Impact of Perfluoroalkyl Substances on Microbial Membranes and Microbial Functions

A Dissertation
SUBMITTED TO THE FACULTY OF
UNIVERSITY OF MINNESOTA
BY

Nicole Jean Mohapp Fitzgerald

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

Dr. Paige Novak & Dr. Matt Simcik

September 2017

Acknowledgements

The completion of my PhD dissertation would not have been possible without the support from my family and friends as well as my advisers. My parents set a great example and encouraged my health and sanity. They both work very hard and were very supportive in my studies, although growing up my mom was constantly trying to get me to prioritize sleep over work. Since we first met in Madison, my husband has provided a strong shoulder to lean on and is always there to help when he is needed. From jumper cables to charge dead moped batteries to lending his car for grocery shopping I could always count on him. Now that I am in graduate school, he has volunteered to listen to nearly all my presentations (often more than once) and has provided unending writing edits. Additionally, my friends (near and far, old and new) provided a listening ear when times were hard and supplied necessary distraction. They have always been there to celebrate minor milestones (birthdays, small laboratory success etc.) Lastly, I would like to acknowledge my advisers Paige and Matt. They were instrumental in the process and remained positive even when progress was painstakingly slow. Their guidance and support made a world of difference.

Dedication

I would like to dedicate my work to my loving husband. Thank you for your unending support and encouragement.

Abstract

Perfluoroalkyl substances (PFAS) are strictly manmade compounds that are ubiquitous in environmental systems as a result of use in many industrial and consumer products. They have amphiphilic properties and are expected to partition into cellular membranes where they may disrupt membrane properties. While PFAS have been associated with a variety of biotic effects including an increased susceptibility to co-contaminants, their primary mechanism of action is unknown. It is also unknown if any observed effects on cellular membranes can be translated to impacts on microbial function. A few studies have cited evidence of altered permeability of biological membranes upon exposure to PFAS, though this has not been conclusively demonstrated. A change in permeability could increase or decrease membrane diffusion, having ramifications on microbial functions including quorum sensing and co-contaminant toxicity. Elevated concentrations of PFAS and co-contaminants are often observed in landfills, wastewater treatment plants, and contaminated sites (i.e. military bases) where microorganisms are critical components of waste treatment systems designed to protect human health. Given the importance of microorganisms in nutrient cycling, I studied the effect of PFAS on microbial function and microbial membrane permeability. Specifically, the effects of PFAS on (1) bacterial membrane partitioning, (2) quorum sensing, and (3) anaerobic digester function were investigated.

Results indicate that PFAS partition into microbial membranes, which leads to increased fluidity and permeability; although these effects on cell membranes did not explain all functional changes observed in this study. PFAS partitioned to model

membranes and bacteria, where accumulation was a product of the functional group and fluorinated chain length. In model membranes, PFAS increased membrane fluidity in a manner dependent on dose and PFAS characteristics (functional group and fluorinated chain length). Functional changes were observed in a pure culture of a quorum-sensing bacteria, *Aliivibrio fischeri*. In this case, cultures that were exposed to PFAS were brighter (enhanced quorum sensing function) after a signaling molecule was amended. Increased luminescence was likely a result of increased membrane permeability, resulting in increased diffusion of the signaling molecule. Effects on luminescence were detected at 10 μg/L in PFAS containing eight fluorinated carbons. Lastly, in a mixed anaerobic digester community, the presence of PFAS and aqueous film forming foam, a product that contains g/L concentrations of PFAS, inhibited methane production and the degradation of a co-contaminant, 2,4-dichlorophenol. In each study, the observed effects were correlated to the functional group and fluorinated chain length and were generally only observed when PFAS was present at concentrations greater than 50 mg/L. Results from this study will help the scientific community better understand the range of microbial effects associated with PFAS exposure and the primary PFAS chemical characteristics associated with effects (i.e. the functional group and chain length).

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

Perfluoroalkyl substances (PFAS) are anthropogenic compounds used in many consumer and industrial products and processes. In domestic products they are primarily used as surface active agents for protection of textiles and food packaging from oil, grease, water, and dirt.[1] They are also widely used in aqueous film-forming foams (AFFF) used to fight hydrocarbon fires at locations such as military bases and airports.[2] In industrial processes they are used to aid in the production of other fluoropolymers such as those used in nonstick cookware, waterproof clothing, electrical wire casing, chemical and fire-resistant tubing, and plumbing seal tape.[3] PFAS are also used as surface agents in high temperature and acid/base applications.[3] Because of health concerns, eight carbon (8C) perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are being phased out. Therefore, the production of PFAS with different configurations and short-chains (<8C), such as perfluorobutane sulfonate (PFBS) and perfluorobutanoic acid (PFBA), has increased.[4,5] For example, an 8C PFAS was phased out of the consumer stain repellant Scotchgard, and was replaced with its 4C homologue.[4,6] The physical-chemical properties of PFAS make them attractive constituents in consumer products, and despite bans on a few compounds it is unlikely that the use of these fluorinated chemicals will stop.

As a result of their extensive use, PFAS are ubiquitous in the environment and can be found at concentrations that span multiple orders of magnitude (Figure 1.1).[7] PFAS are strictly anthropogenic compounds and it is no surprise that the highest concentrations of these compounds are observed in human waste streams (wastewater treatment effluents and landfill leachate).[8–10] The main sources of PFAS are stain

repellent-treated carpets, waterproof apparel, and aqueous film-forming foams.[11] They enter landfills and wastewater plants through household and industrial disposal. Wastewater treatment plants provide a route for PFAS to the environment because PFAS are not easily degraded.[9,12] Some PFAS sorb to wastewater solids and can enter the environment via land application of biosolids.[13] Industrial activity is associated with higher concentrations of PFAS in waste streams and the environment, with direct emissions from products between the years 1970 – 2002 resulting in 450-2,700 tons of PFOS in wastewater streams.[11] The use of high concentrations of PFAS in AFFF also provides a direct route to the environment, as AFFF is sprayed directly over land or water to fight fires.[2] PFAS are therefore found at elevated concentrations in surface and ground waters receiving discharge from areas where these foams were used, such as at airports, Air Force bases and military fire-fighting training areas.[2,14,15] Additionally, PFAS are capable of long range transport and can be found in isolated environments such as the arctic.[7] Concentrated use and transfer of PFAS between products, waste streams, and the environment has resulted in their uneven yet ubiquitous presence.

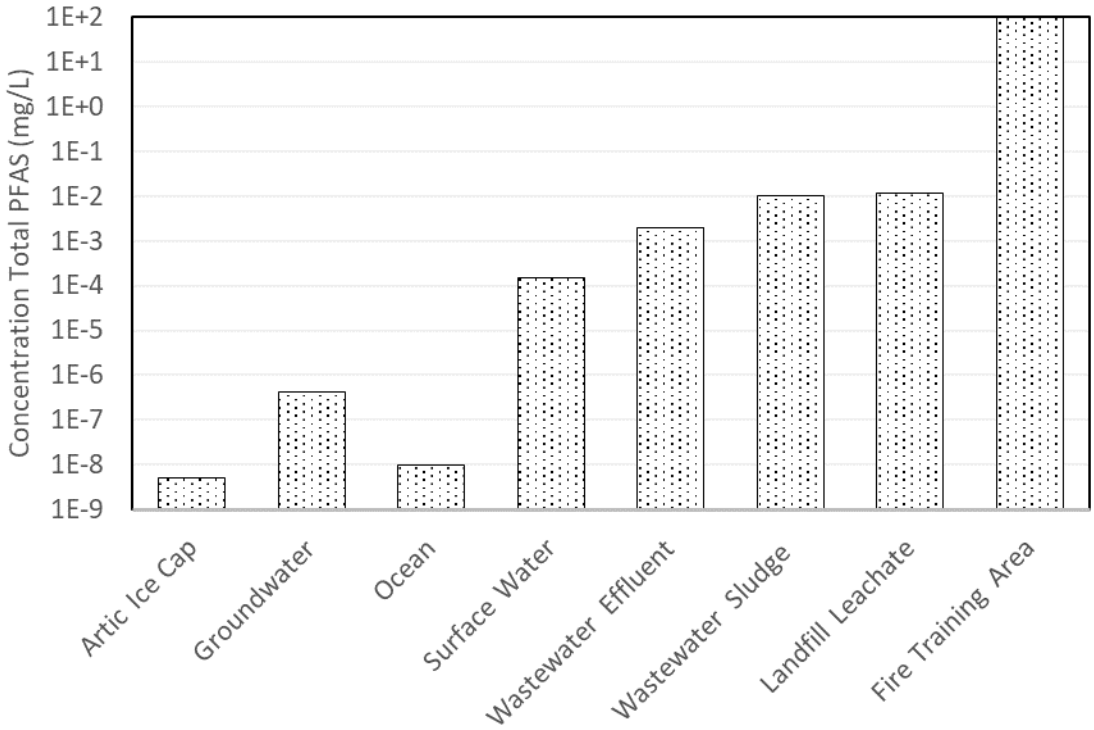


Figure 1.1. Concentration range of PFAS in the environment. Bars represent the range of concentrations detected in each environment; the top of the bar shows maximum concentration reported. Data was summarized from peer reviewed literature.[7–10,13,14,16–22]

PFAS persist in the environment because they are extremely resistant to degradation and are relatively soluble. The carbon-fluorine bond is incredibly strong (105.4 kcal/mol) and imparts stability to the fluorinated chain.[23] In fact, degradation has been studied in processes including photolysis, biodegradation, or exposure to radicals, and there are only a few processes by which defluorination occurs and they often require high energy inputs.[7,16,24–27] The functional group of PFAS, however, can undergo transformation. Precursors such as N-ethyl perfluorooctane sulfonamide acetate, perfluorooctane sulfonamide acetate, 8-2 fluorotelemer alcohol, and N-ethyl perfluorooctane sulfonamide ethyl alcohol have been found to transform to PFOA or

PFOS by means of biotransformation and indirect photolysis.[25,27–32] These transformations have been found to occur in environments such as wastewater and soil.[25,27–32] The chemical properties of PFAS enable them to persist in the environment in dissolved states for long periods of time.

In addition to their persistence in the environment, PFAS have also been found to persist in biota where they are associated with a wide variety of biological effects.[33–40] In humans, PFOS and PFOA serum concentrations are positively associated with serum concentrations of cholesterol and other lipids such as low density lipoprotein cholesterol and triglycerides.[38,41] PFAS have also been shown to bind to the thyroid hormone receptor and disrupt thyroid hormone-mediated pathways.[39,42] In children, the serum concentrations of PFOS and PFOA was negatively correlated with serum antibody levels suggesting reduced immune response to vaccines.[43] Other nonhuman effects that have been observed include the inhibition of gap junction communication in dolphin and rat epithelial cells[44] as well as increased oxygen consumption in muscles[45]. In *Escherichia coli* it has been shown that exposure to PFAS can increase the induction of genes related to membrane damage, oxidative damage, cellular and osmotic damage, and DNA damage.[46] While PFAS have been associated with a wide variety of effects, there is not yet conclusive mechanistic knowledge about how these compounds disrupt biological systems or whether the mechanisms of toxicity and/or damage are similar in different organisms, such as higher organisms versus bacteria.

Bacteria used for crucial nutrient cycling and degradation functions are often exposed to elevated concentrations of PFAS and other pollutants, making it important to understand the details and consequences of PFAS on bacteria.[47] The concentrations of PFAS are often highest in waste streams (Figure 1.1) where bacteria are relied upon to degrade organic waste, produce energy (methane), and cycle nutrients. While humans rely on bacteria to perform such important activities, there are very few studies on the effects of PFAS on bacteria. Indeed, 4-8C PFAS have been shown to cause toxicity to bacteria with EC_{50} values between 35 to 17,520 mg/L depending on the strain and PFAS tested.[24,48,49] Nevertheless, biological effects, such as membrane disruption, changes in membrane permeability, increased amounts of bound extracellular polymeric substance, and competition for binding sites on proteins have been shown to occur at subtoxic levels of PFAS[34,39,50–52]. The objective of this dissertation research was to better understand how PFAS impact microbial communities, both at the level of cell structure and cell function. The specific objectives of each dissertation chapter are summarized below.

Chapter 3 Summary. PFAS partitioning and accumulation in model lipids and bacteria

It is difficult to predict the fate of PFAS in cells because PFAS are both oleophobic and hydrophobic. I hypothesized that PFAS would accumulate in membrane lipids because they have a hydrophilic head and hydrophobic tail. Several studies also have demonstrated increased fluidity upon exposure to PFAS in model membranes.[53–57] Nevertheless, studies have also shown *in vitro* interaction between PFAS and

protein.[39,58–60] My results demonstrated that PFAS accumulated in model phospholipid membranes and bacteria. In liposomes, PFAS exposure decreased zeta potential, while this effect was not observed in bacteria, suggesting that PFAS accumulated into the inner phospholipid membrane as opposed to depositing on the cell surface. The degree of PFAS accumulation in model membranes and bacteria varied with fluorinated chain length and functional group; deposition increased with fluorinated chain length and the presence of a sulfonate functional head group. Additionally, uptake into living and dead bacteria was statistically similar for all PFAS except PFHxS. This suggests that PFAS uptake into prokaryotes was passive. In model membranes, PFAS increased membrane fluidity, which was dependent on PFAS dose, fluorinated chain length, and functional group. Similar to other results, changes in fluidity were greater for sulfonates and increased with fluorinated chain length.

While I designed the study and analyzed results, this study would not have been possible without help from Dr. Andreas Wargenau and Carlise Sorenson. Dr. Wargenau helped to execute initial experiments to measure the impact that PFAS had on model membranes. Ms. Sorenson, executed experiments measuring changes in zeta potential after PFAS exposure. I executed all other experiments (phase transition, partitioning to bacteria).

Chapter 4 Summary. PFAS increase membrane permeability and quorum sensing response in *Aliivibrio fischeri*

Quorum sensing is the manner in which bacteria can assess population density and coordinate function and gene expression. It requires the production and receipt of signaling molecules, often types of acyl homoserine lactones (AHLs).[61,62] In systems utilizing N-(β-ketocaproyl)-L-homoserine lactone as the signaling molecule, the AHL must passively diffuse across the membrane and then interact with a receptor, beginning a regulatory cascade that results in the expression of particular genes.[61–63] Given the hypothesized ability of PFAS to alter membrane properties, the diffusion of AHL may also be modified in the presence of PFAS. It is necessary to understand the effect that PFAS might have on quorum sensing, as processes regulated by quorum sensing can be extremely important, including biofilm formation, antibiotic production, and pathogenicity.[62] Here experiments were performed in pure cultures of well-characterized bacteria to understand whether and how PFAS affected quorum sensing and membrane permeability in *Aliivibrio fischeri*. My results showed, an increase in quorum sensing-mediated luminescence and membrane permeability in *A. fischeri* mutant DC43 following exposure to PFAS. An increase in luminescence was observed in cultures exposed to 10 μg/L PFOS and perfluorononanoate (PFNA). The increase in luminescence was more dramatic as the dose and fluorinated chain length of the PFAS increased, except with PFOS. There was a decrease in luminescence for PFOS at 50 mg/L, at which concentration PFOS appeared to be toxic to *A. fischeri*. The increase in luminescence in the presence of other PFAS was likely a result of increased membrane permeability. This

was supported by results that showed that the rate of diffusion for the membrane semi-permeable dye, 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI), was greater in *A. fischeri* DC 43 exposed to 50 mg/L PFAS.

Chapter 5 Summary. Functional consequences of PFAS and AFFF exposure in anaerobic communities

Literature and experimental evidence suggest that PFAS may increase the toxicity of co-contaminants.[64–66] This effect, however, appears to be dependent on the dose and chemical characteristics of the co-contaminants.[67] Furthermore, little is known about the effect of PFAS and co-contaminants on prokaryotic cells. Because AFFF is an important contaminant and contains a mixture of PFAS as well as other compounds, the goal of this study was to determine the effect PFAS exposure has, in the presence and absence of co-contaminants, on anaerobic community function. In this chapter, experiments were performed with a diluted anaerobic digester community to determine whether PFAS changed important community functions and whether AFFF could be modeled by a single PFAS (e.g., PFOS) Two performance metrics were used to assess community function: methane production and co-contaminant degradation. AFFF and PFAS did in fact effect microbial function in an anaerobic digester community. The reduction in methane production following exposure to AFFF was explainable by the major PFAS component of AFFF, PFOS. Additionally, mixtures containing either AFFF, 50 mg/L PFOS, or 50 mg/L PFOS + 8.8 mg/L PFHxS and the co-contaminant, 2,4-dichlorophenol (DCP) were less toxic than the presence of the PFAS components alone, demonstrating that (1) PFAS

have the ability to alter co-contaminant toxicity and (2) AFFF behavior is similar to that of its major PFAS component, PFOS. Changes in toxicity could be a result of physiological effects or physical chemical effects. In addition to decreasing methane production, AFFF and PFAS inhibited the degradation of DCP. In this case, the degradation inhibition caused by AFFF was not explained by that caused by the major PFAS components of AFFF.

References

(1) Stasinakis, A. S.; Petalas, A. V; Mamais, D.; Thomaidis, N. S. Application of the OECD 301F respirometric test for the biodegradability assessment of various potential endocrine disrupting chemicals. *Bioresour. Technol.* **2008**, *99* (9), 3458–3467 DOI: 10.1016/j.biortech.2007.08.002.

(2) Moody, C. A.; Field, J. A. Perfluorinated Surfactants and the Environmental Implications of Their Use in Fire-Fighting Foams. *Environ. Sci. Technol.* **2000**, *34* (18), 3864–3870 DOI: 10.1021/es991359u.

(3) EPA. *Emerging Contaminants – Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA) At a Glance*; 2012.

(4) Renner, R. The long and the short of perfluorinated replacements. *Environ. Sci. Technol.* **2006**, *40* (1), 12–13 DOI: 10.1021/es062612a.

(5) Wang, Z.; Cousins, I. T.; Scheringer, M.; Hungerbühler, K. Fluorinated alternatives to long-chain perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFSAs) and their potential precursors. *Environ. Int.* **2013**, *60* (2013), 242–248 DOI: 10.1016/j.envint.2013.08.021.

(6) Chu, S.; Letcher, R. J. In vitro metabolic formation of perfluoroalkyl sulfonamides from copolymer surfactants of pre- and post-2002 scotchgard fabric protector products. *Environ. Sci. Technol.* **2014**, *48* (11), 6184–6191 DOI: 10.1021/es500169x.

(7) Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J. Perfluoroalkyl Acids : A Review of Monitoring and Toxicological Findings. *Toxicol. Sci.* **2007**, *99* (2), 366–394 DOI: 10.1093/toxsci/kfm128.

