Determination of per- and polyfluoroalkyl substances (PFAS) in Australian wastewater utilizing targeted and untargeted analysis

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Introduction

In Australia, per- and polyfluoroalkyl substances (PFAS) are a contaminant of emerging concern. PFAS have unique properties that make their use in industry and manufacture desirable [1]. However, PFAS are persistent, mobile, bioaccumulative and potentially toxic to organisms [2-5]. To date over 3000 PFAS have been produced and distributed on the global market [6], but most studies have focused on analysis of a few perfluoro- carboxylic acids(PFCAs) and sulfonic acids (PFSAs). High resolution mass spectrometry is becoming an increasingly useful tool for the screening of unknown PFAS in environmental samples [7-9].

Wastewater treatment plants (WWTP) have been demonstrated as a point source of PFAS pollution to the environment [10]. There are limited studies on newer or alternative classes of fluorinated compounds in wastewater and recycled water. Furthermore, the occurrence data for alternative classes of PFAS in Australian wastewater are currently unavailable, while data for PFCAs and PFSAs are limited [11, 12].

The aim of this study was to employ unique workflows using high resolution mass spectrometry for the comprehensive analysis and identification of PFAS in two Australian wastewater treatment plants.

Materials and methods

All reagents used were of the highest purity available. Methanol ($\geq$ 99.9% Honeywell Burdick and Jackson, USA), ultrapure water (Merck Millipore, USA) and ammonium acetate ($\geq$ 99.9%, Sigma-Aldrich, USA) were used in LC-MS analysis. Linear PFAS Analytical standards and isotopically labeled PFAS were purchased from Wellington laboratories (Guelph, Ontario) as a 2 μg mL^{-1} mixture (PFAC-MXB) and as 50 μg mL^{-1} single compound solutions in methanol (L-PFPeS, L-PFHpS, 6:2 FTs, 8:2 FTS, M3PFBS, M3PFHxS, MPFHxA, M8PFOA, M8PFOS, MPFDA, PFDoA, M6:2 FTS).

Replicate 250 mL grab samples (n=3) were collected in polypropylene bottles pre-rinsed with methanol at an activated sludge treatment plant (WWTP1) and a lagoon based treatment plant (WWTP2) in August 2016 (WWTP1: Influent, primary effluent, secondary effluent, final effluent; WWTP2: recycled water class C, recycled water class A). A second set of final effluent samples (n=3) from both treatment plants were collected in January 2017.

Aliquots of sample were spiked with 10 ng of isotopically labeled PFAS, filtered and subjected to solid phase extraction (SPE) with Oasis WAX 6CC, 150 mg, 30 μm cartridges (Waters corp, USA) using methods similar to those published in Houtz, Sutton [13]. QA/QC for each batch of 9 samples consisted of a blank and lab control sample (spiked with 10 ng native PFAS) prepared in ultrapure water and a matrix sample spiked with 10 ng native PFAS. Linear calibration curves ($r^2 > 0.99$, $\geq$ 5 calibration points) were prepared from linear PFAS isomers and used to calculate total branched plus linear PFAS concentrations.

Targeted quantification was performed using stable isotope dilution on an Agilent Technologies 1290 Infinity II liquid chromatograph with an Agilent Technologies 6470 triple quadropole mass spectrometer (LC-MS/MS) operated in negative electrospray ionization and multiple reaction monitoring mode (Agilent Technologies, Delaware, USA). Chromatographic separation was achieved on a Zorbax Eclipse Plus RRHD C18 column (2.1 x 50 mm, 1.8 μm) with a Zorbax Eclipse Plus C18 column (2.1 x 50 mm, 3.5 μm) installed before the solvent mixer to delay instrument contamination (Agilent Technologies, Delaware, USA).

Untargeted analysis was performed on an Agilent Technologies 1290 Infinity II liquid chromatograph with an Agilent Technologies 6550 quadropole time-of-flight mass spectrometer (LC-QTOF) operated in negative electrospray ionization in all ions MS/MS mode (Agilent Technologies, Delaware, USA). Chromatogrpahic separation was achieved on a Zorbax Eclipse Plus RRHD C18 column (2.1 x 50 mm, 1.8 μm) with a Zorbax Eclipse Plus C18 column (4.6 x 50 mm, 3.5 μm) installed before the solvent mixer to delay instrument

contamination (Agilent Technologies, Delaware, USA).

Further, high resolution data obtained with the LC-QTOF were screened for the presence of 106 PFASs using a custom database with various levels of verification ranging from confirmation of fragments from validated MS/MS spectra to identification of molecular mass and isotope fidelity. Finally, software tools such as MassHunter molecular feature extractor (MFE), mass profile professional (MPP) and molecular structure correlator (MSC) were used to propose and identify fluorinated compounds in the samples not present in the database, and further analysis was performed to verify their identities in the sample extracts.

