Science of the Total Environment 636 (2018) 355–359

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Short Communication

Defluorination of perfluoroalkyl acids is followed by production of monofluorinated fatty acids

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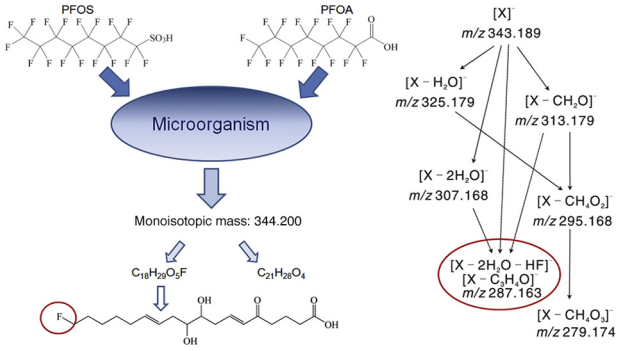
HIGHLIGHTS

- Environmental isolates can reduce concentration of PFOS and PFOA.
- Untargeted LC/MS analysis had confirmed presence of new metabolites.
- Microbial consortia incubated with PFOS and PFOA produced new metabolites.
- Monofluorinated fatty acid is proposed as one of novel biotransformation products.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 December 2017
Received in revised form 16 April 2018
Accepted 18 April 2018
Available online xxxx

Editor: P Holden

Keywords:
Microbial consortium
Monofluorinated fatty acid
Perfluoroalkyl acid
Perfluorooctanesulfonic acid
Perfluorooctanoic acid

ABSTRACT

We investigated the capability of microorganisms isolated from environments polluted with perfluoroalkyl acids (PFAAs) to conduct biotransformation of these emerging pollutants. Two different microbial consortia (chemoorganoheterotrophic bacteria and total yeast and molds) were isolated from two river sediments in Saitama and Osaka, Japan, known for long term pollution with perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). The microbial consortia were incubated in the presence of added PFOS and PFOA, and decreases in concentrations of these compounds were between 46–69% and 16–36%, respectively. Decreases in concentrations were, in part, due to sorption on biomass, but defluorinated PFOS and PFOA products were not detected. However, untargeted analysis suggested the presence of several metabolites found only in samples from consortia with PFOS and PFOA but not in the control samples. Molecular formula candidates were narrowed down to two options, $C_{18}H_{28}O_5F$ and $C_{21}H_{27}O_4$. It was assumed that these formulas were associated with unsaturated monofluorinated fatty acids and hydrocarbons with multiple unsaturated bonds or ring structures.

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https://doi.org/10.1016/j.scitotenv.2018.04.243
0048-9697/

1. Introduction

Technological and scientific progress in the 20th century has led to the development of organic molecules with structures never encountered before in nature. Perfluoroalkyl acids (PFAAs), chemicals with fluorinated alkyl chains, have been extensively used since the 1950s (Buck et al., 2011). Recently, PFAAs have been characterized as persistent, bioaccumulative and toxic, and they are considered to be a potential risk to the environment and humans (Brambilla et al., 2015; Prevedouros et al., 2006). The main representatives of PFAAs are perfluoroalkyl carboxylates (PFCAs) and perfluoroalkyl sulfonates (PFSAs) (Table S1). To date, PFAAs have been detected ubiquitously in the environment of Europe (Kwadijk et al., 2010; Beškoski et al., 2013), northern America (Dinglasan-Panlilio et al., 2014), and Asia (Takemine et al., 2014), including some remote regions (Dietz et al., 2008).

Although the C—F bond is very stable against physical and chemical attack, biochemical dehalogenation of fluoroacetate was first reported 50 years ago (Goldman, 1965). Studies on bacterial degradation of PFAAs were mainly focused on polyfluorinated compounds and especially fluorotelomer alcohols (FTOHs) with alkyl chains of different lengths (Zhang et al., 2013), as well as PFCAs and PFSAs (Parsons et al., 2008). Defluorination was later reported for trifluoroethanesulfonate, 6:2 FTOH (Key et al., 1998), and 8:2 FTOH in sludge and sediment (Dinglasan et al., 2004; Wang et al., 2005). However, one recently published study has found that perfluorooctanoic acid (PFOA) is not biodegradable in a variety of culture conditions (Liou et al., 2010). In contrast, evidence for perfluorooctane sulfonic acid (PFOS) and PFOA degradation is the decrease in their concentrations (Kwon et al., 2014; Mejia Avendaño et al., 2015). The most convincing evidence for biodegradation should be obtained during biotransformation by determining biodegradation products using instrumental analysis such as liquid chromatography/mass spectrometry (LC/MS) and gas chromatography/mass spectrometry (GC/MS), or determining the fate of microbiologically released fluoride ions.

In this study, biotransformations of PFOS and PFOA under aerobic conditions were performed using two different microbial consortia isolated from sediment taken from PFOS- and PFOA-polluted areas. Targeted and untargeted analyses were carried out with LC/MS in order to detect the possible presence of defluorinated PFOS and PFOA products resulting from microbial metabolism.