(8) Busch, J.; Ahrens, L.; Sturm, R.; Ebinghaus, R. Polyfluoroalkyl compounds in

landfill leachates. *Environ. Pollut.* **2010**, *158* (5), 1467–1471 DOI: 10.1016/j.envpol.2009.12.031.

(9) Sinclair, E.; Kannan, K. Mass loading and fate of perfluoroalkyl surfactants in wastewater treatment plants. *Environ. Sci. Technol.* **2006**, *40* (5), 1408–1414.

(10) Zareitalabad, P.; Siemens, J.; Hamer, M.; Amelung, W. Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in surface waters, sediments, soils and wastewater - A review on concentrations and distribution coefficients. *Chemosphere* **2013**, *91* (6), 725–732 DOI: 10.1016/j.chemosphere.2013.02.024.

(11) Paul, A. G.; Jones, K. C.; Sweetman, A. J. A First Global Production , Emission , And Environmental Inventory For Perfluorooctane Sulfonate. **2012**, *43* (2), 386–392.

(12) Xiao, F.; Halbach, T. R.; Simcik, M. F.; Gulliver, J. S. Input characterization of perfluoroalkyl substances in wastewater treatment plants: Source discrimination by exploratory data analysis. *Water Res.* **2012**, *46* (9), 3101–3109 DOI: 10.1016/j.watres.2012.03.027.

(13) Konwick, B. J.; Tomy, G. T.; Ismail, N.; Peterson, J. T.; Fauver, R. J.; Higginbotham, D.; Fisk, A. T. Concentrations and patterns of perfluoroalkyl acids in Georgia, USA surface waters near and distant to a major use source. *Environ. Toxicol. Chem.* **2008**, *27* (10), 2011–2018 DOI: 10.1897/07-659.1.

(14) Arias E, V. a; Mallavarapu, M.; Naidu, R. Identification of the source of PFOS and PFOA contamination at a military air base site. *Environ. Monit. Assess.* **2015**, *187* (1), 4111 DOI: 10.1007/s10661-014-4111-0.

(15) de Solla, S. R.; De Silva, a O.; Letcher, R. J. Highly elevated levels of perfluorooctane sulfonate and other perfluorinated acids found in biota and surface water downstream of an international airport, Hamilton, Ontario, Canada. *Environ. Int.* **2012**, *39* (1), 19–26 DOI: 10.1016/j.envint.2011.09.011.

(16) Rayne, S.; Forest, K. Perfluoroalkyl sulfonic and carboxylic acids: a critical review of physicochemical properties, levels and patterns in waters and wastewaters, and treatment methods. *J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng.* **2009**, *44* (12), 1145–1199 DOI: 10.1080/10934520903139811.

(17) Moody, C. A.; Field, J. A. Determination of Perfluorocarboxylates in Groundwater Impacted by Fire-Fighting Activity. *Environ. Sci. Technol.* **1999**, *33* (16), 2800–2806 DOI: 10.1021/es981355+.

(18) Huset, C. a; Barlaz, M. a; Barofsky, D. F.; Field, J. a. Quantitative determination of fluorochemicals in municipal landfill leachates. *Chemosphere* **2011**, *82* (10), 1380–1386 DOI: 10.1016/j.chemosphere.2010.11.072.

(19) Eggen, T.; Moeder, M.; Arukwe, A. Municipal landfill leachates: a significant source for new and emerging pollutants. *Sci. Total Environ.* **2010**, *408* (21), 5147–5157 DOI: 10.1016/j.scitotenv.2010.07.049.

(20) Loos, R.; Wollgast, J.; Huber, T.; Hanke, G. Polar herbicides, pharmaceutical products, perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and nonylphenol and its carboxylates and ethoxylates in surface and tap waters around Lake Maggiore in Northern Italy. *Anal. Bioanal. Chem.* **2007**, *387* (4), 1469–1478 DOI: 10.1007/s00216-006-1036-7.

(21) Kim, S.-K.; Kannan, K. Perfluorinated acids in air, rain, snow, surface runoff, and lakes: relative importance of pathways to contamination of urban lakes. *Environ. Sci. Technol.* **2007**, *41* (24), 8328–8334.

(22) Nakayama, S.; Strynar, M. J.; Helfant, L.; Egeghy, P.; Ye, X.; Lindstrom, A. B. Perfluorinated compounds in the Cape Fear Drainage Basin in North Carolina. *Environ. Sci. Technol.* **2007**, *41*, 5271–5276 DOI: 10.1021/es070792y.

(23) O'Hagan, D. Understanding organofluorine chemistry. An introduction to the C-F bond. *Chem. Soc. Rev.* **2008**, *37* (2), 308–319 DOI: 10.1039/b711844a.

(24) Ochoa-Herrera, V.; Field, J. A.; Luna-Velasco, A.; Sierra-Alvarez, R. Microbial toxicity and biodegradability of perfluorooctane sulfonate (PFOS) and shorter chain perfluoroalkyl and polyfluoroalkyl substances (PFASs). *Environ. Sci. Process. Impactts* **2016**, *18* DOI: 10.1039/c6em00366d.

(25) Plumlee, M. H.; McNeill, K.; Reinhard, M. Indirect Photolysis of Perfluorochemicals: Hydroxyl Radical-Initiated Oxidation of N-Ethyl Perfluorooctane Sulfonamido Acetate (N-EtFOSAA) and Other Perfluoroalkanesulfonamides. *Environ. Sci. Technol.* **2009**, *43* (20), 3662–3668 DOI: 10.1021/es902634x.

(26) Vaalgamaa, S.; Vähätalo, A. V; Perkola, N.; Huhtala, S. Photochemical reactivity of perfluorooctanoic acid (PFOA) in conditions representing surface water. *Sci. Total Environ.* **2011**, *409* (16), 3043–3048 DOI: 10.1016/j.scitotenv.2011.04.036.

(27) Liou, J. S.-C.; Szostek, B.; DeRito, C. M.; Madsen, E. L. Investigating the biodegradability of perfluorooctanoic acid. *Chemosphere* **2010**, *80* (2), 176–183 DOI: 10.1016/j.chemosphere.2010.03.009.

(28) Nguyen, T. V.; Reinhard, M.; Gin, K. Y.-H. Rate laws and kinetic modeling of N-ethyl perfluorooctane sulfonamidoethanol (N-EtFOSE) transformation by hydroxyl radical in aqueous solution. *Water Res.* **2013**, *47* (7), 2241–2250 DOI: 10.1016/j.watres.2013.01.047.

(29) Dinglasan, M. J. a; Ye, Y.; Edwards, E. a; Mabury, S. a. Fluorotelomer alcohol

biodegradation yields poly- and perfluorinated acids. *Environ. Sci. Technol.* **2004**, *38* (10), 2857–2864.

(30) Lange, C. C.; Lundberg, J. K.; Services, P. A.; Division, S. S. The Aerobic Biodegradation of N -EtFOSE Alcohol by the Microbial Activity Present in Municipal Wastewater Treatment Sludge Table of Contents. **2000**.

(31) Benskin, J. P.; Ikonomou, M. G.; Gobas, F. a P. C.; Begley, T. H.; Woudneh, M. B.; Cosgrove, J. R. Biodegradation of N-ethyl perfluorooctane sulfonamido ethanol (EtFOSE) and EtFOSE-based phosphate diester (SAmPAP diester) in marine sediments. *Environ. Sci. Technol.* **2013**, *47* (3), 1381–1389 DOI: 10.1021/es304336r.

(32) Wang, N.; Szostek, B.; Buck, R. C.; Folsom, P. W.; Sulecki, L. M.; Gannon, J. T. 8-2 Fluorotelomer Alcohol Aerobic Soil Biodegradation: Pathways, Metabolites, and Metabolite Yields. *Chemosphere* **2009**, *75* (8), 1089–1096 DOI: 10.1016/j.chemosphere.2009.01.033.

(33) Jensen, A. A.; Leffers, H. Emerging endocrine disrupters: perfluoroalkylated substances. *Int. J. Androl.* **2008**, *31* (2), 161–169 DOI: 10.1111/j.1365-2605.2008.00870.x.

(34) Luebker, D. J.; Hansen, K. J.; Bass, N. M.; Butenhoff, J. L.; Seacat, A. M. Interactions of fluorochemicals with rat liver fatty acid-binding protein. *Toxicology* **2002**, *176* (3), 175–185.

(35) Melzer, D.; Rice, N.; Depledge, M. H.; Henley, W. E.; Galloway, T. S. Association Between Serum Perfluorooctanoic Acid (PFOA) & Thyroid Disease in the U . S . National Health and Nutrition Examination Survey. *Environ. Health Perspect.* **2010**, *118* (5), 686–692 DOI: 10.1289/ehp.0901584.

(36) Naile, J. E.; Wiseman, S.; Bachtold, K.; Jones, P. D.; Giesy, J. P. Transcriptional effects of perfluorinated compounds in rat hepatoma cells. *Chemosphere* **2012**, *86* (3), 270–277 DOI: 10.1016/j.chemosphere.2011.09.044.

(37) Reistad, T.; Fonnum, F.; Mariussen, E. Perfluoroalkylated compounds induce cell death and formation of reactive oxygen species in cultured cerebellar granule cells. *Toxicol. Lett.* **2013**, *218*, 56–60.

(38) Steenland, K.; Fletcher, T.; Savitz, D. a. Epidemiologic evidence on the health effects of perfluorooctanoic acid (PFOA). *Environ. Health Perspect.* **2010**, *118* (8), 1100–1108 DOI: 10.1289/ehp.0901827.

(39) Weiss, J. M.; Andersson, P. L.; Lamoree, M. H.; Leonards, P. E. G.; Leeuwen, S. P. J. Van; Hamers, T. Competitive Binding of Poly- and Perfluorinated Compounds to the Thyroid Hormone Transport Protein Transthyretin. *Toxicol. Sci.*

2009, *109* (2), 206–216 DOI: 10.1093/toxsci/kfp055.

(40) Yu, W.; Liu, W.; Jin, Y.; Liu, X.-H.; Wang, F.-Q.; Liu, li; Nakayama, S. F. Prenatal and Postnatal Impact of Perfluorooctane Sulfonate (PFOS) on Rat Development : A Cross-Foster Study on Chemical Burden and Thyroid Hormone System. *Environ. Sci. Technol.* **2009**, *43* (21), 8416–8422.

(41) Geiger, S. D.; Xiao, J.; Ducatman, A.; Frisbee, S.; Innes, K.; Shankar, A. The association between PFOA, PFOS and serum lipid levels in adolescents. *Chemosphere* **2014**, *98*, 78–83 DOI: 10.1016/j.chemosphere.2013.10.005.

(42) Ren, X.-M.; Zhang, Y.-F.; Guo, L.-H.; Qin, Z.-F.; Lv, Q.-Y.; Zhang, L.-Y. Structure-activity relations in binding of perfluoroalkyl compounds to human thyroid hormone T3 receptor. *Arch. Toxicol.* **2015**, *89* (2), 233–242 DOI: 10.1007/s00204-014-1258-y.

(43) Grandjean, P.; Andersen, E. W. Serum Vaccine Antibody Concentrations in Children Exposed to Perfluorinated Compounds. *J. Am. Med. Assoc.* **2012**, *307* (4), 391–397.

(44) Hu, W.; Jones, P. D.; Upham, B. L.; Trosko, J. E.; Lau, C.; Giesy, J. P. Inhibition of gap junctional intercellular communication by perfluorinated compounds in rat liver and dolphin kidney epithelial cell lines in vitro and Sprague-Dawley rats in vivo. *Toxicol. Sci.* **2002**, *68* (2), 429–436.

(45) Fernández-Sanjuan, M.; Faria, M.; Lacorte, S.; Barata, C. Bioaccumulation and effects of perfluorinated compounds (PFCs) in zebra mussels (Dreissena polymorpha). *Environ. Sci. Pollut. Res. Int.* **2013**, *20* (4), 2661–2669 DOI: 10.1007/s11356-012-1158-8.

(46) Nobels, I.; Dardenne, F.; De Coen, W.; Blust, R. Application of a multiple endpoint bacterial reporter assay to evaluate toxicological relevant endpoints of perfluorinated compounds with different functional groups and varying ch ... Toxicology in Vitro functional groups and varying chain length. *Toxicol. Vitr.* **2010**, *24*, 1768–1774 DOI: 10.1016/j.tiv.2010.07.002.

(47) Loos, R.; Carvalho, R.; António, D. C.; Comero, S.; Locoro, G.; Tavazzi, S.; Paracchini, B.; Ghiani, M.; Lettieri, T.; Blaha, L.; et al. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. *Water Res.* **2013**, *47* (17), 6475–6487 DOI: 10.1016/j.watres.2013.08.024.

(48) Ding, G.; Peijnenburg, W. J. G. M. Physicochemical Properties and Aquatic Toxicity of Poly- and Perfluorinated Compounds. *Crit. Rev. Environ. Sci. Technol.* **2013**, *43* (6), 598–678 DOI: 10.1080/10643389.2011.627016.

(49) Rosal, R.; Rodea-Palomares, I.; Boltes, K.; Fernández-Piñas, F.; Leganés, F.; Petre, A. Ecotoxicological assessment of surfactants in the aquatic environment: combined toxicity of docusate sodium with chlorinated pollutants. *Chemosphere* **2010**, *81* (2), 288–293 DOI: 10.1016/j.chemosphere.2010.05.050.

(50) Pasquini, L.; Merlin, C.; Hassenboehler, L.; Munoz, J.-F.; Pons, M.-N.; Gorner, T. Impact of certain household micropollutants on bacterial behavior. Toxicity tests/study of extracellular polymeric substances in sludge. *Sci. Total Environ.* **2013**, *463–464* (August 2016), 355–365 DOI: 10.1016/j.scitotenv.2013.06.018.

(51) Hu, W. yue; Jones, P. D.; DeCoen, W.; King, L.; Fraker, P.; Newsted, J.; Giesy, J. P. Alterations in cell membrane properties caused by perfluorinated compounds. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2003**, *135* (1), 77–88 DOI: 10.1016/S1532-0456(03)00043-7.

(52) Liu, W.; Chen, S.; Quan, X.; Jin, Y. Toxic Effect of Serial Perfluorosulfonic and Perfluorocarboxylic Acids On the Membrane System of a Freshwater Alga Measured by Flow Cytometry. *Environ. Toxicol. Chem.* **2008**, *27* (7), 1597–1604.

(53) Matyszewska, D.; Leitch, J.; Bilewicz, R.; Lipkowski, J. Polarization modulation infrared reflection-absorption spectroscopy studies of the influence of perfluorinated compounds on the properties of a model biological membrane. *Langmuir* **2008**, *24* (14), 7408–7412 DOI: 10.1021/la8008199.

(54) Lehmler, H. J.; Xie, W.; Bothun, G. D.; Bummer, P. M.; Knutson, B. L. Mixing of perfluorooctanesulfonic acid (PFOS) potassium salt with dipalmitoyl phosphatidylcholine (DPPC). *Colloids Surfaces B Biointerfaces* **2006**, *51* (1), 25–29 DOI: 10.1016/j.colsurfb.2006.05.013.

(55) Lehmler, H. J.; Bummer, P. M. Mixing of perfluorinated carboxylic acids with dipalmitoylphosphatidylcholine. *Biochim. Biophys. Acta - Biomembr.* **2004**, *1664* (2), 141–149 DOI: 10.1016/j.bbamem.2004.05.002.

(56) Oldham, E. D.; Xie, W.; Farnoud, A. M.; Fiegel, J.; Lehmler, H. Disruption of Phosphatidylcholine Monolayers and Bilayers by Perfluorobutane Sulfonate. *J. Phys. Chem.* **2012**, *116*, 9999–10007.

(57) Matyszewska, D.; Tappura, K.; Ora, G.; Bilewicz, R. Influence of Perfluorinated Compounds on the Properties of Model Lipid Membranes. *J. Phys. Chem.* **2007**, *111*, 9908–9918.

(58) Xia, X.; Dai, Z.; Rabearisoa, A. H.; Zhao, P.; Jiang, X. Comparing humic substance and protein compound effects on the bioaccumulation of perfluoroalkyl substances by Daphnia magna in water. *Chemosphere* **2015**, *119*, 978–986 DOI: 10.1016/j.chemosphere.2014.09.034.

(59) Xia, X.; Rabearisoa, A. H.; Jiang, X.; Dai, Z. Bioaccumulation of Perfluoroalkyl Substances by Daphnia magna in Water with Different Types and Concentrations of Protein. *Environ. Sci. Technol.* **2013**, *47*, 10955–10963.

(60) Jones, P. D.; Hu, W.; De Coen, W.; Newsted, J. L.; Giesy, J. P. Binding of perfluorinated fatty acids to serum proteins. *Environ. Toxicol. Chem.* **2003**, *22* (11), 2639–2649.

(61) Eberl, L. N-acyl homoserinelactone-mediated gene regulation in gram-negative bacteria. *Syst. Appl. Microbiol.* **1999**, *22* (4), 493–506 DOI: 10.1016/S0723-2020(99)80001-0.

(62) Waters, C. M.; Bassler, B. L. Quorum sensing: cell-to-cell communication in bacteria. *Annu. Rev. Cell Dev. Biol.* **2005**, *21*, 319–346 DOI: 10.1146/annurev.cellbio.21.012704.131001.

(63) Kaplan, H. B.; Greenberg, E. P. Diffusion of Autoinducer Is Involved in Regilation of the Vibrio fischeri Luminescence System. *J. Bacteriol.* **1985**, *163* (3), 1210–1214.

(64) Shan, G.; Ye, M.; Zhu, B.; Zhu, L. Enhanced cytotoxicity of pentachlorophenol by perfluorooctane sulfonate or perfluorooctanoic acid in HepG2 cells. *Chemosphere* **2013**, *93* (9), 2101–2107 DOI: 10.1016/j.chemosphere.2013.07.054.

(65) Liu, W.; Zhang, Y.-B.; Quan, X.; Jin, Y.-H.; Chen, S. Effect of perfluorooctane sulfonate on toxicity and cell uptake of other compounds with different hydrophobicity in green alga. *Chemosphere* **2009**, *75* (3), 405–409 DOI: 10.1016/j.chemosphere.2008.11.084.

(66) Jernbro, S.; Rocha, P. S.; Keiter, S.; Skutlarek, D.; Färber, H.; Jones, P. D.; Giesy, J. P.; Hollert, H.; Engwall, M. Perfluorooctane Sulfonate Increases the Genotoxicity of Cyclophosphamide in the Micronucleus Assay with V79 Cells. *Environ. Sci. Pollut. Res. Int.* **2007**, *14* (2), 85–87.

(67) Boltes, K.; Rosal, R.; García-Calvo, E. Toxicity of mixtures of perfluorooctane sulphonic acid with chlorinated chemicals and lipid regulators. *Chemosphere* **2012**, *86* (1), 24–29 DOI: 10.1016/j.chemosphere.2011.08.041.