Results and discussion

At WWTP1, PFCAs and PFSAs were detected in influent and effluent with mean ∑PFAS concentrations ranging from 160 to 173 ng L^{-1} (Figure 1). The compounds PFOS, PFOA, PFHxS, PFHxA, PFPeA and PFBA were detected at all sample locations, and at concentrations ranging from 8.7 to 88.0 ng L^{-1}. PFBS was detected at all sample locations excluding influent and at low concentrations (1.2 to 4.8 ng L^{-1}).
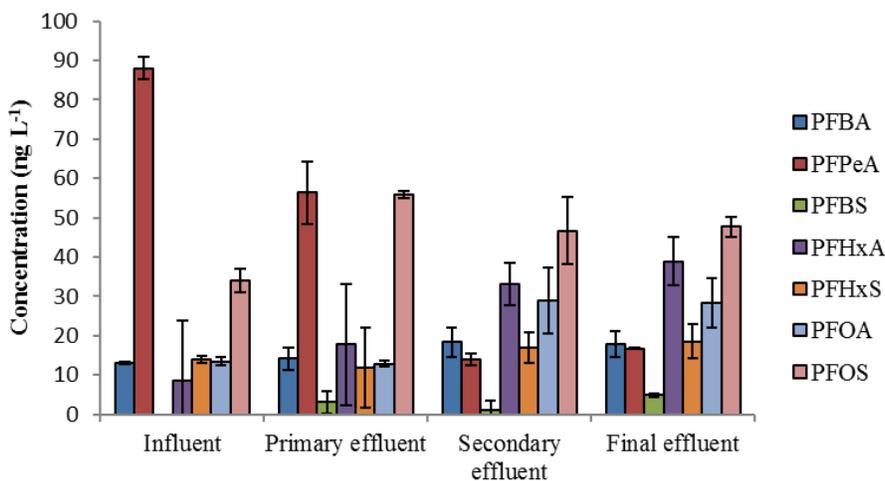

Figure 1. Mean PFAS concentrations at various treatment stages in WWTP1. Error bars represent ± 1 standard deviation

Mean ∑PFAS concentrations in recycled water at WWTP2 were 202 and 210 ng L^{-1} in Class C and Class A water, respectively. PFOS, PFOA, PFHxS, PFHxA, PFPeA, PFBS and PFBA were detected in both Class C and Class A water at concentrations ranging from 5.9 to 66.7 ng L^{-1} (Figure 2). PFOS was the compound found at the highest concentration in both Class C water and Class A water at 52.4 and 66.7 ng L^{-1}, respectively.
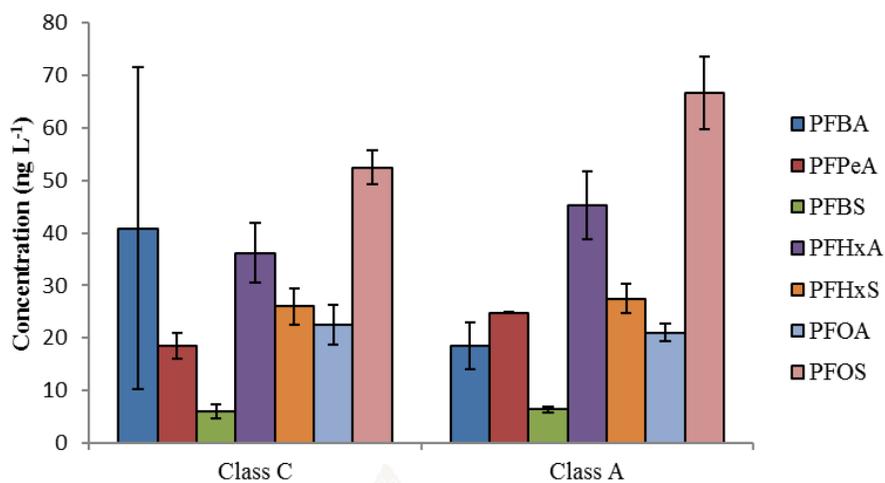

Figure 2. Mean PFAS concentrations in recycled water at WWTP2. Error bars represent ± 1 standard deviation.

Suspect screening using a custom database with MS/MS spectra and retention time data identified with high certainty a range of non-target PFAS in WWTP1. Furthermore, unknowns analysis utilizing the fluorine mass defect identified a number of unidentified fluorinated compounds. Unknown fluorinated compounds contributed 66 to 82% of the total fluorinated compounds in samples at WWTP1. Whereas 6 to 7% of compounds detected were through targeted analysis and 10 to 16% in suspect screening.

