 82% in the Cake stream of DW. However, the long-chain PFAS (≥C7) mass flow distributions in the TWAS, TPS and Cake streams of GDT, DAF and DW processes were found similar ranging between 72% and 112%. Viewed collectively, solid capture alone was not enough to explain the fractionation of long-chain PFAS in solid-liquid separation.

DW processes were compared between the two WWTPs to investigate the effect of this liquid-solid separation process on PFAS in the different plants. While the long-chain PFAS (≥C7) distribution to solid streams (Cake) ranged between 62% and 112% in WWTP-A, this range was 79% and 120% in WWTP-B. Moreover, while the short-chain PFAS (<C7) distribution to liquid streams (Cent) ranged between 10% and 70% in WWTP-A, this range was 31% and 60% in

WWTP-B. These data showed that PFAS distributions to solid and liquid streams of the DW process in WWTP-A and WWTP-B were not substantially different.

The impact of PFAS chain length on the distribution of PFAS between the liquid and solid streams was investigated. From Table 3-7, it can be seen that the PFAS with carbon chain lengths of C3 to C6 were mostly present in higher percentages (between 50% and 86%) in liquid streams, whereas the PFAS with chain lengths higher than C6 were mostly present in higher percentages (between 50% and 121%) in the solid streams. The results demonstrated that as the chain length increased, the PFAS tended to exit in the solid streams (TWAS, TPS and Cake). This could be attributed to the increased hydrophobicity of long-chain PFAS.

This is the first study that has comprehensively studied the fate of PFAS in sludge handling systems by characterizing all liquid and solid streams. Thus, these are the first results that investigated the impact of PFAS properties on PFAS destinations in sludge handling systems. However, Kim et al. (2022) studied the fate of PFAS in wastewater treatment. In their study, it was reported that short-chain PFAAs with a carbon chain length of C3 to C7 were present in greater amounts in wastewater samples, whereas long-chain PFAAs (with a carbon chain length ≥C8) were more dominant in the sludge samples.[74] Viewed collectively, the results of the two studies show that chain length plays an important role in determining the fate of PFAS in wastewater and sludge systems.

PFAS that have different functional groups were also compared to evaluate the effect of functional groups on PFAS destination. PFAA compounds (PFBA (C4), PFHxA (C6) and PFOA (C8)) were compared with the corresponding PFSA compounds (PFBS (C4), PFHxS (C6) and PFOS (C8)) respectively. The average ratio of PFAS mass flow in the solids stream to that of the liquid stream was calculated for each of the compounds over all five liquid-solid separation processes. The ratios were determined to be 0.5 and 0.8 for PFBA and PFBS, 2.9 and 3.9 for PFHxA and PFHxS, 3.8 and 13.5 for PFOA and PFOS. Viewed collectively, the solid/liquid ratios were higher for the PFSA compounds as compared to PFCAs, showing that PFSAs were more prone to binding to solids. These findings showed that the functional group is also a key factor determining PFAS fate in sludge handling systems.

Table 3-7. Distribution of PFAS in outlet streams of liquid-solid separation processes (% of inlet mass flow).

	WWTP-A		WWTP-B		WWTP-B		WWTP-A		WWTP-B	
	GDT		DAF		GDT		DW		DW	
	TWAS-A	Filt-A	TWAS-B	Sub-B	TPS-B	Overf-B	Cake-A	Cent-A	Cake-B	Cent-B
PFBA	33	75	43	60	27	71	30	68	35	60
PFPeA	41	66	52	65	43	53	44	70	58	38
PFHxA	32	74	64	52	60	54	48	58	70	31
PFHpA	61	58	77	18	72	37	67	34	74	36
PFOA	47	55	88	9	71	31	62	29	91	26
PFNA	89	27	95	15	92	10	75	20	80	16
PFDA	375*	68	101	4	92	7	90	3	109	5
PFUdA			104	6	107	12			108	3
PFDoA			80	6	111	4			104	1
PFBS	17	86	46	56	44	55	29	78	55	40
PFHxS	47	68	73	31			78	10	87	34
PFOS	329*	64	89	6	80	18	91	3	92	7
FOSA	71	14	92	6			105	2	111	6
MeFOSAA	89	13	99	4	98	2	112	2	100	3
EtFOSAA	83	21	91	5			101	1	114	8