Chapter 2: Literature Review

2.1 Properties of PFAS

PFAS are considered contaminants of emerging concern (CECs) and have unique surfactant, oleophobic, and hydrophobic properties.[1,2] They are anthropogenic surfactants in which all hydrogen atoms in the alkyl chain have been replaced with fluorine atoms. The carbon–fluorine bond is one of the strongest covalent bonds, making these compounds extremely resistant to degradation.[3] Because of fluorine's electronegativity, the exterior of the molecule has a slight negative charge.[4] PFAS are also unique as they display hydrophobic and oleophobic tendencies. Hydrophobicity of PFAS is positively correlated to chain length (Table 2.1).[5] Additionally, the fluorinated chains impart a stiffness to the molecule.[4] The functional head group is in the anionic form in the environment as a result of the low pK_as of these compounds.[1] These properties of PFAS make them unique and must be taken into account when predicting toxicity and biological effects.

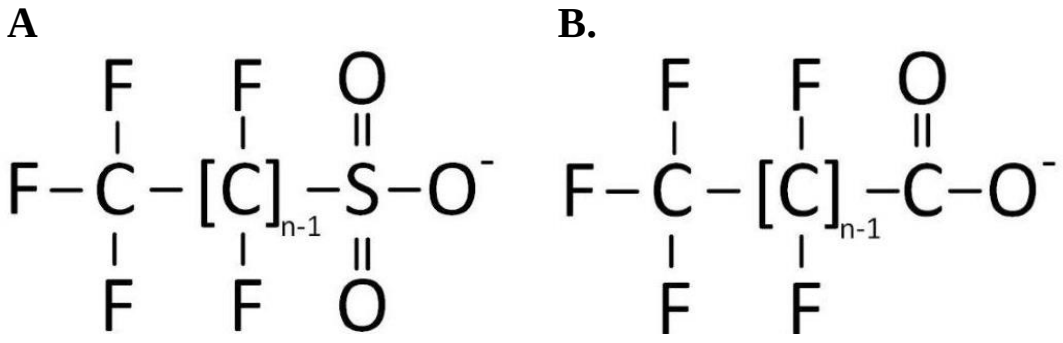


Figure 2.1. Chemical structure of perfluorinated sulfonates (A) and perfluorinated carboxylates (B).

Table 2.1. Octanol-water partitioning coefficients for perfluorinated carboxylates and sulfonates containing varying numbers of perfluorinated carbons.

			log K_{ow}
	PFBS	4C	-0.3
	PFHxS	6C	1.24*
Sulfonates	PFOS	8C	2.45
Carboxylates	PFBA	3C	-0.52
	PFOA	7C	1.92
	PFNA	8C	2.57

*Value calculated in Kim *et.al.*[6]
**All other data obtained experimentally from Jing et. al. [5]

2.2 PFAS Partitioning

PFAS share structural similarities to phospholipids (Figure 2.2), thus it is hypothesized that PFAS will partition into cell membranes.[5] Phospholipids serve as the primary structural component of the cell membrane, providing a selective barrier for cells. Phospholipid membranes prevent charged, polar, and large compounds from entering the cell while allowing water and small neutral organics to pass through (i.e., some signaling molecules).[7] Like phospholipids, PFAS have a hydrophilic head group and a hydrophobic tail structure. PFAS are unique from phospholipids as a result of the electronegativity of their fluorinated tail (Figure 2.3).[4] Because of the charge distribution of PFAS molecules, it is difficult to predict the exact orientation of a PFAS molecule within a biological membrane, but it is hypothesized that they may orient themselves in lipid bilayers and thereby alter lipid interactions within the cell membrane. In fact, PFAS including PFOS, PFOA, and PFBS, have been shown to accumulate in model membranes.[8] While membrane partitioning has been demonstrated, it is important to consider other cellular components that could accumulate PFAS in a more complex whole cell, such as proteins.

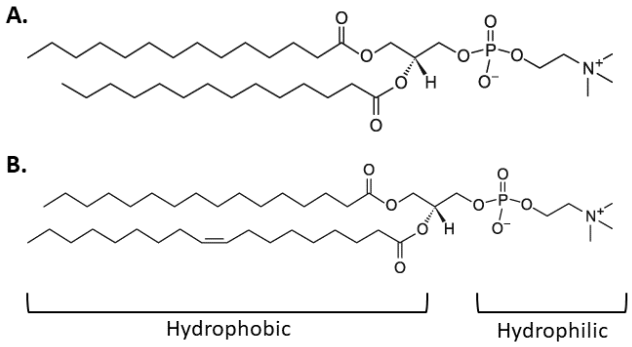


Figure 2.2. Structure of two phospholipids, (A) 1,2-dimyristoyl-sn-glycero-3-phosphocholine and (B) 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine. Chemical structures were obtained from Avanti Polar Lipids, Inc[47].

PFAS have been found to bind proteins. Proteins make up the majority of dry cell mass and are responsible for a wide variety of cellular functions, including transport, catalysis of metabolic reactions, DNA replication, and energy generation via proton translocation and the action of the ATPase.[7] Proteins are composed of amino acid subgroups connected via peptide bonds. The size, shape, and chemical properties of proteins vary and are dependent on the amino acid composition as well as environmental conditions.[7] In vivo, PFAS have been found to bind to serum albumin, the thyroid hormone transporter, and peroxisome proliferator-activated receptors.[9–11] Furthermore, in multicellular organisms, PFAS have been demonstrated to enter and exit cells through organic anion transport proteins, making cell uptake through membrane diffusion less significant.[12] In addition, PFAS partitioning to *Daphnia magna* was decreased when protein albumin was present in solution. Because proteins can be organism-specific, if protein binding is the primary mode of PFAS interaction in an organism, it is difficult to extrapolate PFAS effects between species, and particularly difficult to predict protein-

mediated effects on bacteria. Though more research is needed on protein interactions, it is clear that in certain cases they play a role in PFAS uptake.

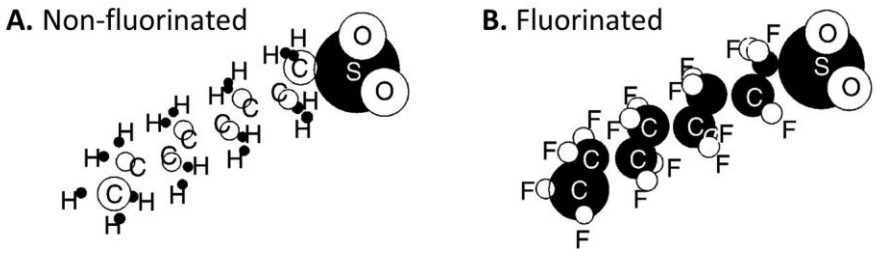


Figure 2.3. Charge distribution on PFOS (B) and its non-fluorinated counterpart n-octanesulfonate (A). White spheres show negative charges and black spheres show positive charges. Sphere size is proportional to charge magnitude. Image modified from Johnson et. al.[4]

Indeed, studies have shown that both protein and phospholipid interactions play a role in PFAS partitioning. A study that modeled the accumulation of PFAS in various organisms, including rats, mice, beluga whales, harbor seals, and herring gulls looked at the contribution of proteins and phospholipids in terms of PFAS partitioning. It found that protein interactions are needed to accurately predict concentrations in the blood, while the phospholipid model component is needed to describe liver accumulation. In the end both terms were needed to accurately describe concentrations throughout the organisms.[12] Partitioning to both lipid and protein components have been demonstrated in multi-cellular organisms, however, it is unknown which area is responsible for effects. Additionally, it is unknown if the models would be as relevant for a single-celled prokaryote.

2.3 PFAS Effects

It is hypothesized that one important way in which PFAS affect organisms is via impacts on the cell membrane. In fact, PFOS, PFOA, and PFBS were shown to decrease the phase transition temperature of a model lipid bilayer, the temperature at which the membrane changes from its gel to fluid state, at concentrations on the order of mg/L.[13–15] This effect was dependent on fluorinated chain length as PFBS had a much smaller effect than PFOS.[13] Alterations in phase transition temperature can occur when phospholipid packing has been disrupted, providing further evidence that PFAS incorporate into and disrupt membrane properties. In another study it was demonstrated that PFAS have a similar impact on lipid bilayers as cholesterol, increasing membrane fluidity and thickness as a result of a decrease in the tilt angle of the acyl chains of the lipids.[16] Additionally, studies using NMR show that PFAS impact the packing and lateral diffusion of phospholipids, in general making the membranes more fluid.[14] Overall, it appears that PFAS increase fluidity of model membranes. It is important to remember, however, that model membranes represent ideal cases.

Attempts have been made to verify these model studies and analyze membrane changes as a result of PFAS exposure in more realistic configurations. Membrane fluidity can be correlated to the diffusion, or leakage, of protons across the membrane; in studies with mitochondria it was demonstrated that PFOS and PFOA increased the intrinsic proton leak of the inner membrane.[17] Additionally, fish leukocytes were used to study the effects of PFOS, perfluorohexane sulfonate (PFHxS), and PFBS on membrane fluidity.[18] No effects were seen for PFBS and PFHxS at mg/L levels; PFOS, however, was shown to increase membrane fluidity in a dose-dependent manner at these higher concentrations

(i.e., mg/L levels).[18] There was also evidence of disrupted membranes in human B-lymphoblastoid cells at sublethal concentrations. Non fluorinated fatty acids did not cause detectable disruption, showing that fluorination in fact changes chemical behavior.[19] Microalga exposed to nontoxic levels of PFOA and PFOS also had more permeable cell membranes, as well as enhanced mitochondrial membrane potential.[20] In contrast, other researchers discovered a decrease in cell membrane permeability of green algae upon exposure to PFOA.[21] Similar to the results observed in model membrane systems, PFAS seem to effect membrane fluidity. The results in real biological systems, however, are not as pronounced or predictable as those observed in model membranes.

While PFAS bind to some proteins, biological effects caused by this phenomenon are less conclusive. PFAS are known to bind to the thyroid hormone transporter and peroxisome proliferator-activated receptor (PPAR). The binding of PFAS to PPAR is hypothesized to cause tumor induction and immune and hormone changes in rats.[11] This mechanism may not be relevant to organisms where peroxisome proliferation is less evident, however.[22] Additionally, effects such as immune suppression and liver toxicity in mice appear to occur, but are independent of PPAR binding.[22] In the case of binding to the thyroid hormone receptor, thyroid hormone imbalances have been reported in animals as a result of PFAS exposure, yet the findings in human studies are less clear.[2] PFAS have also been found to inhibit gap junctional communication in epithelial cells in a dose dependent manner. Effects have been found to be dependent on the fluorinated chain length but independent of the functional group. The authors studying effects on gap junction communication suggested that PFAS interrupt lipid/protein interactions.[23] It was

also demonstrated that PFAS increased the signal received from hydrophobic ligands. It is unknown if a higher number of ligands were able to diffuse through the cell (increased permeability) or if PFAS bound protein receptors and reduced non-target binding of the ligands.[37] Due to protein specificity, it is difficult to draw conclusions between different organisms. It is especially difficult to make conclusions on single-celled prokaryotes. It is unknown if PFAS-protein binding is a mechanism of action in bacteria.

2.4 PFAS in Mixtures

Complicating the task of evaluating toxicity, PFAS are often formulated in unknown mixtures. For example, in Scotchgard, active ingredients (PFAS) are reported to be less than 3%.[24] This means that the vast majority of the product is composed of other chemical compounds. The typical ingredients of AFFF consist of solvents such as diethylene glycol butyl ether, hydrocarbons, and corrosion inhibitors.[25] In addition, it is estimated that between 41-100% of per- and polyfluorinated compounds present in AFFF are precursors to perfluorinated carboxylates and sulfonamides, some of which are unknown.[26] Thus far, perfluorinated compounds including perfluoroalkyl sulfonamido amines, perfluoroalkyl sulfonamide amino carboxylates and fluorotelomer betaines with carbon chains ranging from three to 15 carbons and encompassing over 100 different compounds have been discovered in AFFF.[27,28] To make matters more complicated, formulations can change from year to year and have been shifting towards the inclusion of short-chain compounds as a result of the health and environmental effects associated with long-chain compounds.[26,29,30] It is evident that PFAS will always be associated with a plethora of other co-contaminants, some in quite high concentrations.

In addition to the mixtures present in product formulations containing PFAS, PFAS are also found in environmental matrices where other co-contaminants also persist. In fire training areas and military bases, fuel components and chlorinated solvents can also be present.[25] In wastewater plants artificial sweeteners, corrosion inhibitors, plasticizers and pharmaceuticals can also be found.[31,32] Lastly, in landfills, compounds such as chlorophenols, chlorinated alkyl phosphates, insect repellents, and personal care products are detected at elevated levels.[33] PFAS contamination is therefore often present in environmental matrices with other co-contaminants.

While PFAS have been studied, little is known about their effects in co-contaminant mixtures. The increase in membrane fluidity as a result of exposure to PFAS may indicate that membrane permeability has also been altered. This could alter the bioavailability and cross-membrane diffusion of certain compounds. In fact, in hamster lung V79 cells it was demonstrated that PFOS increased the genotoxicity and uptake of cyclophosphamide, possibly by increasing the membrane permeability.[34] In addition, PFOS increased cellular response to 2,3,7,8-tetrachlorodibenzo-p-dioxin and 17β-estradiol in rat hepatoma cells at a concentration of 0.1 mg/L; the response at higher and lower concentrations of PFOS was reduced.[18] In HepG2 (human liver carcinoma) cells, PFOA and PFOS increase availability, and thus cytotoxicity, of pentachlorophenol (PCP). This was determined through measurements of cell viability and damage coupled with intracellular concentrations of PCP.[35] Studies performed with algae were less conclusive with respect to demonstrating the ability of PFAS to increase cell permeability. In fact, although PFOS increased the toxic effect of PCP to algae, it

decreased the effect of diuron and atrazine. Researchers postulated that the hydrophobicity of these co-contaminants played a role in the compounds' diffusivity through a PFAS-impacted membrane.[36] Similarly, PFOS was shown to behave synergistically (i.e., greater than additive increase in toxicity) with 2,4,6-trichlorophenol and in a ternary combination of 2,4,6-trichlorophenol and triclosan.[37] Nevertheless, the magnitude and occurrence of synergism was dependent on the concentrations of PFOS and co-contaminants and of the chemistry of the co-contaminants.[37] It is unknown if short-chain PFAS are associated with similar changes. It is also unknown if changes are organism or co-contaminant specific. It is evident that PFAS exert an effect on membrane permeability. The details and consequences of this effect have not been clearly shown.

2.5 Relevance to Bacteria

Microbial communities are subjected to elevated concentrations of PFAS, but, little research has been completed to study effects. We rely on bacteria in our waste streams to perform certain functions. For example, in wastewater treatment plants where PFAS have been detected at levels of micrograms per liter, bacteria are relied upon to degrade organics, remove nutrients, and in some cases, create energy.[31,38] Similarly, in landfills, bacteria are needed to degrade organic matter and create energy. Finally, in fire training areas, PFAS are found in combination with fuel components and chlorinated solvents where microbial communities are used to degrade these co-contaminants.[25] The lowest EC_{50} associated with PFAS is 35 mg/L; nevertheless, changes in function have been observed at subtoxic levels, such as increases in bound extracellular polymeric substance

and the enhanced formation of flocs.[39–41] Thus, it is important to be prepared for unexpected effects as a result of the exposure of bacteria to PFAS.

For example, studies have shown that PFAS affect cell uptake of organic compounds in eukaryotes. It is therefore possible that similar effects could occur in microbial communities. In eukaryotes, a predicted change in uptake is a result of changes in cell membrane properties. In prokaryotes, there is evidence that PFAS impact bacterial membranes, which could increase toxicity of co-contaminants and diminish essential microbial functions, such as nutrient cycling. Additionally, PFAS can enter eukaryotic cells through protein transporters and have been found to competitively bind to protein receptors. Changes in analogous systems could also occur in microorganisms, again, impacting nutrient cycling and other metabolic functions. Community shifts upon exposure to PFAS have also been observed in the literature. In a mixed methanogenic community exposed to a PFAS mixture, 65% of operational taxonomic units significantly changed and numbers of *Dehalococcoides* spp. decreased while those of methane-generating *Archaea* increased. In these cultures the degradation of trichloroethene was inhibited (a process performed by *Dehalococcoides* spp.).[42] In anaerobic methanogenic reactors shifts in community structure were also seen after 114 days. While the role of PFOS in this study is less clear, PFOS exposure increased community susceptibility to the antibacterial triclosan,[43] demonstrating that changes in microbial communities caused by PFAS exposure can affect microbial function in unforeseen ways.

Finally, the changes in membrane permeability observed in eukaryotes could alter bacterial communication pathways. Functions such as biofilm formation, antibiotic

production, and pathogenicity are regulated by quorum sensing.[44–46] Certain quorum sensing pathways require that a signaling molecule, often a type of AHL, diffuses passively through bacterial membranes.[44–46] Changes in membrane structure could affect the diffusion of AHL and thus the cell processes regulated via quorum sensing. Indeed, in cultures exposed to high concentrations (>100 mg/L) of a PFAS mixture, the metabolic function of bacteria did not change, but biofilm formation and flocculation of the bacteria was enhanced.[40] While this observed effect was not attributed to changes in AHL diffusion or quorum sensing, biofilm formation is often a process regulated by quorum sensing, raising the question of whether bacterial functions regulated by quorum sensing could be impacted by exposure to PFAS.

2.6 Summary and Research Needs

Microorganisms play a role in a wide range of environmental functions and more research is needed to determine the effects that PFAS could have on microbial communities. This dissertation incorporates a multifaceted approach, targeting mechanistic and functional changes in model lipid bilayers, pure cultures, and mixed communities to identify potential outcomes of PFAS exposure. In the first objective, the mechanism in which PFAS sorb to microbes was elucidated. Results provided information concerning active vs. diffusive uptake as well as information regarding membrane partitioning of PFAS. Alterations to quorum sensing and cell permeability was studied in the second objective to determine the effect that PFAS have on membrane function. Lastly, environmental functional consequences are elucidated through studies of AFFF and PFAS on co-contaminant toxicity and degradation. Overall, this research

helped to bridge the gap between mechanistic changes and functional consequences to PFAS exposure in microbial communities.

References

(1) Ding, G.; Peijnenburg, W. J. G. M. Physicochemical Properties and Aquatic Toxicity of Poly- and Perfluorinated Compounds. *Crit. Rev. Environ. Sci. Technol.* **2013**, *43* (6), 598–678 DOI: 10.1080/10643389.2011.627016.

