Science of the Total Environment 610–611 (2018) 776–785

Contents lists available at ScienceDirect

Science of the Total Environment

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

Effects of microbial activity on perfluorinated carboxylic acids (PFCAs) generation during aerobic biotransformation of fluorotelomer alcohols in activated sludge

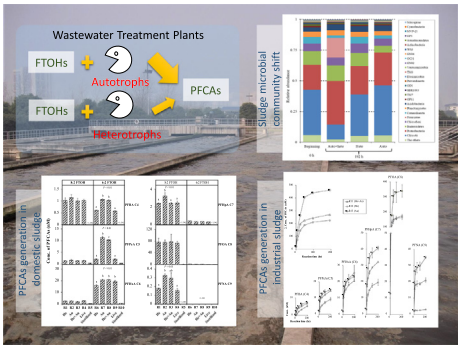
Xiaolong Yu *, Fumitake Nishimura, Taira Hidaka

Department of Environmental Engineering, Graduate School of Engineering, Kyoto University, C1, Kyoto daigaku-Katsura, Kyoto 615-8540, Japan

HIGHLIGHTS

- Microbial activity significantly influenced PFCAs generation from FTOHs in sludge.
- Autotrophs based on ammonia oxidation generate more PFCAs from FTOHs.
- Microbial community in a long-term PFASs-exposed activated sludge is analyzed.
- *Proteobacteria* increased after 8 d at 12 mg/L 8:2 FTOH dose.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 June 2017
Received in revised form 6 August 2017
Accepted 8 August 2017
Available online xxxx

Editor: Jay Gan

Keywords:
FTOHs
PFCAs
Ammonia oxidizing microorganisms
Monooxygenases
Microbial community
Proteobacteria

ABSTRACT

Biotransformation of fluorotelomer alcohols (FTOHs) in wastewater treatment plants (WWTPs) can release toxic intermediates and perfluorinated carboxylic acids (PFCAs) to the aqueous environment. However, little information is known about the role of relevant microbial activity (i.e., autotrophs and/or heterotrophs) in biotransformation of FTOHs. Additionally, the dynamics of microbial community in sludge after exposure to FTOHs remain unclear. In the present research, using domestic and industrial WWTP sludge, we performed lab-scale batch experiments to characterize the FTOHs biodegradation property under aerobic condition. Both heterotrophs and the autotrophs were associated with FTOHs biotransformation. However, the microbial activity influenced PFCAs generation efficiency. Autotrophs based on ammonia oxidation (50 mgN/L) resulted in more effective generation of PFCAs than heterotrophs based on glucose (200 mgC/L) metabolism. Moreover, autotrophs generated more amounts of short-chain PFCAs (carbon number ≤7) than the heterotrophs. The ammonia monooxygenase (AMO) in ammonia oxidizing microorganisms (AOMs) are suggested as responsible for the enhanced generation of PFCAs during FTOHs biotransformation. In the sludge that had been exposed to poly- and perfluorinated alkyl substances in an industrial WWTP, *Chlorobi* was the predominant microorganisms (36.9%), followed by *Proteobacteria* (20.2%), *Bacteroidetes* (11.1%), *Chloroflexi* (6.2%), *Crenarchaeota* (5.6%), *Planctomycetes* (4.2%), and *Acidobacteria* (3.5%). In the present research, the dosed 8:2 FTOH (12.1 mg/L) and its biotransformation products (intermediates and PFCAs) could force a shift in microbial community composition in the sludge. After 192 h, *Proteobacteria* significantly increased and dominated. These results provide knowledge for comprehending the effects of microbial activity on FTOHs biodegradation and the information about interaction between microbial community and the exposure to FTOHs in activated sludge.