* Higher than 150%

3.3.2.4.2. Fermentation

There are two fermentation units in WWTP-B. PFAS enter these units via two inlet streams, namely PS and EW. Hence, the distribution of PFAS mass flows between the outlet streams was calculated as a fraction of the total mass flow of the PS and EW streams and presented in Table 3-8. In both units, similar trends in distribution between the two outlet streams were observed. For example, most of the PFAS with a chain length between C3 and C6 resulted in high presence (>50%) in liquid streams, whereas most of the PFAS with a chain length higher than C6 were present in high percentages (>80%) in the solid streams. As the chain length of PFAS increased, the adsorption affinity to the solids increased. The longer chain PFAS tended to stay in the solid streams (FS-B 1 and FS-B 2). Furthermore, PFSAs (PFBS (63%) and PFOS (85%)) were more prone to binding to solids as compared to their PFCA counterparts (PFBA (33%) and PFOA (70%)). These findings demonstrate that the impacts of chain length and functional group on the destination of PFAS in fermentation processes were consistent with those observed in the other solids-liquid separation processes.

Table 3-8. Distribution of PFAS between outlet streams of fermentation processes (% of inlet mass flow).

	WWTP-B			
	FM 1		**FM 2**	
	FS-B 1	**Super-B 1**	**FS-B 2**	**Super-B 2**
PFBA	25	80	44	88
PFPeA	83	64	46	54
PFHxA	43	40	39	51
PFHpA	86	36	94	54
PFOA	58	46	81	48
PFNA	86	36	97	54
PFDA	119	13	99	8
PFUdA	124	3	110	5
PFDoA	95	12	91	11
PFBS	47	49	80	56
PFOS	88	16	82	19
MeFOSAA	117	5	142	6

3.3.2.5. Overall fate

As previously demonstrated, PFAS were observed to partition differently between the liquid and solid streams of the individual processes in the two WWTPs and hence it was considered important to examine the overall formation, removal and destination of PFAS considering all of the sludge handling processes together. PS and WAS streams were considered as the inlet streams to the sludge handling system in WWTP-A, while the EW streams in the FM units were considered as an additional inlet stream for WWTP-B. The outlet streams consisted of liquid streams from liquid-solid separation processes (i.e., Filt, Cent) and the solids streams (Cake) from dewatering (Table 3-9).

The percent formation of individual PFAS throughout overall sludge handling systems was initially investigated as increases in mass flow through the system influenced the interpretation of the destination of PFAS in outlet streams. From Table 3-9, in WWTP-A, the mass flows of a majority (10/13) of the detected PFAS were observed to increase through the system with the extent of increase ranging between 10% and 140%. In contrast, a smaller fraction (4/15) of the PFAS was observed to have an increase in mass flows in WWTP-B, with the range spanning from 10% to 94%. The highest formation was observed for PFOS and PFDA (>100%) in WWTP-A, and PFOS and PFHxS (>30%) in WWTP-B. These PFAS formations were attributed to the precursor degradation seen in the AD processes of both plants.

The percent removal of individual PFAS throughout overall sludge handling systems was also investigated. The mass flows of small fractions (3/13 and 3/15) of PFAS were observed to decrease in WWTP-A and WWTP-B, respectively. These PFAS were all precursors, namely FOSA, MeFOSAA and EtFOSAA in both plants. The degree of decrease in the mass flows was similar in both WWTPs and ranged between 8% and 30%. The precursor removal could mainly be attributed to the precursor degradation seen in the AD process of the sludge handling systems.

The overall destinations of individual PFAS were also examined to determine if PFAS properties (i.e., carbon chain length) are also important to determine PFAS fate. The overall fractionation of PFAS within each plant was initially investigated and then the two plants were compared. Specifically, within the plants, the total fractionation of PFAS mass flows in the liquid streams was compared with the final solid stream, the Cake. In WWTP-A, the majority of PFAS with chain length ≥C7 were present in the Cake stream at high percentages ranging between 49%

and 227%, while the majority of PFAS with chain length <C7 were present at high percentages in the liquid streams (i.e., Filt, Cent), ranging between 72% and 114%. Similarly, in WWTP-B, while the PFAS with chain length ≥C7 were present in the Cake stream at high percentages ranging between 50% and 115%, the PFAS with chain length <C7 were present at high percentages ranging between 66% and 110% in the liquid streams and recycled back to wastewater treatment processes. The results demonstrate that there was a high degree of recycling of short-chain PFAS to the liquid treatment train. These streams which have relatively low flows and low solids concentrations might be targeted for the application of focused PFAS removal technologies.

Between WWTP-A and WWTP-B, the percent fractionations of long-chain PFAS in the Cake streams (49 - 227% and 50 - 115%, respectively) and short-chain PFAS in the liquid streams (72 - 114% and 66 - 110%, respectively) did not differ substantially. In fact, the fractionation ranges overlapped for most of the long-chain and short-chain PFAS. Only the maximum ranges were found higher in WWTP-A. This might be attributed to the higher SRT of the AD process in WWTP-A, which allowed for an extended period for the degradation process to occur leading to PFAAs formation.