(2) Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J. Perfluoroalkyl Acids : A Review of Monitoring and Toxicological Findings. *Toxicol. Sci.* **2007**, *99* (2), 366–394 DOI: 10.1093/toxsci/kfm128.

(3) O'Hagan, D. Understanding organofluorine chemistry. An introduction to the C-F bond. *Chem. Soc. Rev.* **2008**, *37* (2), 308–319 DOI: 10.1039/b711844a.

(4) Johnson, R. L.; Anschutz, A. J.; Smolen, J. M.; Simcik, M. F.; Penn, R. L. The Adsorption of Perfluorooctane Sulfonate onto Sand, Clay, and Iron Oxide Surface. *J. Chem. Eng. Data* **2007**, *52*, 1165–1170.

(5) Jing, P.; Rodgers, P. J.; Amemiya, S. High lipophilicity of perfluoroalkyl carboxylate and sulfonate: Implications for their membrane permeability. *J. Am. Chem. Soc.* **2009**, *131* (6), 2290–2296 DOI: 10.1021/ja807961s.

(6) Kim, M.; Li, L. Y.; Grace, J. R.; Yue, C. Selecting reliable physicochemical properties of perfluoroalkyl and polyfluoroalkyl substances (PFASs) based on molecular descriptors. *Environ. Pollut.* **2015**, *196*, 462–472 DOI: 10.1016/j.envpol.2014.11.008.

(7) Madigan, M.; Marktinko, J.; Dunlap, P.; Clark, D. *Brock Biology of Microorganisms*, 13th Editi.; Espinoza, D., Cook, K., Cutt, S., Hutchinson, E., Cogan, D., Marcus, E., Wagner, A., Eds.; Benjamin Cummings: Indianapolis, Indiana, 2006.

(8) Lehmler, H. J.; Xie, W.; Bothun, G. D.; Bummer, P. M.; Knutson, B. L. Mixing of perfluorooctanesulfonic acid (PFOS) potassium salt with dipalmitoyl phosphatidylcholine (DPPC). *Colloids Surfaces B Biointerfaces* **2006**, *51* (1), 25–29 DOI: 10.1016/j.colsurfb.2006.05.013.

(9) Jones, P. D.; Hu, W.; De Coen, W.; Newsted, J. L.; Giesy, J. P. Binding of perfluorinated fatty acids to serum proteins. *Environ. Toxicol. Chem.* **2003**, *22* (11), 2639–2649.

(10) Weiss, J. M.; Andersson, P. L.; Lamoree, M. H.; Leonards, P. E. G.; Leeuwen, S.

P. J. Van; Hamers, T. Competitive Binding of Poly- and Perfluorinated Compounds to the Thyroid Hormone Transport Protein Transthyretin. *Toxicol. Sci.* **2009**, *109* (2), 206–216 DOI: 10.1093/toxsci/kfp055.

(11) Luebker, D. J.; Hansen, K. J.; Bass, N. M.; Butenhoff, J. L.; Seacat, A. M. Interactions of fluorochemicals with rat liver fatty acid-binding protein. *Toxicology* **2002**, *176* (3), 175–185.

(12) Ng, C. a; Hungerbuhler, K. Bioaccumulation of Perfluorinated Alkyl Acids : Observations and Models. *Environ. Sci. Technol.* **2014**, *48*, 4637–4648 DOI: 10.1021/es404008g.

(13) Oldham, E. D.; Xie, W.; Farnoud, A. M.; Fiegel, J.; Lehmler, H. Disruption of Phosphatidylcholine Monolayers and Bilayers by Perfluorobutane Sulfonate. *J. Phys. Chem.* **2012**, *116*, 9999–10007.

(14) Matyszewska, D.; Tappura, K.; Ora, G.; Bilewicz, R. Influence of Perfluorinated Compounds on the Properties of Model Lipid Membranes. *J. Phys. Chem.* **2007**, *111*, 9908–9918.

(15) Xie, W.; Ludewig, G.; Wang, K.; Lehmler, H.-J. Model and cell membrane partitioning of perfluorooctanesulfonate is independent of the lipid chain length. *Colloids Surf. B. Biointerfaces* **2010**, *76* (1), 128–136 DOI: 10.1016/j.colsurfb.2009.10.025.

(16) Matyszewska, D.; Leitch, J.; Bilewicz, R.; Lipkowski, J. Polarization modulation infrared reflection-absorption spectroscopy studies of the influence of perfluorinated compounds on the properties of a model biological membrane. *Langmuir* **2008**, *24* (14), 7408–7412 DOI: 10.1021/la8008199.

(17) Starkov, a a; Wallace, K. B. Structural determinants of fluorochemical-induced mitochondrial dysfunction. *Toxicol. Sci.* **2002**, *66* (2), 244–252.

(18) Hu, W. yue; Jones, P. D.; DeCoen, W.; King, L.; Fraker, P.; Newsted, J.; Giesy, J. P. Alterations in Cell Membrane Properties Caused by Perfluorinated Compounds. *Comp. Biochem. Physiol. Part C* **2003**, *135*, 77–88.

(19) Levitt, D.; Liss, A. Perflourinated Fatty Acids Alter Merocyanine 540 Dye Binding to Plasma Membranes. *J. Toxicol. Environ. Health* **1987**, *20*, 303–316.

(20) Liu, W.; Chen, S.; Quan, X.; Jin, Y. Toxic Effect of Serial Perfluorosulfonic and Perfluorocarboxylic Acids On the Membrane System of a Freshwater Alga Measured by Flow Cytometry. *Environ. Toxicol. Chem.* **2008**, *27* (7), 1597–1604.

(21) Xu, D.; Li, C.; Chen, H.; Shao, B. Cellular response of freshwater green algae to perfluorooctanoic acid toxicity. *Ecotoxicol. Environ. Saf.* **2013**, *88*, 103–107 DOI:

10.1016/j.ecoenv.2012.10.027.

(22) Steenland, K.; Fletcher, T.; Savitz, D. a. Epidemiologic evidence on the health effects of perfluorooctanoic acid (PFOA). *Environ. Health Perspect.* **2010**, *118* (8), 1100–1108 DOI: 10.1289/ehp.0901827.

(23) Hu, W.; Jones, P. D.; Upham, B. L.; Trosko, J. E.; Lau, C.; Giesy, J. P. Inhibition of gap junctional intercellular communication by perfluorinated compounds in rat liver and dolphin kidney epithelial cell lines in vitro and Sprague-Dawley rats in vivo. *Toxicol. Sci.* **2002**, *68* (2), 429–436.

(24) Chu, S.; Letcher, R. J. In vitro metabolic formation of perfluoroalkyl sulfonamides from copolymer surfactants of pre- and post-2002 scotchgard fabric protector products. *Environ. Sci. Technol.* **2014**, *48* (11), 6184–6191 DOI: 10.1021/es500169x.

(25) Moody, C. A.; Field, J. A. Perfluorinated Surfactants and the Environmental Implications of Their Use in Fire-Fighting Foams. *Environ. Sci. Technol.* **2000**, *34* (18), 3864–3870 DOI: 10.1021/es991359u.

(26) Houtz, E. F.; Higgins, C. P.; Field, J. A.; Sedlak, D. L. Persistence of perfluoroalkyl acid precursors in AFFF-impacted groundwater and soil. *Environ. Sci. Technol.* **2013**, *47* (15), 8187–8195 DOI: 10.1021/es4018877.

(27) Agostino, L. A. D.; Mabury, S. A. Identification of Novel Fluorinated Surfactants in Aqueous Film Forming Foams and Commercial Surfactant Concentrates. *Environ. Sci. Technol.* **2014**, *48*, 121–129.

(28) Backe, W. J.; Day, T. C.; Field, J. A. Zwitterionic, cationic, and anionic fluorinated chemicals in aqueous film forming foam formulations and groundwater from U.S. military bases by nonaqueous large-volume injection HPLC-MS/MS. *Environ. Sci. Technol.* **2013**, *47* (10), 5226–5234 DOI: 10.1021/es3034999.

(29) Renner, R. The long and the short of perfluorinated replacements. *Environ. Sci. Technol.* **2006**, *40* (1), 12–13 DOI: 10.1021/es062612a.

(30) Wang, Z.; Cousins, I. T.; Scheringer, M.; Hungerbühler, K. Fluorinated alternatives to long-chain perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFSAs) and their potential precursors. *Environ. Int.* **2013**, *60* (2013), 242–248 DOI: 10.1016/j.envint.2013.08.021.

(31) Loos, R.; Carvalho, R.; António, D. C.; Comero, S.; Locoro, G.; Tavazzi, S.; Paracchini, B.; Ghiani, M.; Lettieri, T.; Blaha, L.; et al. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. *Water Res.* **2013**, *47* (17), 6475–6487 DOI: 10.1016/j.watres.2013.08.024.

(32) Clarke, B. O.; Smith, S. R. Review of "emerging" organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. *Environ. Int.* **2011**, *37* (1), 226–247 DOI: 10.1016/j.envint.2010.06.004.

(33) Eggen, T.; Moeder, M.; Arukwe, A. Municipal landfill leachates: a significant source for new and emerging pollutants. *Sci. Total Environ.* **2010**, *408* (21), 5147–5157 DOI: 10.1016/j.scitotenv.2010.07.049.

(34) Jernbro, S.; Rocha, P. S.; Keiter, S.; Skutlarek, D.; Färber, H.; Jones, P. D.; Giesy, J. P.; Hollert, H.; Engwall, M. Perfluorooctane Sulfonate Increases the Genotoxicity of Cyclophosphamide in the Micronucleus Assay with V79 Cells. *Environ. Sci. Pollut. Res. Int.* **2007**, *14* (2), 85–87.

(35) Shan, G.; Ye, M.; Zhu, B.; Zhu, L. Enhanced cytotoxicity of pentachlorophenol by perfluorooctane sulfonate or perfluorooctanoic acid in HepG2 cells. *Chemosphere* **2013**, *93* (9), 2101–2107 DOI: 10.1016/j.chemosphere.2013.07.054.

(36) Liu, W.; Zhang, Y.-B.; Quan, X.; Jin, Y.-H.; Chen, S. Effect of perfluorooctane sulfonate on toxicity and cell uptake of other compounds with different hydrophobicity in green alga. *Chemosphere* **2009**, *75* (3), 405–409 DOI: 10.1016/j.chemosphere.2008.11.084.

(37) Boltes, K.; Rosal, R.; García-Calvo, E. Toxicity of mixtures of perfluorooctane sulphonic acid with chlorinated chemicals and lipid regulators. *Chemosphere* **2012**, *86* (1), 24–29 DOI: 10.1016/j.chemosphere.2011.08.041.

(38) Zareitalabad, P.; Siemens, J.; Hamer, M.; Amelung, W. Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in surface waters, sediments, soils and wastewater - A review on concentrations and distribution coefficients. *Chemosphere* **2013**, *91* (6), 725–732 DOI: 10.1016/j.chemosphere.2013.02.024.

(39) Rosal, R.; Rodea-Palomares, I.; Boltes, K.; Fernández-Piñas, F.; Leganés, F.; Petre, A. Ecotoxicological assessment of surfactants in the aquatic environment: combined toxicity of docusate sodium with chlorinated pollutants. *Chemosphere* **2010**, *81* (2), 288–293 DOI: 10.1016/j.chemosphere.2010.05.050.

(40) Weathers, T. S.; Higgins, C. P.; Sharp, J. O. Enhanced Biofilm Production by a Toluene-Degrading Rhodococcus Observed after Exposure to Perfluoroalkyl Acids. *Environ. Sci. Technol.* **2015**, *49*, 5458–5466 DOI: 10.1021/es5060034.

(41) Pasquini, L.; Merlin, C.; Hassenboehler, L.; Munoz, J.-F.; Pons, M.-N.; Gorner, T. Impact of certain household micropollutants on bacterial behavior. Toxicity tests/study of extracellular polymeric substances in sludge. *Sci. Total Environ.* **2013**, *463–464* (August 2016), 355–365 DOI: 10.1016/j.scitotenv.2013.06.018.

(42) Weathers, T. S.; Harding-Marjanovic, K.; Higgins, C. P.; Alvarez-Cohen, L.; Sharp, J. O. Perfluoroalkyl Acids Inhibit Reductive Dechlorination of Trichloroethene by Repressing Dehalococcoides. *Environ. Sci. Technol.* **2016**, *50*, 240–248 DOI: 10.1021/acs.est.5b04854.

(43) Mcnamara, P. J.; Lapara, T. M.; Novak, P. J. The Effect of Perfluorooctane Sulfonate , Exposure Time , and Chemical Mixtures on Methanogenic Community Structure and Function. *Microbiol. Insights* **2015**, *8* (S2), 1–7 DOI: 10.4137/MBI.S31345.TYPE.

(44) Greenberg, E. P. Quorum sensing in bacteria : the LuxR-LuxI MINIREVIEW Quorum Sensing in Bacteria : the LuxR-LuxI Family of Cell Density-Responsive Transcriptional Regulatorst. *J. Bacteriol.* **1994**, *176* (2), 269–275.

(45) Eberl, L. N-acyl homoserinelactone-mediated gene regulation in gram-negative bacteria. *Syst. Appl. Microbiol.* **1999**, *22* (4), 493–506 DOI: 10.1016/S0723-2020(99)80001-0.

(46) Boyer, M.; Wisniewski-Dyé, F. Cell-cell signalling in bacteria: not simply a matter of quorum. *FEMS Microbiol. Ecol.* **2009**, *70* (1), 1–19 DOI: 10.1111/j.1574-6941.2009.00745.x.

(47) Avanti Polar Lipids, Inc. avantilipids.com (accessed Sep 10, 2017).

Chapter 3: PFAS Partitioning and Accumulation in Model Lipid Bilayers and Bacteria

Introduction

Perfluoroalkyl substances (PFAS) are ubiquitous in the environment and are associated with a variety of effects in different organisms.[1–3] In humans, PFAS serum concentrations have been correlated with thyroid disease[4] and decreased fecundity[5]. In children, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) serum concentrations were negatively correlated with serum antibody concentrations for routine vaccinations, indicating a decrease in immune response.[6] In addition, PFAS have been associated with an inhibition in gap junction communication in rat and dolphin cell lines.[7] The effects of PFAS are less studied in prokaryotes, though at concentrations of 22-110 mg/L they increased floc formation of *Rhodococcus jostii*[8] and inhibited the microbial degradation of trichloroethylene by *Dehalococcoides mccartyi* enrichments[9,10]. At lower concentrations, 100 μg/L, PFAS increased bacterial mobility in a model aquifer system.[11] It is clear that PFAS affect a variety of biota, yet their mechanism of action, and whether it is similar in prokaryotes and eukaryotes, remains unknown.

PFAS have surfactant properties, suggesting that they may partition into cell membranes and impact biological function by altering membrane properties.[12–16] Studies have shown that PFOS, perfluorobutane sulfonate (PFBS), and PFOA partition into phospholipids bilayers and increase fluidity at mg/L to g/L concentrations.[12,14,17,18] It has also been determined that this increase in fluidity is dose dependent for PFOS from 10 to 200 mg/L.[12] Little research has been performed on the partitioning and subsequent biological effect of PFAS with six or fewer fluorinated carbons, so-called short-chain PFAS; nevertheless, one study showed that PFBS did partition to phospholipid

membranes and increased fluidity at concentrations of 450 to 3,600 mg/L.[14] Experimental evidence has also demonstrated that partitioning of PFAS to membranes can have biological effects. In mitochondria, exposure to either 41 mg/L PFOA or 5 mg/L PFOS increased the proton leakage across the mitochondrial membrane as a result of increased membrane fluidity and permeability.[15] Changes in membrane properties are particularly important because they can affect the toxicity of co-contaminants. In fact, rat hepatoma cells exposed to 0.01 mg/L PFOS had a greater response to 2,3,7,8-tetrachlorodibenzodioxin, likely a result of increased cell permeability to this dioxin congener.[19] Additionally, exposure to either 50 mg/L PFOS or 41 mg/L PFOA were found to increase the uptake and toxicity of pentachlorophenol in liver cells.[20] In green algae, such effects were not as consistent, with exposure to 10-40 mg/L PFOS increasing the toxicity and uptake of pentachlorophenol, but decreasing the toxicity and uptake of atrazine and diuron.[21] Because so little research has been performed on the impact of PFAS on prokaryotes, it is unclear if similar lipid partitioning, and as a result, biological effects, will occur with these simpler organisms.

While the potential for PFAS to change membrane properties in model systems and eukaryotes is apparent, PFAS have also been found to partition to proteins. It is well known that PFAS bind to several sites on serum albumin.[22,23] They have also been found to be transported into cells by fatty acid binding liver proteins[5,24] as well as organic anion transporters[5,22]. The affinity of PFAS to protein was also evident when PFAS accumulation was studied in *Daphnia magna;* the presence of albumin in solution decreased PFAS partitioning to *Daphnia magna*, showing that preferential PFAS binding

to albumin occurred.[25] Similarly, a study modeling PFAS bioaccumulation in different organisms found that it was equally important to include protein and phospholipid partitioning descriptors to accurately describe bioaccumulation.[22] The interactions of PFAS and protein adds a level of uncertainty with respect to how PFAS will partition in prokaryotes and the subsequent effects that may result, particularly given the differences in prokaryote and eukaryote cell structures and the variation within proteins of different organisms.

Bacteria are critical for many ecological functions and may behave differently than higher organisms with respect to PFAS accumulation. Bacteria are often exposed to elevated concentrations of PFAS in engineered systems where we rely on their metabolic functions to treat waste. Additionally, industries are switching to the use of more short-chain PFAS. [26,27] The effects of these short-chain PFAS are less studied and understood, particularly in prokaryotes, leaving an important knowledge gap. The effect of PFAS functional group is also unclear, with current health standards treating PFOS and PFOA as equivalent, while experimental observations show that these two PFAS cause biological effects at different concentrations.[6,15,17,24] To address some of these knowledge gaps, particularly as they pertain to prokaryotes, this study focused on the partitioning behavior of PFAS in model (liposomes) and real bacteria (Gram positive and negative) and investigated some of the resulting effects as a function of PFAS functional group and fluorinated chain length.