* Corresponding author.
E-mail address: yu.xiaolong.76c@st.kyoto-u.ac.jp (X. Yu).

http://dx.doi.org/10.1016/j.scitotenv.2017.08.075
0048-9697/

1. Introduction

The anthropogenic chemicals of poly- and perfluorinated alkyl substances (PFASs; C_nF_{2n+1}-R) have been widely incorporated into consumer products due to their advantages of hydrophobic and lipophobic nature (Lindstrom et al., 2011). Because the carbon–fluorine bonds are extremely inert, PFASs are resistant to degradation (O'Hagan, 2008). Among the thousands of PFASs, the perfluoalkyl carboxylic acids (PFCAs; $C_nF_{2n+1}COOH$) group is one of the best known. Numerous epidemiological studies have revealed the detrimental health effects associated with exposure to long-chain (carbon number ≥8) PFCAs (Lau et al., 2004; Steenland et al., 2010; Vieira et al., 2013; Yang et al., 2017). The large-scale application and the persistent characteristics of PFCAs have made them broadly detected in soil, water, sediment, plants, animals, and humans.

The fluorotelomer alcohols (FTOHs; $C_nF_{2n+1}CH_2CH_2OH$, n = 4–16, or 4:2–16:2 FTOHs) have been used as building blocks in the synthesis of various fluorotelomer-based surfactants (FTSs). Therefore, FTOHs may coexist as impurities in consumer products (Dinglasan-Panlilio and Mabury, 2006; Yuan et al., 2016). During a product's life cycle, FTSs can be decomposed from the products and released into the environment. The released FTSs serve as a long-term source of FTOHs (B. Li et al., 2017a; L. Li et al., 2017b). It has been recognized that FTOHs are known precursors of PFCA (Frömel and Knepper, 2010; Liu and Mejia Avendaño, 2013; Parsons et al., 2008; Washington and Jenkins, 2015). The biotransformation of 8:2 FTOH ($F(CF_2)_8CH_2CH_2OH$) and 6:2 FTOH ($F(CF_2)_6CH_2CH_2OH$) has been widely investigated in mixed microbial systems like soil (Liu et al., 2010; J Liu et al., 2007; Wang et al., 2009), sediment (Dinglasan et al., 2004; Zhao et al., 2013a), and activated sludge (Wang et al., 2005a, 2005b; Zhang et al., 2013). In activated sludge, the published results suggested that the microorganisms seemed to perform efficient FTOHs biodegradation and PFCAs generation under aerobic conditions (Wang et al., 2005b; Yu et al., 2016; Zhang et al., 2013; Zhao et al., 2013b). It prompts us to hypothesize that certain indigenous aerobic microbial consortia existing in activated sludge may possess catabolic enzymes that are capable of performing efficient FTOHs biotransformation.

For the activated sludge process in a wastewater treatment plant (WWTP), it is known that conventional pollutants (i.e., ammonia, organic compounds, etc.) are removed as primary substrates (growth substrates) via the enzymatic metabolism of microorganisms. For micropollutants (i.e., pharmaceuticals, estrogens, pesticides, FTOHs, etc.), biotransformation probably takes place via a cometabolism process. In the cometabolism process, the microbes cannot benefit from biotransforming micropollutants, but they can use the non-specific enzymes that are induced by the growth substrates to occasionally attack and degrade the micropollutants (Fernandez-Fontaina et al., 2014). Since the organic and inorganic conventional pollutants existing in sewage may be metabolized by heterotrophs and autotrophs, respectively, both heterotrophs and autotrophs are able to use the enzymes expressed during primary substrates oxidation to cometabolize (biotransform) micropollutants like FTOHs in activated sludge. However, previous researches on FTOHs biotransformation in activated sludge mostly focused on exploring biodegradation pathways and identifying biotransformed products; little information is known about the role of relevant microbial activity (i.e., autotrophs and/or heterotrophs) in the aerobic biotransformation of FTOHs. It is important to clarify the ways in which bioactivities are included in FTOHs biodegradation because it can provide practical knowledge applicable to the control of PFCAs pollution in WWTPs.