2. Materials and methods

2.1. Standards, chemicals, and reagents

PFOS (>98% purity) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). PFOA (96% purity, 99.9% by GC area) was obtained from Sigma-Aldrich (St. Louis, MO). Mass-labeled PFOS and PFOA internal standards used for quantitative targeted analysis by LC/MS (MPFAC-MXA) were obtained from Wellington Laboratories (Guelph, Ontario, Canada). All solvents and reagents were HPLC grade. Ultra-pure water was used throughout the study. The Retention Gap Technique was used to effectively reduce the blank level, thus avoiding any increase of blank level from solvent and the LC system (Ezaki et al., 2009).

2.2. Sediments used for isolation of microbial consortia

For isolation of microbial consortia, two sediments were used. One was taken from Motokoyama River in Saitama, Japan (Site A), known for PFOS long term pollution (Zushi et al., 2011). It was a composite sediment sample collected from two locations which were both known for long term pollution: 36°15′08″N, 139°10′25″E and 36°14′13″N, 139°12′36″E. Another was taken from Ajifu Waterway, Osaka, Japan (34°45′24″N, 135°33′31″E, Site B, Beškoski et al., 2017), which was known for long term PFOA pollution (Saito et al., 2004). Sediments were collected from the river bottom surfaces 10 cm below water level, using a sediment sampler, and were stored in sterile glass jars at 4 °C. PFAAs in sediment samples were analyzed as previously described (Beškoski et al., 2013). Both deposited sediments were predominantly composed of silt, with some sand and traces of clay, and they can be classified as silty sand.

2.3. Preparation of microbial consortia and extraction of PFAAs

Two microbial communities were enriched and isolated from both Site A and B sediments. Consortia of chemoorganoheterotrophic bacteria (CB) were isolated and enriched using Bushnell Haas medium supplemented with glucose (hereinafter referred to as A-CB and B-CB depending on the site) (Table S2). Consortia of yeast and molds (YM) were isolated and enriched using Malt extract broth (Sigma-Aldrich) (A-YM and B-YM). In all media, PFOS and PFOA were added to stimulate the growth of zymogenous microorganisms and to inhibit the growth of microorganisms sensitive to PFAAs. Dimethyl sulfoxide (DMSO) solutions of PFOS and PFOA at 2 mg mL^{-1} were respectively added to the sediments from Site A and Site B so that the concentration was adjusted to 1 μg mL^{-1}. Enriched microorganisms were grown on PFAA-free media prior to inoculation of model systems. The number of microorganisms in the biotic test (BT) and biotic control (BC) was determined by plating appropriate serial dilutions on agar plates incubated at 28 °C. The media used were: Nutrient Agar (Sigma-Aldrich) for CB and Malt Extract Agar for YM. Each experimental setup included three model systems: BT, abiotic control (AC), and BC (Table S2). All model systems were initially 100 mL of liquid held in 500 mL Erlenmeyer flasks with starting concentrations of PFAAs of 1 μg mL^{-1}. Experiments were performed at 28 °C at 200 rpm. All incubations were completed in triplicate. The pH and the number of microorganisms in each model system were measured at the beginning of the experiment and every 7 days during 28 days. From all model systems, 5 mL aliquots were centrifuged, and 50 μL of 1% formic acid were added to the supernatants. The addition of formic acid was found to increase recovery and improve the chromatographic peak shape of each analyte. Labeled 10 μL of $^{13}C_8$PFOS and $^{13}C_8$PFOA (100 ng mL^{-1} in methanol) was added as a syringe spike. PFAAs were extracted from the supernatants with solid phase extraction (SPE) cartridges (Presep PFC II, Wako Pure Chemical Industries, Ltd., Osaka, Japan) which were preconditioned with 10 mL of 0.1% methanolic ammonia, 10 mL of methanol, 15 mL of Milli-Q water and 5 mL of 1% formic acid. After the analyte-loading, the cartridges were washed with 10 mL of Milli-Q water. The glass beaker was rinsed with 5 mL of 0.1% methanolic ammonia; the rinsing solution was directly used for elution of PFAAs from the cartridge. The eluate was concentrated to 1 mL by using a gentle nitrogen stream and filtered before analysis.

2.4. LC/MS analysis

LC/MS quantitative analysis was conducted in accordance with the previously described procedure (Beškoski et al., 2013; Table S3). For untargeted analysis, an Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA), which had a mass accuracy of 5 ppm, was used (Table S4).

2.5. Structural elucidation of biotransformation products

To identify potential transformation products, mass spectra ranging from *m/z* 200–3000 were acquired with a resolving power of 100,000 at *m/z* 200 at a scan rate of 1 Hz. Ions detected only in BT samples were drawn from all ions detected. All ion fragmentation (AIF) mode at 25 eV was used to acquire accurate masses of fragment ions and to complement determination of the elemental composition. Determination of the elemental composition from accurate masses was carried out with Thermo Fisher XCalibur Software.