Methods

Chemicals

The effect of fluorinated chain length and functional group was examined by performing experiments with three perfluorinated carboxylates and three perfluorinated sulfonates of varying fluorinated chain lengths. Perfluorobutanoate (PFBA, CAS 375-22-4), perfluorooctane (PFOA, CAS 335-67-1), perfluorononanoate (PFNA, CAS 375-95-1), perfluorobutane sulfonate (PFBS, CAS 375-73-5), perfluorohexane sulfonate (PFHxS, CAS 355-46-4), and perfluorooctane sulfonate (PFOS, CAS 1763-23-1), containing 3, 7, 8, 4, 6, and 8 perfluorinated carbons, respectively, were tested. PFOS was purchased from Santa Cruz Biotechnology; all other PFAS were purchased from Sigma Aldrich. Stock solutions of PFAS were prepared in methanol and stored at -20°C. Analytical standards for all six PFAS and mass-labeled standards (sodium perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonate, sodium perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonate, sodium perfluoro-[$^{13}C_8$]octanesulfonate, perfluoro-n-[2,3,4-$^{13}C_3$]butanoic acid, perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid, and perfluoro-n-[$^{13}C_9$]nonanoic acid) were purchased from Wellington Labs. Analytical and mass labeled standards were prepared by transferring purchased standards into a 10-mL volumetric flask via a pasteur pipette. The remaining volume was filled with methanol. The final solution was transferred to a capped serum vial and stored at -20 °C. The phospholipids, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) in chloroform at 25 mg/mL and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) in chloroform at 25 mg/mL, were purchased from Avanti Polar Lipids, Inc. and were >99% pure. Lipid solutions were stored at -20 °C.

Culture preparation

In this research both Gram positive (*Staphylococcus epidermidis* ATCC 12228) and Gram negative bacteria (*Aliivibrio fischeri* mutant strain DC43 from Dr. Eric Stabb[28]) were evaluated. Source liquid cultures were started from cultures grown on solid Luria-Bertani-Salt (LBS) media[29]. Source liquid cultures were grown for 24 hrs in liquid LBS medium and used to inoculate experimental cultures. Bacteria used in partitioning experiments were inoculated with 10 mL of source culture and grown for 48 hrs in 1 L LBS, stirred on a magnetic stir plate. Bacteria used in zeta potential experiments were inoculated with 0.5 mL of source culture and grown for 18 hrs in 50 mL LBS, shaken at 150 rpm. *S. epidermis* was grown at 37°C and *A. fischeri* was grown at room temperature (21-22°C).

Liposome Preparation

To prepare liposomes, 1 mL of POPC dissolved in chloroform was aliquoted to an ashed serum vial and chloroform was evaporated overnight under a stream of pre-purified nitrogen. POPC was re-suspended in 5 mL phosphate buffer (pH 7.4, 4.9 g/L NaCl, 1.19 g/L K_2HPO_4, 0.29g/L KH_2PO_4) filtered with a 0.22-μm membrane (pre-rinsed with phosphate buffer) and extruded 15 times through a 100-nm polycarbonate membrane using a Mini Extruder (Avanti Polar Lipids Inc.) to form liposomes. After extrusion, liposomes were diluted in filtered phosphate buffer to a concentration of 1 mg/mL. Size distribution was verified using a ZetaPALS Potential analyzer (Brookhaven Instruments).

Liposomes prepared for use on the quartz crystal microbalance with dissipation monitoring (QCM-D) were prepared with DMPC following the procedure of Wargenau and Tufenkji.[30]

Experimental Design

Determination of PFAS Partitioning to Bacteria. Sorption of PFBA, PFOA, PFNA, PFBS, PFHxS, and PFOS to both live and dead bacteria was measured to determine if PFAS uptake was active or passive. After 48 hrs of growth, bacteria were washed twice with phosphate buffer by centrifugation at 2,500 rcf before their final resuspension in 800 mL phosphate buffer. Ten mL of the washed bacteria were aliquoted into 15-mL sterile polypropylene centrifuge tubes. Half of the bacteria aliquots were killed via the addition of 50 mM sodium azide. Each PFAS was added to the washed bacteria (live and dead) at a target concentration of 750 μg/L in quadruplicate. PFAS-free controls were included to verify the lack of PFAS contamination.

PFAS were added via methanol stock and PFAS-free controls received methanol only. Methanol represented less than 0.25% of the total volume and was added such that each treatment received equivalent amounts of methanol. Bacteria were shaken on an orbital rotator at 18 rpm for 20 hours, after which the solid and liquid fractions were separated by centrifugation at 2,500 rcf for 15 min. A 0.5-mL sample was taken of the supernatant and the rest was decanted.

The total mass of PFAS was measured by repeating the experiment in duplicate with biomass-free controls. Samples (0.5-mL) were collected before and after mixing.

Effect of PFAS on Zeta Potential. Surface accumulation of PFAS on bacteria and liposomes was determined through zeta potential measurements. Bacteria were grown in quadruplicate and each replicate was washed three times with phosphate buffer and resuspended in an equal volume (50 mL) of phosphate buffer. Replicate cultures were then aliquoted (3 mL) into test tubes and amended in duplicate with either methanol (control) or 50 mg/L PFBS, PFHxS, PFOS, PFBA, PFOA, or PFNA in a methanol stock. Equal quantities of methanol were added to all treatments and represented less than 0.25% of the total volume. Bacteria were incubated at room temperature with PFAS for 20 min prior to zeta potential measurements. A 25 mL sample was taken of each replicate culture to measure the suspended solids concentration.

The surface accumulation of PFAS on liposomes was tested in a similar manner. Briefly, liposomes in phosphate buffer were aliquoted (3 mL) into test tubes. PFAS was added via methanol stock and all treatments, including a PFAS-free control, contained equivalent amounts of methanol. The concentration of liposomes was equivalent to the mass of POPC present, 1 mg/mL.

PFAS Incorporation into Model Lipid Bilayers and Impact on Phase Transition. A Q-Sense E1 QCM-D coupled with a quartz crystal with silica coating (QSX303) was used to determine the extent of PFAS deposition into lipid bilayers and the subsequent effect on phase transition temperature. The lipid bilayer was formed through the fusion of liposomes onto the silica-coated quartz crystal surface. Liposomes were prepared as described in Wargenau and Tufenkji[31] and were suspended in Tris buffer (10 mM, 100 mM NaCl, pH 7.5). This suspension was passed through the QCM-D

chamber at 0.2 mL/min for 7-9 min to promote lipid deposition. Deionized water and liposome-free Tris buffer were then passed through the QCM-D chamber at 0.2 mL/min for 5 min each to collapse remaining liposomes and form a mostly liposome free suspended lipid bilayer within the chamber. Changes in frequency and dissipation following membrane deposition were 25-26 Hz and $< 0.5 \times 10^{-6}$, respectively.

PFAS deposition and the subsequent effect on phase transition were measured consecutively. To begin, an initial methanol-only control experiment was performed with an equivalent quantity of methanol to be used in PFAS-addition experiments to monitor the effect that methanol had on each bilayer. Tris buffer amended with methanol was pumped through the chamber for 5 min. After 5 min, the pump was turned off and the bilayer was equilibrated for 15 min at 30°C. At this time, any changes in frequency as a result of methanol deposition was recorded. After equilibration, the temperature cycle began; temperature was first decreased to 15°C, held at 15°C for 25 min, and then returned to 30°C. All temperature changes occurred at a rate of 0.3°C/min.

Following the first temperature cycle, PFAS was added to Tris buffer via methanol stock. Experiments were performed with PFBS (50 mg/L), PFOS (0.1 mg/L, 0.3 mg/L, 1 mg/L, 5 mg/L, and 50 mg/L), PFBA (50 mg/L), PFOA (1 mg/L, 50 mg/L), and PFNA (1 mg/L, 50 mg/L). Tris containing PFAS was pumped through the chamber for 15 min and frequency changes caused by PFAS accumulation in the bilayer were recorded. The suspended lipid bilayer was equilibrated with PFAS for 1 hr prior to temperature cycling. After equilibration, the temperature was cycled as described above.

Analytical Methods

PFAS Analysis. PFAS in liquid media collected during the partitioning experiments (0.5 mL) were diluted with a 0.5-mL addition of methanol (Optima Grade, Fischer Scientific) for analysis. Following dilution, 50 µL of the mass-labeled standard corresponding to the analyte of interest was added to each sample. More details are found in Appendix A.

Table 3.1. Mass labeled standards used for PFAS analysis. Concentration is concentration after addition to the sample.

PFAS	Internal Standard	m/z	Mass-Labeled Ion
PFBS	Sodium perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonate	299	302
PFHxS	Sodium perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonate	399	402
PFOS	Sodium perfluoro-[$^{13}C_8$]octanesulfonate	499	507
PFBA	Perfluoro-n-[2,3,4-$^{13}C_3$]butanoic acid	213	217
PFOA	Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid	413	417
PFNA	Perfluoro-n-[$^{13}C_9$]nonanoic acid	463	468

PFAS concentrations were determined using a Hewett Packard 1100 HPLC equipped with a Hewett Packard 1100 mass spectrometer. Chromatography was performed through a 50 X 2.1 mm with 3-µm particle size BetaSil C_{18} column (Thermo Scientific) downstream of a 50 X 21 mm 3-µm particle Luna Omega column (Phenomenex). A 10-µL injection volume was used and the mobile phase gradient involved (A) 2 mM ammonium acetate in 10% methanol (Optima Grade, Fischer Scientific) and (B) 2 mM ammonium acetate in 100% methanol (Optima Grade, Fischer Scientific) solution. The linear gradient was 0 min (22% B), 3 min (67% B), 8-15 min

(100% B), 20-25 min (22% B). The mass spectrometer was operated in negative electrospray ionization mode with a capillary voltage of 4,000 V, a dry gas flow of 8 L/min, and a nebulizer pressure of 35 psig. Ions were detected in selective ion monitoring mode according to the m/z reported in Table 3.1. Methanol blanks were run between each sample and an analytical standard was run every 6 samples. Experimental and method blanks were run every 8 samples. The concentration of a given PFAS was calculated using the response factor method.

***Zeta Potential Analysis*.** Electrophoretic mobility was measured using a ZetaPALS Potential Analyzer (Brookhaven) and translated to zeta potential via the Smoluchowski method.[32] For bacteria measurements, settings were as follows: five runs, ten cycles, angle 90°, dust cutoff 30, concentration 0.5 mg/L, and particle size 300 nm. Particle diameter and suspended solids concentration were determined before analysis using bacterial cultures prepared under the same conditions. The settings for liposome analysis were similar, except that the concentration was 1 mg/mL and particle size was 100 nm. Zeta potential values calculated during the 10 cycles were evaluated for increasing or decreasing trends and if detected the measurement would be repeated.

***Suspended Solids Analysis*.** Suspended solids of bacteria cultures used in zeta potential experiments were measured using washed (100 mL deionized water) and dried (105°C) Whatman® Glass microfiber filters, Grade GF/A. Bacterial cultures in phosphate buffer (25 mL) were filtered through the washed and dried filters and then dried at 105°C overnight. Totals solids concentrations were determined as the difference between the mass after filtration of the bacteria solution and the initial mass of the washed and dried

filter divided by the volume of bacteria solution filtered. The suspended solids of bacterial cultures used in partitioning experiments were measured similarly, but with approximately 50 mL bacteria solution filtered through a 0.22-µm Durapore filter (Millipore).

Data Analysis

K_d Analysis. Solid-water partitioning is described by the distribution ratio, K_d. The equation used to determine this partitioning constant is given below. The mass of the bacteria was determined through suspended solids measurements.

$$K_d\,(L\cdot kg^{-1}) = \frac{C_s\,(mg\cdot kg^{-})}{C_w\,(mg\cdot L^{-})}$$

C_s = solids concentration

C_w = aqueous concentration

The aqueous loss method was used to calculate K_d. The aqueous concentration was measured and the mass of PFAS that partitioned to the solids was determined by subtracting the mass of PFAS in the aqueous phase from the total mass of PFAS added. Negative K_d values were excluded in analysis. Differences between live and dead partitioning were tested for each PFAS treatment using the two-sided Student t-test with Welch's correction. Partitioning between PFOS and PFNA was also compared via two-sided Student t-test with Welch's correction. The relationship between fluorinated chain length and K_d was quantified using Spearman correlations. K_d values for living and dead bacteria were combined to calculate correlations between fluorinated chain lengths and the comparison between PFOA and PFNA.

Zeta Potential. Average zeta potentials in bacteria and liposome experiments were compared between PFAS treatments and the respective PFAS-free controls using

the Student t-test. Spearman correlations were used to determine if there was a relationship between zeta potential and fluorinated chain length.

QCM-D Analysis. All QCM-D analysis was based on the normalized 11th harmonic overtone as described in Wargenau and Tufenkji.[31] Frequency changes as a result of mass addition is described by the Sauerbrey equation.[33] Phase transitions were identified by the first-order time derivative of the normalized resonance frequency. To this end, a linear baseline correction was determined for the frequency data for the respective methanol control temperature ramps and applied to both the methanol control and subsequent PFAS data set. The phase transition temperature was determined with a dissipation-temperature calibration curve as described in Wargenau and Tufenkji.[31]

Results and Discussion

PFAS partitioned to liposomes and bacteria (Table 3.2, Figure 3.1), but only changed the zeta potential of the liposomes (Figure 3.1). This indicates that PFAS likely incorporated into the lipid membranes of the bacteria and did not associate with the bacterial surface to the degree that the zeta potential was affected. Other than to exclude the cell surface, the location of PFAS accumulation within bacterial cells was irresolvable. The incorporation of PFAS into the inner bacterial membrane would not be expected to change the zeta potential because of the presence of the cell wall, teichoic acids, and proteins in Gram positive bacteria and the cell wall and outer membrane in Gram negative bacteria.[32,34] Other researchers have also shown that exposure to 100 μg/L PFOA did not change the zeta potential of bacteria.[11] Changes in zeta potential of bacteria have been reported for cationic compounds that are known to attack the outer membrane, such as polymyxin B

and CTAB, though exposure for at least 2 hrs was necessary to induce these changes.[35] Cranberry extracts have also been found to decrease the negativity of bacteria, with the likely site of action the outer membrane of Gram negative bacteria.[36,37] In addition, incorporation of PFAS in living and dead bacteria was statistically equivalent, except for PFHxS, indicating that PFAS diffusion and incorporation is primarily a passive process (Table 3.1). Active uptake of PFAS has been observed in eukaryotic cells.[5,22,24] It is possible that PFHxS is able to compete for binding sites on transport proteins while the other PFAS do not.

Table 3.2. K_d (L/kg) describing PFAS partitioning to bacteria. Average is listed with standard error of the mean.

			Gram -			Gram +		
			Live	Dead	*p* value	Live	Dead	*p* value
	PFBS	4C	1,200 ± 300	1,100 ± 370	0.98	730 ± 240*	770 ± 74	0.88
	PFHxS	6C	1,600 ± 220	810 ± 90	0.03	1,600 ± 82	1,700 ± 210	0.74
Sulfonates	PFOS	8C	3,100 ± 610	2,700 ± 230	0.57	850 ± 270	1,400 ± 170	0.16
Carboxylates	PFOA	7C	1,200 ± 300**	700 ± 51**	0.37	350 ± 33*	590 ± 92	0.08
	PFNA	8C	1,100 ± 130	1,500 ± 480	0.52	950 ± 250	400 ± 280	0.33

*n=3

*n=2

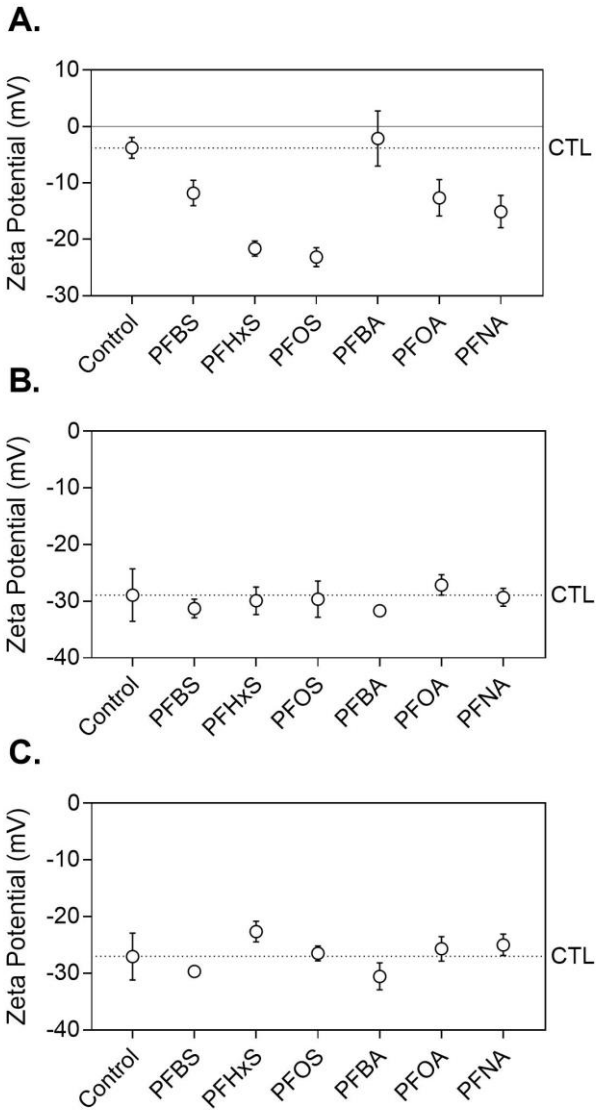


Figure 3.1. The zeta potential in PFAS treatments for (A) liposome, (B) Gram negative bacteria, and (C) Gram positive bacteria. The error bars depict standard deviation and the dashed line marked CTL is the average of the control treatments.

The passive incorporation of PFAS within the cell membrane is also supported by the incorporation of PFAS within model membranes where they altered lipid bilayer fluidity (Figure 3.2, Figure 3.3, Table 3.3). Indeed, in experiments with model membranes, PFAS deposition was not associated with increased dissipation, suggesting that PFAS accumulated within the lipid bilayer rather than depositing on the surface of the bilayer. An increase in dissipation can be a result of increased surface roughness, which can be caused by compounds extruding out of the bilayer.[38–40] In addition, our observations of phase transition changes with PFAS deposition also supports the integration of PFAS into the lipid bilayer where lipid-lipid interactions were altered. Partitioning into the bilayer was also rapid (Figure 3.2) and reversible. Deposition and fluidity increases amplified with PFAS exposure concentration (Table 3.4), which, along with the rapid and reversible deposition, suggests a passive equilibrium-type process. This behavior is also consistent with the similar partitioning values with live and dead bacteria discussed above (Table 3.2). It is likely that all PFAS, regardless of chain length and functional group, disrupt membranes via a similar mechanism of action. While PFBS and PFBA showed a very small effect on phase transition, the effect had similar signatures (i.e., phase transition broadened and shifted to lower temperatures as with the long-chain PFOS, PFOA, and PFNA). Thus, while short-chain PFAS (i.e. PFBA, PFBS) were less accumulative and had less of an effect on cell membrane fluidity, they did accumulate into lipids and were able to change model membrane characteristics.

Table 3.3. PFAS accumulation and affect on the main phase transition temperature in a model DMPC bilayer.