In all samples from August 2016, the compound 6:2 fluorotelomer sulfonate (6:2 FTS) was identified in suspect screening (EIC 426.96790) with a high relative response. In final effluent samples collected January 2017 at WWTP1 and WWTP2, the compound 6:2 FTS was quantified at mean concentrations of 8.3 and 23.8 ng L^{-1}, respectively (Figure 3). At WWTP2, the highest PFAS concentrations were PFOS>PFOA>6:2 FTS, indicating 6:2 FTS as an important PFAS contributor at this WWTP.
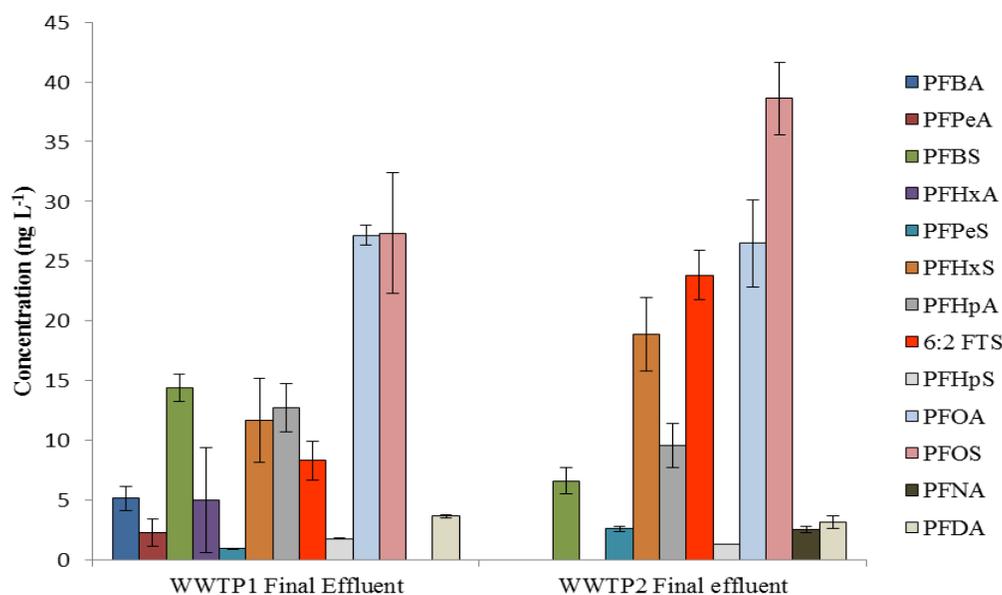

Figure 3. Mean PFAS concentrations measured in WWTP1 and WWTP2 final effluent January 2017. Error bars represent ± 1 standard deviation

The ∑PFAS (excluding 6:2 FTS) at WWTP1 was lower relative to August 2016 in final effluent (173.1 vs 112.1 ng L^{-1}). Unpaired *t*-tests showed significantly higher concentrations ($p<0.05$) of PFBA, PFHpA, PFHxA, PFHpA and PFOS in August 2016 samples. In samples from January 2017, PFBS concentrations were significantly higher ($p<0.05$), and there was a greater number of compounds present. Temporal variation of PFAS was seen in San Francisco by Houtz, Sutton [13] in sampling campaigns separated by 5 years. However, in the current study, temporal variation at the scale observed may be a result of several factors such as seasonal fluxes. Further research into the sources of PFAS to these two WWTPs may provide insight into the mechanisms of the observed temporal PFAS variation.

Worldwide it is accepted that conventional wastewater treatment processes fail to remove PFAS [10]. The two Australian WWTPs studied display this trend, with PFAS persisting through the wastewater treatment process to final effluent and recycled water. As PFAS are present in effluent from both WWTPs, PFAS are likely entering the receiving environment at substantial levels (high ng L^{-1} to µg L^{-1}). This is important in an Australian context as WWTP effluent is not yet widely monitored as a source of PFAS to the environment. Current Australian research and government efforts have been focused on the impact of aqueous film forming foams (AFFF) at fire training facilities, defence force sites and airports [14, 15].

A large portion of the fluorinated compounds in the two WWTPs studied are not yet identified. Furthermore, the combination of targeted and untargeted analysis can be a powerful tool for understanding PFAS in effluent and recycled water. As demonstrated in this study, screening of nontarget PFAS with high resolution mass spectrometry can be used to refine targeted analysis methods. This is particularly relevant in Australia due to the finding that appreciable levels of the compound 6:2 FTS were present in WWTP effluent. High levels of 6:2 FTS

and PFOS are generally associated with AFFF use sites [16]. If influent containing AFFF is reaching these two WWTPs, that receive primarily domestic sewage, this may imply that AFFF use is having further reaching not yet considered impacts.

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