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Investigating the biodegradability of perfluorooctanoic acid

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ABSTRACT

Perfluorooctanoic acid (PFOA) is an industrial chemical that has become disseminated globally in aquatic and terrestrial habitats, humans, and wildlife. Understanding PFOA's biodegradability (susceptibility to microbial metabolic attack) is a crucial element in developing an informed strategy for predicting and managing this compound's environmental fate. Reasoning that PFOA might be susceptible to reductive defluorination by anaerobic microbial communities, we embarked on a 2-phase experimental approach examining the potential of five different microbial communities (from a municipal waste-water treatment plant, industrial site sediment, an agricultural soil, and soils from two fire training areas) to alter PFOA's molecular structure. A series of primarily anaerobic incubations (up to 259 d in duration) were established with acetate, lactate, ethanol, and/or hydrogen gas as electron donors and PFOA (at concentrations of 100 ppm and 100 ppb) as the electron acceptor. Cometabolism of PFOA during reductive dechlorination of trichloroethene (TCE) and during reduction of nitrate, iron, sulfate, and methanogenesis were also examined. Endpoints of potential PFOA transformation included release of fluoride and detection of potential transformation products by LC/Orbitrap MS and LC/accurate radioisotope counting in a ^{14}C radiotracer study. The strongest indication of PFOA transformation occurred during its potential cometabolism at the 100 ppb concentration during reductive dechlorination of TCE. Despite an extensive search for transformation products to corroborate potential cometabolism of PFOA, we were unable to document any alteration of PFOA's chemical structure. We conclude that, under conditions examined, PFOA is microbiologically inert, hence environmentally persistent.

1. Introduction

Perfluoroalkyl compounds (PFCs) are chemicals that have valuable properties for myriad industrial and consumer applications (Kissa, 2001). Thus, PFCs have been produced and emitted to the environment (i.e., soils, sediments, waters, and the atmosphere) for more than 50 years (Olsen et al., 2005; Prevedouros et al., 2006). Like many industrial chemicals (e.g., chlorofluorocarbons; Rowland, 1995), the ecological, toxicological, and environmental impacts of PFCs were unanticipated. International and national regulations, such as REACH and TSCA (Muir and Howard, 2006), were not in place during the early-to-mid 20th century; therefore, through routine global production and use in commerce and industry, PFCs have been widely disseminated in the environment. PFCs have been detected in many habitats, including indoor and outdoor air, house dust, drinking water, food, human blood, breast milk, and wildlife (Houde et al., 2006; Skutlarek et al., 2006; Barber et al., 2007; Kärrman et al., 2007; Lau et al., 2007).

Perfluorooctanoic acid (PFOA) is a PFC that has received a great deal of ecological and toxicological attention. PFOA's global presence is mechanistically linked to the compound's volatility, aqueous solubility, and other partitioning characteristics (Prevedouros et al., 2006). PFOA's limited use in industry is to solubilize fluoromonomers during manufacture of fluoropolymers: PFOA occurs at trace levels in these products (Prevedouros et al., 2006). Thus, PFOA's widespread environmental occurrence is not necessarily quantitatively commensurate with the compound's direct industrial production. Among the hypotheses advanced to explain PFOA's environmental ubiquity is its possible formation as a breakdown product from precursors including raw materials (Prevedouros et al., 2006) and high-molecular-weight polymers (Dinglasan et al., 2004; Russell et al., 2008; van Zelm et al., 2008; Washington et al., 2009).

Within the current structure of regulations aimed at environmental protection, commercial chemicals are managed based on three key traits: persistence, bioaccumulation, and toxicity (Muir and Howard, 2006). Among this trio of properties, persistence is particularly influential because if a chemical does not persist, its potential bioaccumulation and toxicity are less significant. By some criteria, direct detection of a contaminant in environmental

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samples is evidence for persistence. But detection should not be mistaken for static non-reactivity. Instead, detection provides evidence that, for a given sampled environment, dynamic rates of chemical destruction do not match or exceed the rate of production. Thus, independent determination of the mechanisms of both production and destruction of an environmental contaminant is crucial for understanding and predicting contaminant fate.

Biodegradation (simplification of molecular structure, usually via microbial processes) is a major pathway that leads to the destruction of organic environmental pollutants in soils, waters, and sediments (Madsen, 1991). Although a great deal of current effort in biodegradation research is directed toward developing analytical and biomarker-based strategies for proving that potential biodegradation reactions actually occur in field sites (National Research Council, 1993, 2000: Griebler et al., 2004), the first step toward establishing field criteria for biodegradation reactions is enrichment culturing, aimed at discovery of potential microbial metabolic processes (Madsen, 2005); in this case ones that can alter or destroy pollutants. Perhaps because of widespread acknowledgement of the stability of the C—F bond (Key et al., 1997), to date the number of studies examining biodegradation and transformation of PFCs has been limited. In an early study, Key et al. (1998) reported that perfluorooctane sulfonate was chemically inert under aerobic conditions that allowed a bacterium to utilize difluoromethane sulfonate as a sulfur source. Based on in vitro and in vivo studies, there is no evidence that PFOA is metabolized in mammals (Kennedy et al., 2004; Kemper and Nabb, 2005). At least five studies have examined potential aerobic microbial transformation of 8–2 fluorotelomer alcohol [8–2 FTOH, $F(CF_2)_7CH_2CH_2OH$)] (Dinglasan et al., 2004; Wang et al., 2005a,b, 2009; Liu et al., 2007). The emerging consensus is that PFOA is a common transformation product (with a yield as high as 25%; Wang et al., 2009) and that other transformation products (including ones indicative of minor defluorination; Wang et al., 2005a) can be detected. Liu et al. (2007) suggested that cometabolism was the physiological mechanism of PFOA formation from 8–2 FTOH. Though an OECD respirometric procedure has found PFOA to be inert to aerobic biodegradation (Stasinakis et al., 2008), to our knowledge a thorough investigation of PFOA biodegradation (anaerobic and aerobic) has not been reported. Washington et al. (2009) added acrylate-linked fluorotelemer to soil (2000 ppm) and incubated microcosms under undefined anaerobic/aerobic conditions for 546 d. In this poorly replicated study (six microcosms total), in which abiotic controls were not prepared and the authors admitted that "unacceptable extraction artifacts" were found mid-experiment, degradation of PFOA to 2H-PFOA was suggested by a reportedly quantitative change in the LC/MS/MS peak area of 2H-PFOA. The current investigation utilized a broad suite of enrichment culturing procedures in which distinctive physiological conditions were imposed upon an array of microbial inocula to explore the possibility that PFOA is a readily biodegradable or cometabolizable compound.