	Sulfonates			Carboxylates				
	4C - PFBS	8C - PFOS		3C - PFBA	7C - PFOA		8C - PFNA	
Conc. PFAS (mg/L)	50	1	50	50	1	50	1	50
PFAS Accumulation (ng/cm^2)	*	18	40	*	22	*	4.8	24
Δ Phase Transition (°C)	0.8	2.7	**	< 0.1	**	< 0.1	1.5	**

* below detection limit

** no phase transition peaks detected

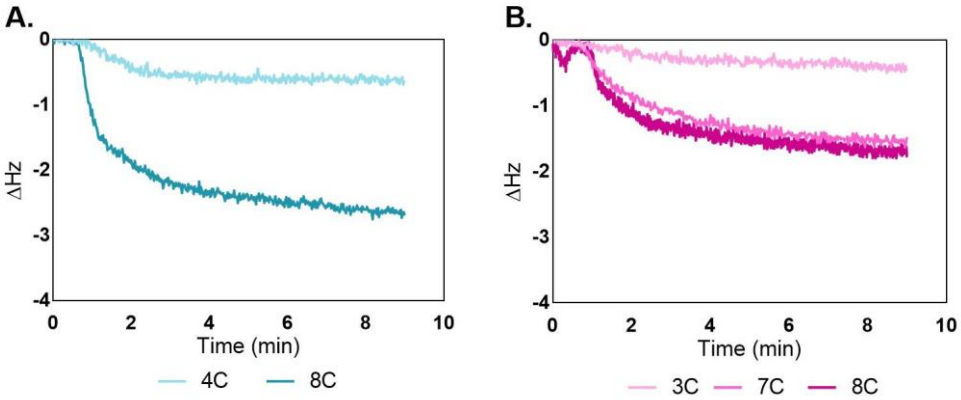


Figure 3.2. Deposition of (A) perfluorinated sulfonates and (B) perfluorinated carboxylates on DMPC bilayer.

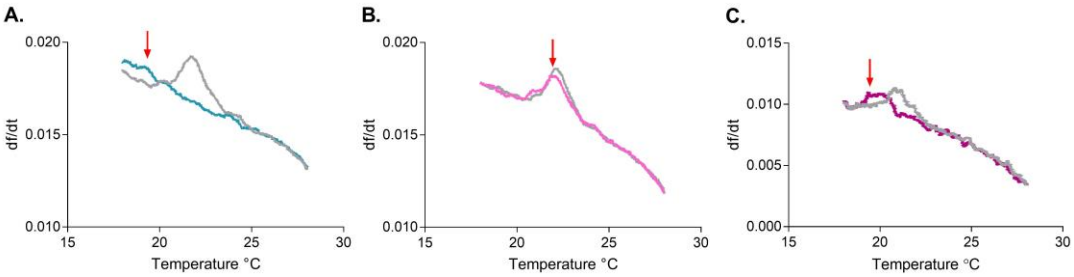


Figure 3.3. Phase transitions in bilayers exposed to 1 mg/L (A) PFOS, (B) PFOA, and (C) PFNA. Grey lines show transition in methanol-control and the colored lines show transition in PFAS-exposed bilayer. Red arrows indicate phase transition peaks in bilayers exposed to PFAS.

Table 3.4. Accumulation and affect of varying PFOS concentrations on the main phase transition temperature in DMPC bilayers.

	PFOS Concentration (mg/L)				
	50	5	1	0.3	0.1
PFAS Accumulation (ng/cm^2)	40	31	18	5	*
Δ Phase Transition (°C)	**	**	2.7	1	0.6

* below detection limit

** no phase transition peaks detected

Fluorinated chain length was positively correlated to zeta potential (p=0.08, Figure A.1) and effects on membrane fluidity. While, PFAS with longer chain lengths had larger distribution ratios (K_d), there was not a statistically significant correlation between fluorinated chain length and distribution ratio (Figure A.2). This could be a result of the large variation in measured values. In all experiments (bacteria partitioning, zeta potential, and QCM-D deposition) long-chain PFAS partitioned or incorporated to a greater extent, likely a result of hydrophobicity increases with chain length.[3] This mirrors what has been seen in the literature and is again consistent with PFAS partitioning in a passive manner. In phospholipid bilayers, partition coefficients of 4C PFBS were reported between 4.9×10^2 and 8.2×10^2, while the partition coefficient reported for 8C PFOS is two orders of magnitude greater at 5.7×10^4, showing PFOS has a greater affinity for lipid than its 4C homologue.[12,14] Increases in membrane fluidity were likewise related to fluorinated chain length, with 8C PFAS (PFOS and PFNA) causing the largest increases in fluidity, followed by the 7C PFOA. This observation is likely facilitated by greater membrane incorporation of the more hydrophobic long-chain PFAS. Literature studies have also demonstrated that lipid bilayer and membrane fluidity or permeability

increases are often a function of fluorinated chain length. It has been demonstrated that PFOS increased membrane fluidity in fish leukocytes in a dose-dependent manner at concentrations of 5 mg/L and higher, while 6C PFHxS and 4C PFBS did not.[41] PFOS was also shown to partition into dipalmitoylphosphatidylcholine (DPPC) membranes and interrupt phase transition at concentrations as low as 10 mg/L.[12] In another study, PFBS was observed to cause disruption in phosphatidylcholine monolayers, albeit at much higher concentrations (>450 mg/L)[14] than those studied herein (50 mg/L).

Differences in PFAS functional groups had a less dramatic effect on partitioning. Nevertheless, sulfonates had a greater affinity for model membranes and bacteria. In partitioning experiments, PFOS accumulated into Gram negative bacteria more than PFNA ($p<0.01$), though there was no difference in accumulation in Gram positive bacteria ($p=0.92$). In experiments with model membranes, PFOS also incorporated at a greater extent than PFNA, altering the zeta potential to a larger degree ($p<0.01$) and showing greater accumulation in suspended lipid bilayers. Additionally, in suspended lipid bilayers, PFOS caused the greatest increase in fluidity and caused effects at the lowest concentrations (Figure 3.3). At 1 mg/L PFAS addition, PFNA only caused a slight increase in membrane fluidity, while the phase transition was not detected in bilayers exposed to PFOS, showing that bilayer fluidity was significantly impacted, even at this lower concentration. While the octanol-water partitioning constants for PFNA and PFOS are nearly equivalent (2.45 vs 2.57),[16] this differential effect based on functional group may be a result of the increased size, electronegativity, or hydrogen bond donating ability of sulfonate as compared to carboxylate.[42,43] This may result in sulfonated PFAS

disrupting the hydrogen bonding and dipole interactions of phospholipids to a greater extent and may make the fluorinated chain slightly less lipophilic and better able to interact with fatty acid chains. The effect of functional group is less clear in the literature, with few studies comparing PFAS based on fluorinated chain length (e.g., PFOS vs. PFNA) rather than carbon number (e.g., PFOS vs. PFOA)[15,44,45].

When anticipating negative effects of PFAS on prokaryotes, long-chain PFAS (7-8C) appear to be more problematic. PFBA and PFBS concentrations must be significantly higher than PFOS and PFOA to exhibit effects on membrane fluidity. Nevertheless, with industry using more short-chain PFAS and their greater mobility,[26,27] it is possible that bacteria could be impacted by these compounds in some environments, such as those impacted by landfill leachate. In addition, short-chain PFAS are often detected in combination with long-chain PFAS. It is possible that their presence may enhance the action of long-chain PFAS, although this has not been addressed experimentally. Lastly, sulfonates and carboxylates partition differently and effect membrane fluidity differently. Restrictions on these compounds that treat them equivalently need to be rethought.

Acknowledgements

We would like to thank Dr. Eric Stabb for the strain of *A. fischeri*. We would also like to thank Dr. Joel Pederson at the University of Wisconsin – Madison for use of his QCM-D. Dr. Lisa Prevette at the University of Saint Thomas was also instrumental in assisting with liposome formation and analysis. This research was supported by the Environment and Natural Resources Trust Fund as recommended by the Legislative Citizen Commission on Minnesota Resources.

References

(1) Schultz, M. M.; Barofsky, D. F.; Field, J. a. Fluorinated Alkyl Surfactants. *Environ. Eng. Sci.* **2003**, *20* (5), 487–501 DOI: 10.1089/109287503768335959.

(2) EPA. *Emerging Contaminants – Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA) At a Glance*; 2012.

(3) Ding, G.; Peijnenburg, W. J. G. M. Physicochemical Properties and Aquatic Toxicity of Poly- and Perfluorinated Compounds. *Crit. Rev. Environ. Sci. Technol.* **2013**, *43* (6), 598–678 DOI: 10.1080/10643389.2011.627016.

(4) Melzer, D.; Rice, N.; Depledge, M. H.; Henley, W. E.; Galloway, T. S. Association Between Serum Perfluorooctanoic Acid (PFOA) & Thyroid Disease in the U . S . National Health and Nutrition Examination Survey. *Environ. Health Perspect.* **2010**, *118* (5), 686–692 DOI: 10.1289/ehp.0901584.

(5) Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J. Perfluoroalkyl Acids : A Review of Monitoring and Toxicological Findings. *Toxicol. Sci.* **2007**, *99* (2), 366–394 DOI: 10.1093/toxsci/kfm128.

(6) Grandjean, P.; Andersen, E. W. Serum Vaccine Antibody Concentrations in Children Exposed to Perfluorinated Compounds. *J. Am. Med. Assoc.* **2012**, *307* (4), 391–397.

(7) Dong, Y.; Gusti, A. R.; Zhang, Q.; Xu, J.; Zhang, L. Identification of Quorum-Quenching N -Acyl Homoserine Lactonases from Bacillus Species Identification of Quorum-Quenching N -Acyl Homoserine Lactonases from Bacillus Species. *Appli. Environ. Microbiol.* **2002**, *68* (4), 1754–1759 DOI: 10.1128/AEM.68.4.1754.

(8) Weathers, T. S.; Higgins, C. P.; Sharp, J. O. Enhanced Biofilm Production by a Toluene-Degrading Rhodococcus Observed after Exposure to Perfluoroalkyl Acids. *Environ. Sci. Technol.* **2015**, *49*, 5458–5466 DOI: 10.1021/es5060034.

(9) Harding-Marjanovic, K. C.; Yi, S.; Weathers, T. S.; Sharp, J. O.; Sedlak, D. L.; Alvarez-Cohen, L. Effects of Aqueous Film-Forming Foams (AFFFs) on Trichloroethene (TCE) Dechlorination by a Dehalococcoides mccartyi -Containing Microbial Community. *Environ. Sci. Technol.* **2016**, *50*, 3352–3361 DOI: 10.1021/acs.est.5b04773.

(10) Weathers, T. S.; Harding-Marjanovic, K.; Higgins, C. P.; Alvarez-Cohen, L.; Sharp, J. O. Perfluoroalkyl Acids Inhibit Reductive Dechlorination of Trichloroethene by Repressing Dehalococcoides. *Environ. Sci. Technol.* **2016**, *50*, 240–248 DOI: 10.1021/acs.est.5b04854.

(11) Wu, D.; Tong, M.; Kim, H. Influence of Perfluorooctanoic Acid on the Transport and Deposition Behaviors of Bacteria in Quartz Sand. *Environ. Sci. Technol.* **2016**, *50* (5), 2381–2388 DOI: 10.1021/acs.est.5b05496.

(12) Lehmler, H. J.; Xie, W.; Bothun, G. D.; Bummer, P. M.; Knutson, B. L. Mixing of perfluorooctanesulfonic acid (PFOS) potassium salt with dipalmitoyl phosphatidylcholine (DPPC). *Colloids Surfaces B Biointerfaces* **2006**, *51* (1), 25–29 DOI: 10.1016/j.colsurfb.2006.05.013.

(13) Lehmler, H. J.; Bummer, P. M. Mixing of perfluorinated carboxylic acids with dipalmitoylphosphatidylcholine. *Biochim. Biophys. Acta - Biomembr.* **2004**, *1664* (2), 141–149 DOI: 10.1016/j.bbamem.2004.05.002.

(14) Oldham, E. D.; Xie, W.; Farnoud, A. M.; Fiegel, J.; Lehmler, H. Disruption of Phosphatidylcholine Monolayers and Bilayers by Perfluorobutane Sulfonate. *J. Phys. Chem.* **2012**, *116*, 9999–10007.

(15) Starkov, a a; Wallace, K. B. Structural determinants of fluorochemical-induced mitochondrial dysfunction. *Toxicol. Sci.* **2002**, *66* (2), 244–252.

(16) Jing, P.; Rodgers, P. J.; Amemiya, S. High lipophilicity of perfluoroalkyl carboxylate and sulfonate: Implications for their membrane permeability. *J. Am. Chem. Soc.* **2009**, *131* (6), 2290–2296 DOI: 10.1021/ja807961s.

(17) Matyszewska, D.; Tappura, K.; Ora, G.; Bilewicz, R. Influence of Perfluorinated Compounds on the Properties of Model Lipid Membranes. *J. Phys. Chem.* **2007**, *111*, 9908–9918.

(18) Xie, W.; Ludewig, G.; Wang, K.; Lehmler, H.-J. Model and cell membrane partitioning of perfluorooctanesulfonate is independent of the lipid chain length. *Colloids Surf. B. Biointerfaces* **2010**, *76* (1), 128–136 DOI: 10.1016/j.colsurfb.2009.10.025.

(19) Hu, W. yue; Jones, P. D.; DeCoen, W.; King, L.; Fraker, P.; Newsted, J.; Giesy, J. P. Alterations in cell membrane properties caused by perfluorinated compounds. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2003**, *135* (1), 77–88 DOI: 10.1016/S1532-0456(03)00043-7.

(20) Jernbro, S.; Rocha, P. S.; Keiter, S.; Skutlarek, D.; Färber, H.; Jones, P. D.; Giesy, J. P.; Hollert, H.; Engwall, M. Perfluorooctane Sulfonate Increases the Genotoxicity of Cyclophosphamide in the Micronucleus Assay with V79 Cells. *Environ. Sci. Pollut. Res. Int.* **2007**, *14* (2), 85–87.

(21) Liu, W.; Zhang, Y.-B.; Quan, X.; Jin, Y.-H.; Chen, S. Effect of perfluorooctane sulfonate on toxicity and cell uptake of other compounds with different hydrophobicity in green alga. *Chemosphere* **2009**, *75* (3), 405–409 DOI:

10.1016/j.chemosphere.2008.11.084.

(22) Ng, C. a; Hungerbuhler, K. Bioaccumulation of Perfluorinated Alkyl Acids : Observations and Models. *Environ. Sci. Technol.* **2014**, *48*, 4637–4648 DOI: 10.1021/es404008g.

(23) Jones, P. D.; Hu, W.; De Coen, W.; Newsted, J. L.; Giesy, J. P. Binding of perfluorinated fatty acids to serum proteins. *Environ. Toxicol. Chem.* **2003**, *22* (11), 2639–2649.

(24) Luebker, D. J.; Hansen, K. J.; Bass, N. M.; Butenhoff, J. L.; Seacat, A. M. Interactions of fluorochemicals with rat liver fatty acid-binding protein. *Toxicology* **2002**, *176* (3), 175–185.

(25) Xia, X.; Rabearisoa, A. H.; Jiang, X.; Dai, Z. Bioaccumulation of Perfluoroalkyl Substances by Daphnia magna in Water with Different Types and Concentrations of Protein. *Environ. Sci. Technol.* **2013**, *47*, 10955–10963.

(26) Wang, Z.; Cousins, I. T.; Scheringer, M.; Hungerbuehler, K. Hazard assessment of fluorinated alternatives to long-chain perfluoroalkyl acids (PFAAs) and their precursors: Status quo, ongoing challenges and possible solutions. *Environ. Int.* **2015**, *75* DOI: 10.1016/j.envint.2014.11.013.

(27) Renner, R. The long and the short of perfluorinated replacements. *Environ. Sci. Technol.* **2006**, *40* (1), 12–13 DOI: 10.1021/es062612a.

(28) Colton, D. M.; Stabb, E. V.; Hagen, S. J. Modeling analysis of signal sensitivity and specificity by Vibrio fischeri LuxR variants. *PLoS One* **2015**, *10* (5), 1–21 DOI: 10.1371/journal.pone.0126474.

(29) Stabb, E. V.; Reich, K. A.; Ruby, E. G. Vibrio fischeri genes hvnA and hvnB encode secreted NAD+-glycohydrolases. *J. Bacteriol.* **2001**, *183* (1), 309–317 DOI: 10.1128/JB.183.1.309-317.2001.

(30) Wargenau, A.; Tufenkji, N. Direct Detection of the Gel-Fluid Phase Transition of a Single Supported Phospholipid Bilayer Using Quartz Crystal Microbalance with Dissipation Monitoring. *Anal. Chem.* **2014**, *86*, 8017–8020.

(31) Wargenau, A.; Tufenkji, N. Direct Detection of Gel-Fluid Phase Transition of a Single Supported Phospholipid Bilayer Using Quartz Crystal Microbalance with Dissipation Monitoring. *Anal. Chem.* **2014**.

(32) Wilson, W. W.; Wade, M. M.; Holman, S. C.; Champlin, F. R. Status of methods for assessing bacterial cell surface charge properties based on zeta potential measurements. *J. Microbiol. Methods* **2001**, *43*, 153–164.

(33) Rodahl, M.; Kasemo, B. On the measurement of thin liquid overlayers with the quartz-crystal microbalance. *Sensors Actuators A Phys.* **1996**, *54* (1), 448–456 DOI: 10.1109/SENSOR.1995.721939.

(34) Madigan, M.; Marktinko, J.; Dunlap, P.; Clark, D. *Brock Biology of Microorganisms*, 13th Editi.; Espinoza, D., Cook, K., Cutt, S., Hutchinson, E., Cogan, D., Marcus, E., Wagner, A., Eds.; Benjamin Cummings: Indianapolis, Indiana, 2006.

(35) Halder, S.; Yadav, K. K.; Sarkar, R.; Mukherjee, S.; Saha, P.; Haldar, S.; Karmakar, S.; Sen, T. Alteration of Zeta potential and membrane permeability in bacteria: a study with cationic agents. *Springerplus* **2015**, *4* (1), 672 DOI: 10.1186/s40064-015-1476-7.

(36) Liu, Y.; Black, M. A.; Caron, L.; Camesano, T. A. Role of Cranberry Juice on Molecular-Scale Surface Characteristics and Adhesion Behavior of Escherichia coli. **2005** DOI: 10.1002/bit.20675.

(37) Habash, M. B.; Mei, H. C. Van Der; Busscher, H. J.; Reid, G. Adsorption of urinary components influences the zeta potential of uropathogen surfaces. **2000**, *19*, 13–17.

(38) Svedhem, S.; Dahlborg, D.; Ekeroth, J.; Kelly, J.; Höök, F.; Gold, J. In situ peptide-nodified supported lipid bilayers for controlled cell attachment. *Langmuir* **2003**, *19* (6), 6730–6736.