In the present research, the sludge collected from an industrial WWTP had long been exposed to PFASs including FTOHs. Within the sludge, rapid PFCAs generation was observed during 8:2 FTOH biodegradation under an aerobic condition (Yu et al., 2016). We suggest that effective FTOHs biotransformation may be related to the heterogeneous microorganism population in the sludge. Additionally, researches have revealed that high concentration of PFASs can lead to a shift in microbial compositions and reduce microbial community diversity in sediment (Sun et al., 2016; Zhang et al., 2017) and soil (B. Li et al., 2017a). However, in WWTPs, the effect of FTOHs on microbial community remains unknown. The clarification on interaction between FTOHs biotransformation and microbial community changes can provide information for better understanding the microbial toxicity of PFASs in WWTPs.

Therefore, the aims of the present research were 1) to explore the effects of autotrophic and heterotrophic bioactivities on PFCAs generation property during FTOHs biotransformation within activated sludge; and 2) to investigate the microbial community of the industrial WWTP activated sludge and estimate the impact of 8:2 FTOH biodegradation on microbial community in the sludge. In the present research, activated sludges collected from domestic and industrial WWTPs were used to perform lab-scale batch experiments under designed conditions of microbial activity. Moreover, a comparatively high concentration of 8:2 FTOH (12.1 mg/L) was dosed into the sludge to mimic the environmental relevant extreme condition. After exposure to high 8:2 FTOH concentration, the 8:2 FTOH and a set of new compounds (intermediates and PFCAs) may have impacted the original microbial community in the sludge. The microbial analysis of the sludge was performed to clarify the heterogeneous microorganism populations and the shift in microbial community composition after exposure to 8:2 FTOH.

2. Materials and methods

2.1. Chemicals

The PFASs treated in the present study are listed in Table S1a, along with basic information about chemical names, acronyms, and molecular structures. Reagents of 6:2 FTOH (1H, 1H, 2H, 2H-Tridecafluoro-1-n-octanol, CAS#647-42-7, >98.0% pure) and 8:2 FTOH (1H, 1H, 2H, 2H-Heptadecafluoro-1-decanol, CAS#678-39-7, >96.0% pure) were purchased from Tokyo Chemical Industry (Tokyo, Japan). A standard solution used for quantifying PFCAs (PFAC-MXB) containing perfluorobutanoic acid (C4, PFBA), perfluoropentanoic acid (C5, PFPeA), perfluorohexanoic acid (C6, PFHxA), perfluoroheptanoic acid (C7, PFHpA), perfluorooctanoic acid (C8, PFOA), and perfluorononanoic acid (C9, PFNA) (as well as others PFCAs not discussed in this paper) was purchased from Wellington Laboratories Japan Inc. (manufactured in Guelph, Canada). A standard solution of mass-labeled PFCAs used as a surrogate (MPFAC-MXA) containing perfluoro-n-[$^{13}C_4$]butanoic acid (MPFBA), perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid (MPFHxA), perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid (MPFOA), and perfluoro-n-[1,2,3,4,5-$^{13}C_5$]nonanoic acid (MPFNA) (as well as others mass-labeled PFCAs not discussed in this paper) was also purchased from Wellington Laboratories Japan Inc. (manufactured in Guelph, Canada). The purity of the individual chemicals in PFAC-MXB and MPFAC-MXA was >98%. Perfluoro-n-[$^{13}C_8$]octanoic acid (M8PFOA) used as a syringe spike was purchased from Wellington Laboratories Japan Inc. (manufactured in Guelph, Canada). The other organic media used for sample pretreatment, and the inorganic chemicals used for sludge cultivation, are detailed in Table S1b.

2.2. Activated sludge collection and culture preparation

A return activated sludge (denoted "sludge-A") was collected from a domestic WWTP in Kyoto, Japan. A second activated sludge (denoted "sludge-B") was collected from an industrial WWTP in Kobe, Japan; this WWTP received dyeing wastewater containing PFASs. Our previous research found that sludge-B was acclimated to FTOHs biotransformation (Yu et al., 2016). Therefore, sludge-B was selected in the present research to represent an efficient FTOH-biodegradable sludge medium. Both of the activated sludge were collected using 10 L rinsed stainless steel containers and transported to the laboratory within 2 h. The sludge preparation was initiated in the same day when the sludge was