2. Materials and methods

2.1. Chemicals and environmental samples

PFOA (96%) was purchased from Sigma–Aldrich. PFOA (99.9%) and ^{14}C-PFOA (ammonium salt, ^{14}C-carboxyl group, 99.3% radiopurity, 57 mCi mmol^{-1}) were provided by N. Wang, DuPont Haskell Global Centers for Health and Environmental Sciences, Newark DE. All other chemicals were reagent grade (Sigma Aldrich). Environmental samples used as inoculum sources in the biodegradation experiments were aseptically gathered (sterile spatula) placed in 0.5 L sterilized canning jars (filled to the brim), stored on ice in the field, and maintained at 4 °C before being transferred to an anaerobic hood (Coy Laboratories, Grass Lake, MI), where samples were degassed and dispensed as slurries in biodegradation assays. Soils and sludges were gathered from: the Ithaca sewage treatment plant; a water-saturated drainage ditch adjacent to the DuPont Chambers Works waste treatment facility in Salem County, New Jersey, previously shown to carry out reductive dechlorination (Fung et al., 2009); the Cornell agricultural field station (Collamer silt loam, Ithaca, NY), the Ithaca fire training facility, and the Rochester, NY fire training facility (the latter two sites were chosen due to potential contamination with fluorinated fire retardant chemicals).

2.1.1. Biodegradation microcosms

Serum bottle-based biodegradation assays utilized the procedure of Yager et al. (1997), Tanner (2002) and Liou et al. (2008). The experimental designs implemented appear in Table 1. The majority of treatments were in triplicate (160-mL serum bottles with 100 mL of media; live ± PFOA and abiotic controls, autoclaved for 1 h). For the ^{14}C-PFOA experiments, 15-mL serum bottles were utilized (50% O_2-free N_2 headspace, 50% inoculated anaerobic test medium) with non-radioactive PFOA and ^{14}C-PFOA ($\sim$4.5 μCi mL^{-1} test medium) to give a final concentration of 100 mg PFOA L^{-1} medium.

To establish the various terminal electron-accepting processes, we used standard anaerobic procedure (Tanner, 2002; Liou et al., 2008). The anaerobic mineral salts buffer (plus vitamins and trace minerals; Tanner, 2002) was used as diluents for the various inoculum sources (5% wt/volume) with addition of electron donors (10 mM sodium acetate ± 40 mM sodium lactate or 0.6 mM ethanol or 2 atm H_2) or electron acceptors [O_2 as air headspace or O_2-free N_2 headspace in each serum bottle with additions of 30 mM nitrate or 4 mg mL^{-1} FeOOH or 10 mM sulfate or 0.4 mM trichloroethene (TCE) or no addition (for the methanogenic treatment)].

Samples (1.0 mL) were periodically removed from each serum bottle, placed in 4-mL glass vials sealed with Al-backed caps, immediately mixed with an equal volume of methanol and stored at -20 °C until analyzed (see below). Accumulated batches of samples from serum vials were analyzed for concentrations of PFOA, ^{14}C-PFOA, fluoride, nitrate, sulfate, and potential PFOA transformation products. Headspace gases were sampled with a gas-tight syringe (250 μL) and analyzed for TCE, vinyl chloride and methane. In the radiotracer study, dissolved ^{14}C activity in the anaerobic medium and in the 0.4 N KOH solution retrieved from the internal reservoir to trap $^{14}CO_2$ were determined by scintillation counting.

To assay potential microbial inhibition by PFOA, triplicate serum-bottle assays inoculated with 5% Ithaca sewage were prepared, as above. Anaerobic preparations (±100 ppm PFOA) were assayed for methanogenesis. Aerobic preparations containing 15 ppm naphthalene were sampled as above and analyzed by high-performance liquid chromatography (HPLC). After filtration through nylon acrodisc filters (0.45 μm; Acrodisc 13 mm Syringe filter, Gelman, Ann Arbor, MI), naphthalene was separated using PAH-Hypersil column (Keystone Scientific, 150 × 4.6 mm at room temperature and at a Shimadzu Sil-10AD auto injector with a Waters model 590 HPLC pump. Methanol–water (1:1) was the mobile phase at a flow rate of 1.5 mL min^{-1}. The eluent was monitored by UV VIS detection (Shimadzu SPD-10A UV–VIS) at 340 nm. Quantification was by comparison to authentic standards.

2.2. Analytical procedures

2.2.1. PFOA by LC/conductivity

PFOA separation was achieved using a Waters model 590 HPLC pump in isocratic mode, and an Acclaim Surfactant column (4.6 × 150 mm, 5 μm, 120 Å packing; Dionex). Mobile phase was 50% methanol/50%10 mM sodium borate applied at a flow rate of