(39) Reimhult, E.; Höök, F.; Kasemo, B. Intact vesicle adsorption and supported biomembrane formation from vesicles in solution: Influence of surface chemistry, vesicle size, temperature, and osmotic pressure. *Langmuir* **2003**, *19* (5), 1681–1691 DOI: 10.1021/la0263920.

(40) Reimhult, E.; Larsson, C.; Kasemo, B.; Höök, F. Simultaneous surface plasmon resonance and quartz crystal microbalance with dissipation monitoring measurements of biomolecular adsorption events involving structural transformations and variations in coupled water. *Anal. Chem.* **2004**, *76* (24), 7211–7220 DOI: 10.1021/ac0492970.

(41) Hu, W. yue; Jones, P. D.; DeCoen, W.; King, L.; Fraker, P.; Newsted, J.; Giesy, J. P. Alterations in Cell Membrane Properties Caused by Perfluorinated Compounds. *Comp. Biochem. Physiol. Part C* **2003**, *135*, 77–88.

(42) Schwarzenbach, R.; Gschwend, P.; Imboden, D. *Environmental Organic Chemistry*; 2003.

(43) Gilli, P.; Pretto, L.; Bertolasi, V.; Gilli, G. Predicting Hydrogen-Bond Strengths from Acid{\textminus}Base Molecular Properties. The p KaSlide Rule: Toward

the Solution of a Long-Lasting Problem. *Acc. Chem. Res.* **2009**, *42* (1), 33–44.

(44) Shan, G.; Ye, M.; Zhu, B.; Zhu, L. Enhanced cytotoxicity of pentachlorophenol by perfluorooctane sulfonate or perfluorooctanoic acid in HepG2 cells. *Chemosphere* **2013**, *93* (9), 2101–2107 DOI: 10.1016/j.chemosphere.2013.07.054.

(45) Nobels, I.; Dardenne, F.; De Coen, W.; Blust, R. Application of a multiple endpoint bacterial reporter assay to evaluate toxicological relevant endpoints of perfluorinated compounds with different functional groups and varying ch ... Toxicology in Vitro functional groups and varying chain length. *Toxicol. Vitr.* **2010**, *24*, 1768–1774 DOI: 10.1016/j.tiv.2010.07.002.

Chapter 4: PFAS Increase Membrane Permeability and Quorum Sensing Response in *Aliivibrio fischeri*

Introduction

Perfluoroalkyl substances (PFAS) are found in the environment at a wide range of concentrations and in various media.[e.g., 1,2] PFAS are present at the highest concentrations, up to mg/L levels, near areas contaminated with aqueous film forming foams.[3,4] They are also detected in wastewater and landfill leachate at levels up to μg/L.[5–9] Background levels of PFAS in surface waters are lower, typically at the ng/L level, but can be orders of magnitude greater in areas effected by industrial activity.[10–13] PFAS contamination is predicted to persist for many years as a result of the strength of the carbon – fluorine bond and the subsequent resistance of PFAS to degradation.[14–16] Bacteria are present in each of these environments where we depend on them for nutrient cycling, contaminant degradation, and human health. It is therefore important to understand the effect PFAS that have on bacteria at a range of concentrations.

PFAS have been shown to alter cell membrane properties and in certain cases increase membrane permeability. Recently, the PFAS perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluorobutane (PFBA), perfluorooctane (PFOA), and perfluorononanoate (PFNA) were shown to incorporate into model lipid bilayers and increase their fluidity (Chapter 3). PFOS had the greatest effect on model lipid bilayers and was found to affect phase transition at a concentration of 0.1 mg/L (Chapter 3). PFOA and PFOS exposure also increased the membrane fluidity in mitochondria, increasing the intrinsic proton leak at concentrations of 41 and 5 mg/L, respectively.[17] Researchers also showed that cell membrane permeability to other compounds was altered by PFAS exposure, with the response of

breast cancer cells to estradiol increasing in the presence of 0.1 mg/L PFOS[18]. In addition, exposure to 50 mg/L PFOS or 41 mg/L PFOA increased the membrane permeability and toxicity of pentachlorophenol in liver cells.[19] Similarly, exposure of lung cells to 12 mg/L PFOS increased the uptake and genotoxicity of cyclophosphamide.[20] While an increase in cell permeability following PFAS exposure has been consistently observed, Boltes and coworkers found that PFOS exposure both decreased and increased the toxicity of co-contaminants, in part, as a function of co-contaminant chemistry.[21] Therefore, though some PFAS increase cell membrane permeability, it is not clear which membrane diffusion-related cell functions are vulnerable to changes upon PFAS exposure and under what conditions.

The quorum sensing response in bacteria is a function of diffusion. Quorum sensing can be described as a process by which bacteria communicate, with many quorum sensing pathways requiring that signaling molecules diffuse passively through the bacterial cell membrane to reach a receptor within the cell.[e.g., 22–25] Quorum sensing plays a role in a variety of bacterial processes, such as biofilm formation, pathogenicity, antibiotic production, and bioluminescence.[22–25] PFAS-induced changes in membrane permeability and subsequent changes in the quorum sensing response could have wide-ranging ramifications for PFAS-impacted environments. Nevertheless, the effects of PFAS exposure on quorum sensing have not been studied.

Although PFAS exposure increases cell membrane permeability in eukaryotes, it is unknown if these effects are also observed in bacteria. It is also unknown if changes in cell membrane permeability can result in changes in bacteria function, such as functions

tied to the quorum sensing response. While little is known regarding the effect of PFAS on bacteria, bacteria can be exposed to elevated concentrations of PFAS in a variety of environments. Therefore, this study was designed to determine the potential for PFAS to affect bacterial cell membrane permeability and quorum sensing. Bacteria were exposed to a range of PFAS and PFAS concentrations, some environmentally relevant, to determine the potential for environmental significance.

Methods

Chemicals. Six different PFAS were used to study effects of fluorinated chain length and functional group: three carboxylates and three sulfonates. The carboxylates tested were the 4-carbon (3C, 3 fully fluorinated carbons) perfluorobutane (PFBA, CAS 375-22-4), the 8-carbon (7C, 7 fully fluorinated carbons) perfluorooctane (PFOA, CAS 335-67-1), and the 9-carbon (8C, 8 fully fluorinated carbons) perfluorononanoate (PFNA, CAS 375-95-1). The sulfonates used were the 4C (4 fully fluorinated carbons) perfluorobutane sulfonate (PFBS, CAS 375-73-5), 6C (6 fully fluorinated carbons) perfluorohexane sulfonate (PFHxS, CAS 355-46-4), and 8C (8 fully fluorinated carbons) perfluorooctane sulfonate (PFOS, CAS 1763-23-1). Other than PFOS, all PFAS were purchased from Sigma Aldrich. PFOS was purchased from Santa Cruz Biotechnology. The signaling molecule, N-(β-ketocaproyl)-L-homoserine lactone (CAS 143537-62-6), a type of acyl homoserine lactone (AHL), was purchased from Cayman Chemical.

Cell Cultures. *Aliivibrio fischeri* mutant DC43 was obtained from Dr. Eric Stabb and was the sole species used in this study.[26] *A. fischeri* DC43 is active in quorum sensing and responds to the diffusion of AHL molecules into the cell by luminescing.[26]

This mutant was selected because, while it is able to respond to the signaling molecule N-(β-ketocaproyl)-L-homoserine lactone, it is unable to produce it.[26] This allowed for tighter control over the concentration of signaling molecule in experiments. *A. fischeri* DC43 was cultured in photobacterium broth.[27] The broth was sterilized by autoclave and then filtered through a sterile 0.22-μm polycarbonate filter to remove particulates. Cultures were inoculated via a transfer (1% by volume) of a 24-hr culture of *A. fischeri* DC43.

Luminescence and Metabolism. *A. fischeri* DC43 used in experiments were grown in 1 mL of broth in 10-mL glass tubes in the presence of 10 μg/L – 50 mg/L PFBS, PFBA, PFHxS, PFOA, PFOS, or PFNA for 30 hours at room temperature while shaken at 150 rpm. A negative control (PFAS-free) was grown and tested with each experiment. PFAS were added via methanol stock and all treatments contained equal amounts of methanol, with control cultures receiving methanol only. Between 13 to 15 replicates were used for each treatment.

Bacteria were aliquoted into two 96-well plates, a white clear-bottomed plate and a black clear-bottomed plate. Each well received 200 μL of bacteria. Metabolism was measured in the black plate via the reduction of resazurin dye to the fluorescent resorufin[28]. Briefly, resazurin was added to a final concentration of 40 μM and plates were incubated for 45 min at room temperature. Fluorescence was read with a Synergy H1 microplate reader. Excitation wavelength was set to 550 nm and emission wavelength was at 590 nm.

Luminescence and optical density were measured in the white plates. AHL was added to a final concentration of 1,000 nM and bacteria were incubated at room temperature for one hr. Luminescence was measured using a Synergy H1 plate reader. The instrument was set to scale to high wells (high wells designated as control wells) and read with a 2-s read speed and a read height of 1 mm. Optical density was measured at 600 nm in the white 96-well plates for normalization purposes.

***Dye Permeability*.** Bacteria were grown in 6 mL of broth in 30-mL glass test tubes in the presence either of 50 mg/L PFBS, PFBA, PFHxS, PFOA, PFOS, or PFNA. A control (PFAS-free) was also grown and tested in each experiment. PFAS were added via methanol stock and all treatments contained equivalent amounts of methanol, with the controls receiving methanol only. After growth, bacteria were centrifuged at 2,500 rcf and rinsed twice with phosphate buffered saline (PBS, 8 g/L NaCl, 0.2 g/L KCl, 1.42 g/L Na_2HPO_4, 0.24 g/L KH_2PO_4, pH 7.0) containing 50 mg/L of the corresponding PFAS before final resuspension in PFAS-amended PBS. Optical density at 600 nm was recorded for the suspensions.

Cell permeability was measured by use of the dye 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI). DAPI is a membrane semi-permeable dye that fluoresces when it binds to DNA. The diffusion of DAPI into the PFAS-exposed bacteria or PFAS-free control bacteria was monitored via its fluorescent signal. Briefly, 2 mL of bacteria suspension was combined with 1 mL of a PBS solution containing DAPI, so that the final concentration of DAPI was 4 μg/mL. The fluorescent signal was measured 10 min after exposure. The remainder of the bacterial suspension was set aside to record optical

density and lyse cells. The maximum fluorescence in each sample was determined for normalization purposes after lysing the bacteria with 5 freeze thaw cycles to release all of the DNA present and make it available for DAPI binding. Lysed bacteria were similarly exposed to DAPI for 10 min before recording the signal. Fluorescence was measured with a Shimadzu RF-5301PC spectrofluorometer, with an excitation wavelength of 340 ± 1.5 nm and an emission wavelength of 488 ± 1.5 nm.

***Data Analysis*.** The data describing luminescence and metabolic activity were processed in the same manner. Luminescence and fluorescence in each well were first normalized by the optical density of the culture in that well. The values were averaged and 95% confidence intervals were determined using a Student's t-test with Welch's correction. These values were then normalized by the value obtained for the PFAS-free control in the same 96-well plate and the error propagated. If values were significantly different from 1.0, they were considered to be statistically different than the control. Values are presented as normalized fluorescence or luminescence as a percent of the control, with 100% being equivalent to the control (*i.e.*, no effect). In the case of metabolism, a change in the normalized fluorescence greater than or less than 100% indicated an increase or decrease in metabolism, respectively, with a decrease (<100%) in fluorescence being suggestive of possible toxicity. In the case of luminescence, a change in the normalized luminescence greater than or less than 100% indicated an increase or decrease in the quorum sensing response, respectively, again, with a decrease (<100%) in luminescence also being suggestive of possible toxicity.

For the permeability experiment, live cell fluorescence was normalized by the fluorescence in that same sample after cell lysis. Values were averaged for each treatment and Student t-tests with Welch's corrections were performed between each treatment and the control.

Results & Discussion

Effect of PFAS Exposure on A. fischeri Metabolic Activity and Growth. Growth and metabolism were monitored to assess bacterial toxicity or activity indicative of uncoupling or proton leakage upon PFAS exposure. In the case of metabolism and the sulfonated PFAS, higher concentrations (mg/L) of the 4C and 6C compounds increased metabolism (Figure 4.1), perhaps as a result of proton leakage as has been seen with mitochondrial membranes.[17,18,29] Exposure to the 8C PFOS, however, significantly decreased metabolism at 50 mg/L, suggesting that at this high concentration, PFOS was toxic to *A. fischeri*. For the carboxylated PFAS, there was no metabolic indication of toxicity, but increases in metabolism were observed for the 8C PFNA at 0.3-50 mg/L (Figure 4.1). Growth of *A. fischeri* was variably affected by PFAS exposure (Figure 4.1), with little overall impact. Statistically significant decreases in OD_{600} were observed with exposure to the 4C sulfonate and 3C and 8C carboxylates and statistically significant increases in OD_{600} were observed with exposure to certain concentrations of the 8C sulfonate and 7C and 8C carboxylates. It is not clear why these differences in growth were observed with PFAS exposure, though overall growth changes were small.

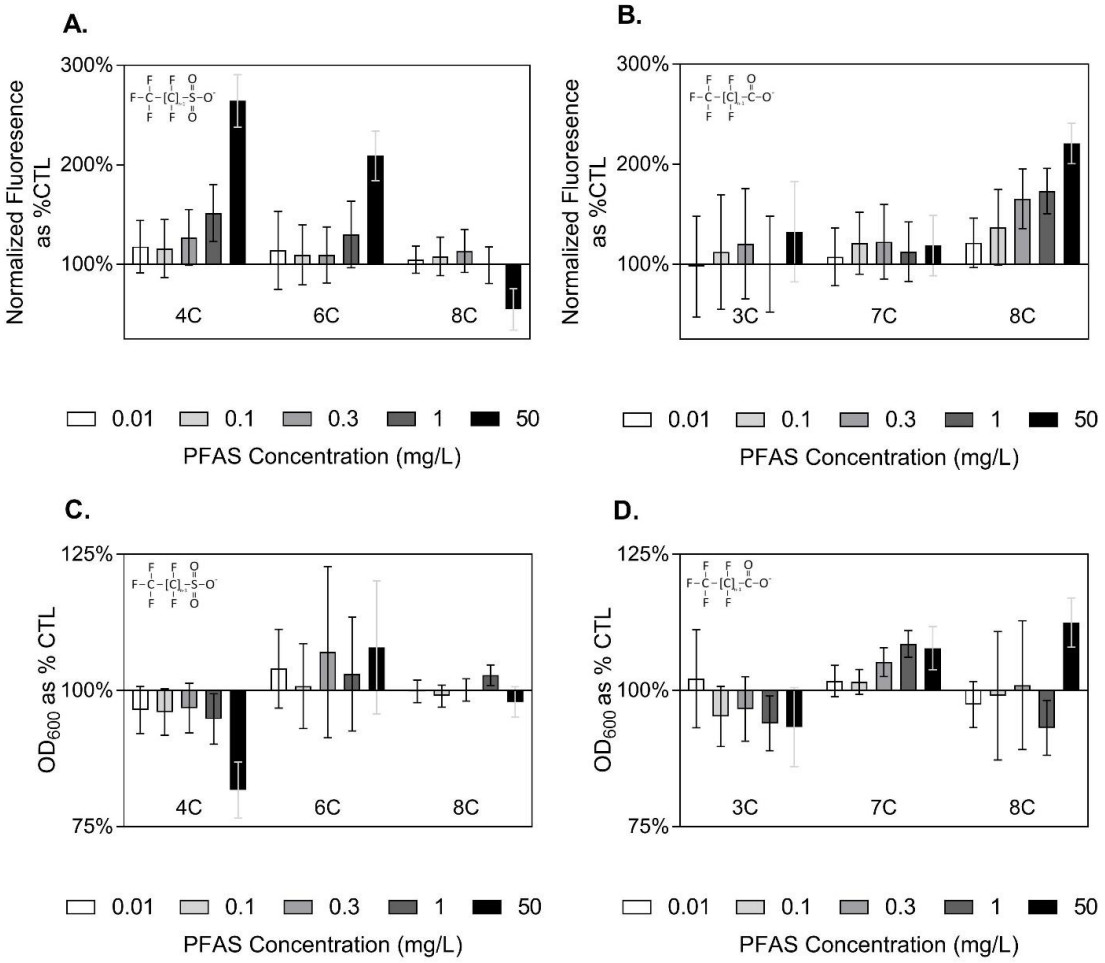


Figure 4.1. Metabolism and growth in *A. fischeri* DC43 exposed to varying concentrations of PFAS. (A) and (B) show metabolic data of the fluorinated sulfonates and fluorinated carboxylates, respectively. The y-axis shows resorufin fluorescence, produced as bacteria respire and reduce resazurin. Fluorescence is normalized to the corresponding control. Values greater than 100% represent an increase in respiration. (C) and (D) show growth data of the fluorinated sulfonates and fluorinated carboxylates, respectively. The y-axis shows optical density as % of corresponding control cultures; values greater than 100% indicate greater growth in treatments versus the controls. Error bars are 95% confidence intervals

Quorum Sensing. We hypothesized based on the literature, that exposure to PFAS would increase the quorum sensing response in bacteria. [19,20,30] This was in fact observed with *A. fischeri* DC43 (Figure 4.2). The quorum sensing response, luminescence, increased as PFAS fluorinated chain length and concentration increased. This was especially evident with the carboxylates (Figure 4.2). This effect was also observed with the sulfonates, with the 6C, PFHxS, causing more luminescence than the 4C, PFBS, at equivalent concentrations. Exposure to the 8C PFOS resulted in greater luminescence at concentrations less than 50 mg/L; nevertheless, the overall trend was masked at 50 mg/L, likely as a result of toxicity (shown as a decrease in metabolism in Figure 4.1.A). The observation of longer-chain PFAS having a greater biological effect is supported by the literature. For example, in model membranes, PFAS partitioning and increases in membrane fluidity were greater as the fluorinated chain length increased (Chapter 3). Others have also observed that at mg/L levels, membrane permeability and fluidity increased in PFAS-exposed algae and mitochondria as fluorinated chain length increased.[29–32] Our results are particularly important because few functional effects have been documented with the short-chain, 4C and 6C, PFAS.[30,31] Additionally, the results presented herein also demonstrate that luminescence increased in PFOS- and PFNA-amended cultures at concentrations as low as 10 μg/L, a concentration that has not been previously linked to ecological effects but is frequently detected in human serum[33–35] and highly contaminated environments such as landfills[5–9].