Table 3-9. Distribution of PFAS between outlet streams of the overall sludge handling systems (% of inlet mass flow).

	Outlet Streams							
	WWTP-A			WWTP-B				
	Filt-A	Cent-A	Cake-A	Sub-B	Overf-B	Super-B	Cent-B	Cake-B
PFBA	32	83	37	21	23	27	17	10
PFPeA	52	60	34	20	19	12	24	37
PFHxA	65	41	39	10	15	12	7	54
PFHpA	32	14	50	9	5	10	9	50
PFOA	51	23	49	6	4	8	13	66
PFNA	20	22	84	9	5	10	9	50
PFDA	35	6	194	2	1	2	4	97
PFUdA				3	2	1	3	78
PFDoA				2	2	3	1	85
PFBS	52	56	50	22	26	33	33	16
PFHxS	40	32	93	35			31	115
PFOS	29	4	227	4	3	2	7	101
FOSA	9	1	67	7			3	74
MeFOSAA	10	1	68	3	1	1	2	65
EtFOSAA	8	0	62	5			4	60

3.4. Conclusion

This study examined the fate of PFAS by assembling PFAS mass balance at two sludge handling systems in Canada. The study was conducted at full-scale WWTPs by including all main and side streams (solid and liquid streams) to achieve full mass balance closures. To the best of our knowledge, this is the first study to investigate the fate of PFAS at the full trains of sludge handling processes. Some of the major findings were:

- PFOS was the most abundant compound in both solid and liquid streams with concentrations reaching up to 12.9 µg/kg and 53 ng/L, respectively. This was followed by PFDA (8.1 µg/kg) in solid and PFHxA (42.1 ng/L) in liquid streams.
- Physical treatment processes such as liquid-solid separation and sludge blending did not cause major formation or removal in almost 92% of individual target PFAS in both WWTPs. These findings demonstrated that these types of physical processes, with short SRTs, did not cause major formation or removal of PFAS.
- Although being a biological process, FM did not cause a significant difference in the majority of mass flows of target PFAS (16/24) in WWTP-B due to having short SRT (4 days) compared to AD (ranges between 20 and 45 days).
- AD process resulted in mass flow increases for a majority (13/22) of PFAAs in both plants, with the highest increases in PFBA, PFHxA, PFOA, PFBS and PFHxS between the range of 50% and 70% in WWTP-A and the range of 30% and 50% in PFBA, PFOA, PFBS, PFHxS and PFOS in WWTP-B. The higher formation percentages seen in WWTP-A were attributed to longer SRT (45 days) as compared to WWTP-B (20 days). It was concluded that the SRT may be a key factor affecting PFAS fate in sludge handling systems
- AD process also resulted in mass flow decreases in all the precursors detected in the samples (FOSA, MeFOSAA and EtFOSAA) between the range of 29% and 47% in WWTP-A and 20% and 32% in WWTP-B. The higher removal percentages seen in WWTP-A were also attributed to longer SRT.
- For the PFAS destinations in liquid-solid separation processes, PFAS with chain lengths of C3 to C6 mostly presented high percentages (between 50% and 86%) in liquid streams, whereas the PFAS with chain lengths higher than C6 mostly presented high percentages (between 50% and 121%) in solid streams. Moreover, the average ratio of PFSA mass flow in the solids stream

to that of the liquid stream was mostly 1.4 to 3 times higher than that of PFCA, showing that the sulfonate group is more prone to binding the solids than carboxylate. These results showed that carbon chain length and functional groups are the key factors determining PFAS fate in sludge handling systems.

- In the overall sludge handling systems, the mass flows of all of PFAA (10/10) entering WWTP-A increased (between 10% and 140%), and only a small fraction (4/12) of PFAAs mass flows increased in WWTP-B (between 10% and 94%). The highest increases were observed for PFOS and PFDA (>100%) in WWTP-A, and PFOS and PFHxS (>30%) in WWTP-B. Furthermore, the decrease in the mass flows of precursors ranged between 10% and 30% in both WWTPs. The removal of precursors and formation of PFAAs were attributed to the biological degradation of precursors in both plants.
- In the overall sludge handling systems, the majority of PFAS with chain length ≥C6 stayed in the solid streams and ended up in Cake, while that of PFAS with chain length <C6 stayed in the liquid streams and recycled back to wastewater treatment processes. This trend also showed that while the recycling liquid streams might be targeted for the application of short-chain PFAS removal technologies, the solid streams might be the focus point for the application of long-chain PFAS removal technologies.

Chapter 4

Conclusions and Recommendations

This research characterized the concentrations of PFAS throughout the sludge handling systems of two full-scale WWTPs in Canada and provided insight into the underlying mechanisms affecting their fate in these systems. The concentration data will support regulators with the establishment of regulatory guidelines. Furthermore, the results of the PFAS fate analysis will inform decision-making for the development of effective approaches to biosolids management.

A novel and improved analytical method was developed to accurately quantify the concentrations of 24 PFAS compounds in solid streams, namely sludges and cakes, associated with sludge handling processes. This innovative method was devised in response to the inadequate applicability of previously reported analytical methodologies when dealing with a diverse range of samples. Specifically, an adsorbent formulation consisting of 1,000 mg Envi-Carb, 500 mg PSA and 500 mg C18 was developed for the cleanup of methanolic biosolids extracts to remove background interferences. The developed formula was found to be effective over a diverse range of sludges and biosolids. It should also be noted that this formulation can be modified for different samples to obtain an optimal trade-off between chromatogram quality and PFAS recovery.

A modification of US EPA Method 1633 was developed and validated for the analysis of PFAS in recycled liquid streams. The original method was developed to analyze wastewater samples with TSS concentrations up to 250 mg/L. As the samples collected from the liquid streams of sludge handling processes contained TSS concentrations ranging between 100 and 7,000 mg/L, ultracentrifugation and extraction steps were added to the sample preparation step to first separate the solids from the samples and then quantify the PFAS adsorbed on them. This modification allowed the reliable quantification of PFAS in samples with TSS concentrations up to 7,000 mg/L.

PFOS, which has drawn the most attention due to its toxicity and bioaccumulative nature, was found to be the most abundant PFAS with concentrations as high as 12.9 µg/kg and 53 ng/L in solid and liquid samples, respectively. The data on PFOS along with other abundant PFAS (i.e., PFOA, PFDA, PFHxA) will be of interest to policymakers that are developing regulations on biosolids disposition.

The fate of PFAS at two full-scale sludge handling systems (WWTP-A and WWTP-B) in Canada was investigated through mass balance analysis. Mass balance closures through individual processes and overall systems were evaluated. In both systems, a majority of individual PFAS mass flows did not significantly change through the liquid-solid separation and blending processes, showing that these types of physical processes with short SRTs did not cause major formation or removal of PFAS. AD, on the other hand, resulted in mass flow increases for a majority (13/22) of the individual PFAAs in both plants (in the range of 50 - 70% in WWTP-A, and 30 - 50% in WWTP-B), while the mass flows of the detected precursors decreased (in the range of 29 - 47% and 20 - 32% in WWTP-A and WWTP-B, respectively). The degradation of PFAS precursors and formation of PFAAs were attributable to biodegradation. While the FM process at WWTP-B is also a biological process, there was no significant change in a majority (16/24) of mass flows of target PFAS. This lack of precursor degradation and PFAAs formation observed in FM was attributed to the short SRT (4 days) of this process (compared to the longer SRTs of 45 and 20 days for the AD processes in WWTP-A and WWTP-B, respectively). Overall, it was concluded that biological processes led to the degradation of PFAS precursors and the formation of PFAAs, and the SRT of these processes was a key factor affecting the degree of formation and removal.

The distribution of PFAS between solid and liquid streams was studied to characterize the destination of PFAS throughout the sludge handling systems. PFAS with carbon chain lengths of C3 to C6 were mostly present (between 50% and 86%) in the liquid streams, whereas PFAS with chain lengths higher than C6 were mostly present (between 50% and 121%) in the solid streams. In light of these results, the recycling of liquid streams from the sludge handling processes might be targeted for the application of short-chain PFAS removal technologies, while the solid streams might be the focus point for the application of long-chain PFAS removal technologies. Furthermore, PFSAs were found to be 1.4 to 4 times more prone to binding to solids as compared to their PFCAs counterparts (i.e., PFBS vs PFBA and PFOS vs PFOA), showing that carbon chain length and functional groups are key factors determining PFAS fate in sludge handling systems.

In the future, it is recommended that the robustness of the analytical method developed here should be assessed over a broader range of samples to validate the analytical method with sludges and biosolids that have different characteristics. Further, additional sludge handling systems (i.e., composting and alkaline stabilization) could be studied with a similar approach to characterize the

fate of PFAS in other common sludge handling processes. Finally, solid/liquid partition coefficients (K_d) could be characterized and compared between different sludge handling processes and systems. The availability of partitioning coefficients will facilitate the development of models for predicting the fate of PFAS in sludge handling systems.

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