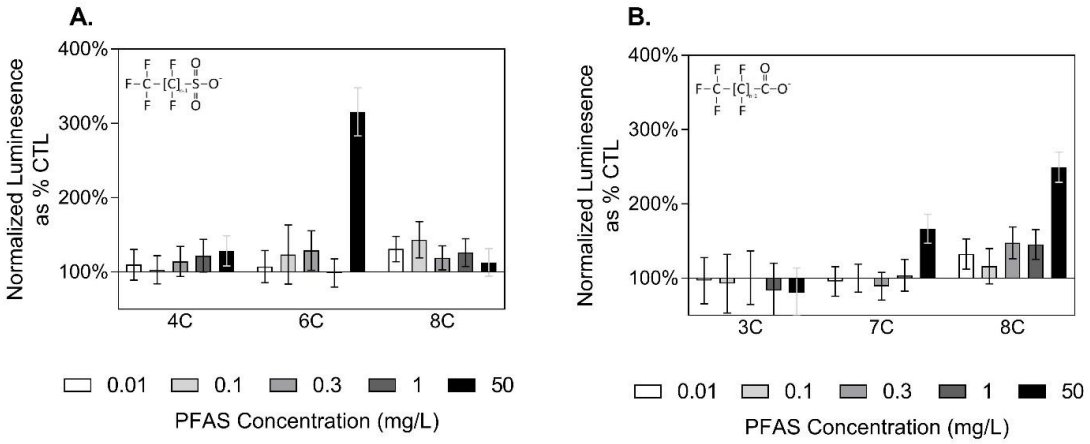


Figure 4.2. Luminescence after addition of AHL in *A. fischeri* DC43 exposed to varying concentrations of PFAS for (A) fluorinated sulfonates and (B) fluorinated carboxylates. The y-axis shows normalized luminescence, with values greater than 100% representing increases in the quorum sensing response of a given treatment as compared to the control. Error bars are 95% confidence intervals.

The PFAS functional group also played a role in the impact of PFAS on the bacterial quorum sensing response (Figure 4.2). The sulfonates had a greater effect on the quorum sensing response at short-chain lengths and appeared to become toxic as chain length and concentration increased (Figure 4.1). The literature is unclear concerning the effect of PFAS functional group on biological function. A study measuring gene expression in *Escherichia coli* K-12 derivatives found slightly more evidence of membrane damage when the bacteria were exposed to sulfonated PFAS versus carboxylated PFAS.[32] Nevertheless, sulfonates and carboxylates with the same fluorinated chain length were not compared.[32] PFOS, when compared to PFOA, also caused greater cellular uptake and toxicity of pentachlorophenol in liver cells, yet again, the compounds do not have the same fluorinated chain length, with PFOS having 8 fully fluorinated carbons and PFOA only having 7.[19] In our previous work, sulfonated PFAS

partitioned to a greater degree into model lipid bilayers than carboxylated PFAS. While only a biological model, these results support our observations of impact on quorum sensing and suggest that sulfonated PFAS could impact biological function more significantly than carboxylated PFAS. The reasons for this difference merit more research.

Dye Permeability. The observed increase in the quorum sensing response could be a result of several different mechanisms, only one of which is an increase in cell membrane permeability. Indeed, while the quorum sensing pathway utilized by *A. fischeri* requires AHL to passively disuse through the membrane, there are other steps prior to luminescence, such as the activation of the transcriptional regulator and gene expression.[e.g., 36] To determine if membrane permeability was in fact increased as a result of PFAS exposure, experiments were also performed to directly measure membrane permeability in live PFAS-exposed *A. fischeri* DC43.

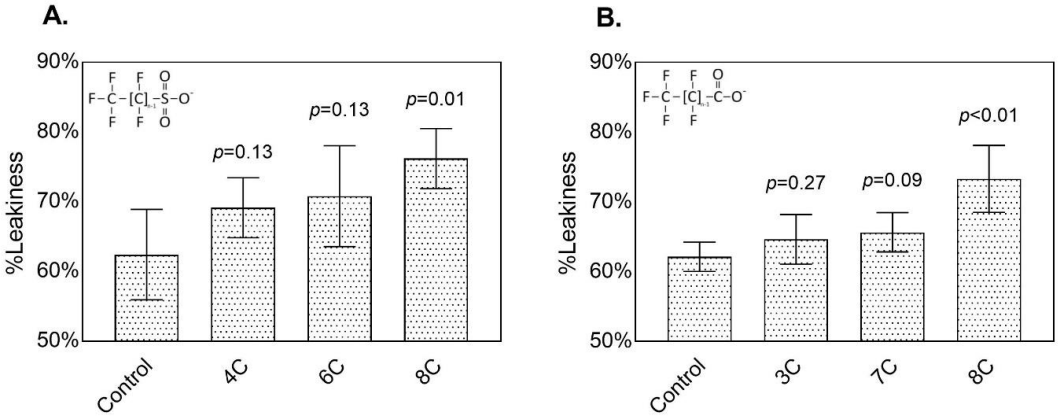


Figure 4.3. Permeability of membrane semi-permeable dye, DAPI in cultures exposed to (A) fluorinated sulfonates and (B) fluorinated carboxylates. Error bars show standard deviation of four replicate samples. Percent leakiness is defined as the fluorescence after a 10-min DAPI exposure period normalized to the fluorescence after a 10-min DAPI exposure after the same bacteria were lysed via 5 freeze-thaw cycles.

Live *A. fischeri* DC43 exposed to 50 mg/L of PFOS or PFNA were significantly more permeable to the membrane semi-permeable dye, DAPI, than unexposed bacteria (Figure 4.3). Similar results were observed for the PFAS with shorter fluorinated chains, though the increase in cell permeability was only significant with 87% confidence in bacteria exposed to PFBS and PFHxS and with 91% confidence in bacteria exposed to PFOA. This trend mimics that observed with luminescence, with live cell permeability to DAPI increasing with increasing fluorinated chain lengths. Differences in the effect of sulfonated versus carboxylated PFAS were not observed, however (Figure 4.3). These results support the hypothesis that an increase in the quorum sensing response in *A. fischeri* DC43 upon PFAS exposure is a result of increased bacterial cell membrane permeability.

Environmental Significance. Our results show that bacteria exhibit a more sensitive quorum sensing response after exposure to PFAS at concentrations that have been detected in the environment. Exposure to PFOS and PFNA increased bacterial response to the signaling molecule at concentrations as low as 10 μg/L. While this concentration is not detected in all environmental matrices, it can be detected in areas that are impacted by industrial activity or fire-fighting activities.[3,4,13,37] Additionally, as mentioned above, the mean concentration of PFOS in human serum is comparable to 10 μg/L and occupationally exposed workers can have concentrations almost 100 times greater.[33–35] In these environments, processes regulated by quorum sensing, such as biofilm production and pathogenicity, could be altered as a result of PFAS exposure.

Other processes mediated by permeability, such as toxicity, could also be effected. The implications of altered quorum sensing pathways and permeability have the potential to be extensive.

More research is needed to determine in which environments bacterial exposure to PFAS are functionally important. This is especially true for mixed cultures in complex matrices and in systems in which bacterial pathogenicity could be important. While *A. fischeri* utilizes a signaling molecule that is membrane permeable, some bacteria utilize signaling compounds for quorum sensing that must be actively transported into the cell.[24] In these cases, quorum sensing responses may not be effected by PFAS in the same way. Additionally, it is important to note that other compounds present in the environment could interfere with the effect of PFAS. For instance, certain bacteria can produce lactonases to degrade AHLs[38,39] and these bacteria may negate the impact of PFAS on quorum sensing. Mixtures of PFAS may behave differently and alter observed effects as well. Future work is needed to determine the factors affecting the environmental relevance of this PFAS-mediated bacterial response.

Acknowledgements

I would like to thank Dr. Eric Stabb for the *A. fischeri* mutant. We would also like to thank Dr. Lisa Peterson for use of the plate reader and Dr. Elizabeth Wattenburg for use of the spectrofluorometer. The work was supported by the Environment and Natural Resources Trust Fund as recommended by the Legislative Citizen Commission on Minnesota Resources.

References

(1) Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J. Perfluoroalkyl Acids : A Review of Monitoring and Toxicological Findings. *Toxicol. Sci.* **2007**, *99* (2), 366–394 DOI: 10.1093/toxsci/kfm128.

(2) Rayne, S.; Forest, K. Perfluoroalkyl sulfonic and carboxylic acids: a critical review of physicochemical properties, levels and patterns in waters and wastewaters, and treatment methods. *J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng.* **2009**, *44* (12), 1145–1199 DOI: 10.1080/10934520903139811.

(3) Moody, C. A.; Field, J. A. Determination of Perfluorocarboxylates in Groundwater Impacted by Fire-Fighting Activity. *Environ. Sci. Technol.* **1999**, *33* (16), 2800–2806 DOI: 10.1021/es981355+.

(4) Arias E, V. a; Mallavarapu, M.; Naidu, R. Identification of the source of PFOS and PFOA contamination at a military air base site. *Environ. Monit. Assess.* **2015**, *187* (1), 4111 DOI: 10.1007/s10661-014-4111-0.

(5) Sinclair, E.; Kannan, K. Mass loading and fate of perfluoroalkyl surfactants in wastewater treatment plants. *Environ. Sci. Technol.* **2006**, *40* (5), 1408–1414.

(6) Huset, C. a; Barlaz, M. a; Barofsky, D. F.; Field, J. a. Quantitative determination of fluorochemicals in municipal landfill leachates. *Chemosphere* **2011**, *82* (10), 1380–1386 DOI: 10.1016/j.chemosphere.2010.11.072.

(7) Eggen, T.; Moeder, M.; Arukwe, A. Municipal landfill leachates: a significant source for new and emerging pollutants. *Sci. Total Environ.* **2010**, *408* (21), 5147–5157 DOI: 10.1016/j.scitotenv.2010.07.049.

(8) Busch, J.; Ahrens, L.; Sturm, R.; Ebinghaus, R. Polyfluoroalkyl compounds in landfill leachates. *Environ. Pollut.* **2010**, *158* (5), 1467–1471 DOI: 10.1016/j.envpol.2009.12.031.

(9) Loos, R.; Carvalho, R.; António, D. C.; Comero, S.; Locoro, G.; Tavazzi, S.; Paracchini, B.; Ghiani, M.; Lettieri, T.; Blaha, L.; et al. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. *Water Res.* **2013**, *47* (17), 6475–6487 DOI: 10.1016/j.watres.2013.08.024.

(10) Zareitalabad, P.; Siemens, J.; Hamer, M.; Amelung, W. Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in surface waters, sediments, soils and wastewater - A review on concentrations and distribution coefficients. *Chemosphere* **2013**, *91* (6), 725–732 DOI: 10.1016/j.chemosphere.2013.02.024.

(11) Kim, S.-K.; Kannan, K. Perfluorinated acids in air, rain, snow, surface runoff, and

lakes: relative importance of pathways to contamination of urban lakes. *Environ. Sci. Technol.* **2007**, *41* (24), 8328–8334.

(12) Nakayama, S.; Strynar, M. J.; Helfant, L.; Egeghy, P.; Ye, X.; Lindstrom, A. B. Perfluorinated compounds in the Cape Fear Drainage Basin in North Carolina. *Environ. Sci. Technol.* **2007**, *41*, 5271–5276 DOI: 10.1021/es070792y.

(13) Konwick, B. J.; Tomy, G. T.; Ismail, N.; Peterson, J. T.; Fauver, R. J.; Higginbotham, D.; Fisk, A. T. Concentrations and patterns of perfluoroalkyl acids in Georgia, USA surface waters near and distant to a major use source. *Environ. Toxicol. Chem.* **2008**, *27* (10), 2011–2018 DOI: 10.1897/07-659.1.

(14) Plumlee, M. H.; McNeill, K.; Reinhard, M. Indirect Photolysis of Perfluorochemicals: Hydroxyl Radical-Initiated Oxidation of N-Ethyl Perfluorooctane Sulfonamido Acetate (N-EtFOSAA) and Other Perfluoroalkanesulfonamides. *Environ. Sci. Technol.* **2009**, *43* (20), 3662–3668 DOI: 10.1021/es902634x.

(15) Liou, J. S.-C.; Szostek, B.; DeRito, C. M.; Madsen, E. L. Investigating the biodegradability of perfluorooctanoic acid. *Chemosphere* **2010**, *80* (2), 176–183 DOI: 10.1016/j.chemosphere.2010.03.009.

(16) O'Hagan, D. Understanding organofluorine chemistry. An introduction to the C-F bond. *Chem. Soc. Rev.* **2008**, *37* (2), 308–319 DOI: 10.1039/b711844a.

(17) Starkov, a a; Wallace, K. B. Structural determinants of fluorochemical-induced mitochondrial dysfunction. *Toxicol. Sci.* **2002**, *66* (2), 244–252.

(18) Hu, W. yue; Jones, P. D.; DeCoen, W.; King, L.; Fraker, P.; Newsted, J.; Giesy, J. P. Alterations in cell membrane properties caused by perfluorinated compounds. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2003**, *135* (1), 77–88 DOI: 10.1016/S1532-0456(03)00043-7.

(19) Shan, G.; Ye, M.; Zhu, B.; Zhu, L. Enhanced cytotoxicity of pentachlorophenol by perfluorooctane sulfonate or perfluorooctanoic acid in HepG2 cells. *Chemosphere* **2013**, *93* (9), 2101–2107 DOI: 10.1016/j.chemosphere.2013.07.054.

(20) Jernbro, S.; Rocha, P. S.; Keiter, S.; Skutlarek, D.; Färber, H.; Jones, P. D.; Giesy, J. P.; Hollert, H.; Engwall, M. Perfluorooctane Sulfonate Increases the Genotoxicity of Cyclophosphamide in the Micronucleus Assay with V79 Cells. *Environ. Sci. Pollut. Res. Int.* **2007**, *14* (2), 85–87.

(21) Boltes, K.; Rosal, R.; García-Calvo, E. Toxicity of mixtures of perfluorooctane sulphonic acid with chlorinated chemicals and lipid regulators. *Chemosphere* **2012**, *86* (1), 24–29 DOI: 10.1016/j.chemosphere.2011.08.041.

(22) Eberl, L. N-acyl homoserinelactone-mediated gene regulation in gram-negative bacteria. *Syst. Appl. Microbiol.* **1999**, *22* (4), 493–506 DOI: 10.1016/S0723-2020(99)80001-0.

(23) Waters, C. M.; Bassler, B. L. Quorum sensing: cell-to-cell communication in bacteria. *Annu. Rev. Cell Dev. Biol.* **2005**, *21*, 319–346 DOI: 10.1146/annurev.cellbio.21.012704.131001.

(24) Boyer, M.; Wisniewski-Dyé, F. Cell-cell signalling in bacteria: not simply a matter of quorum. *FEMS Microbiol. Ecol.* **2009**, *70* (1), 1–19 DOI: 10.1111/j.1574-6941.2009.00745.x.

(25) Galloway, W. R. J. D.; Hodgkinson, J. T.; Bowden, S. D.; Welch, M.; Spring, D. R. Quorum Sensing in Gram-Negative Bacteria : Small-Molecule Modulation of AHL and AI-2 Quorum Sensing Pathways. *Chem. Rev.* **2011**, *111* (1), 28–67.

(26) Colton, D. M.; Stabb, E. V.; Hagen, S. J. Modeling analysis of signal sensitivity and specificity by Vibrio fischeri LuxR variants. *PLoS One* **2015**, *10* (5), 1–21 DOI: 10.1371/journal.pone.0126474.

(27) 38719 Photobacterium Broth.

(28) Maisuria, V. B.; Hosseinidoust, Z.; Tufenkji, N. Polyphenolic Extract from Maple Syrup Potentiates Antibiotic Susceptibility and Reduces Biofilm Formation of Pathogenic Bacteria. No. 514, 1–6.

(29) Kleszczyński, K.; Składanowski, A. C. Mechanism of cytotoxic action of perfluorinated acids. I. alteration in plasma membrane potential and intracellular pH level. *Toxicol. Appl. Pharmacol.* **2009**, *234* (3), 300–305 DOI: 10.1016/j.taap.2008.10.008.

(30) Hu, W. yue; Jones, P. D.; DeCoen, W.; King, L.; Fraker, P.; Newsted, J.; Giesy, J. P. Alterations in Cell Membrane Properties Caused by Perfluorinated Compounds. *Comp. Biochem. Physiol. Part C* **2003**, *135*, 77–88.

(31) Liu, W.; Chen, S.; Quan, X.; Jin, Y. Toxic Effect of Serial Perfluorosulfonic and Perfluorocarboxylic Acids On the Membrane System of a Freshwater Alga Measured by Flow Cytometry. *Environ. Toxicol. Chem.* **2008**, *27* (7), 1597–1604.

(32) Nobels, I.; Dardenne, F.; De Coen, W.; Blust, R. Application of a multiple endpoint bacterial reporter assay to evaluate toxicological relevant endpoints of perfluorinated compounds with different functional groups and varying ch ... Toxicology in Vitro functional groups and varying chain length. *Toxicol. Vitr.* **2010**, *24*, 1768–1774 DOI: 10.1016/j.tiv.2010.07.002.

(33) Olsen, G. W.; Burris, J. M.; Ehresman, D. J.; Froehlich, J. W.; Seacat, A. M.;

Butenhoff, J. L.; Zobel, L. R. Half-life of serum elimination of perfluorooctanesulfonate,perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. *Environ. Health Perspect.* **2007**, *115* (9), 1298–1305 DOI: 10.1289/ehp.10009.

(34) Kuklenyik, Z.; Reidy, J. A.; Caudill, S. P.; Tully, J. S.; Needham, L. L. Serum Concentrations of 11 Polyfluoroalkyl Compounds in the U.S. Population : Data from the National Health and Nutrition Examination Survey (NHANES) 1999 - 2000. *Environ. Sci. Technol.* **2007**, *41*, 2237–2242.

(35) Calafat, A. M.; Wong, L.-Y.; Kuklenyik, Z.; Reidy, J. a; Needham, L. L. Polyfluoroalkyl chemicals in the U.S. population: data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000. *Environ. Health Perspect.* **2007**, *115* (11), 1596–1602 DOI: 10.1289/ehp.10598.

(36) Boyer, M.; Wisniewski-Dyé, F. Cell-cell signalling in bacteria: not simply a matter of quorum. *FEMS Microbiol. Ecol.* **2009**, *70* (1), 1–19 DOI: 10.1111/j.1574-6941.2009.00745.x.

(37) Xiao, F.; Simcik, M. F.; Halbach, T. R.; Gulliver, J. S. Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in soils and groundwater of a U.S. metropolitan area: Migration and implications for human exposure. *Water Res.* **2015**, *72*, 64–74 DOI: 10.1016/j.watres.2014.09.052.

(38) Dong, Y.; Gusti, A. R.; Zhang, Q.; Xu, J.; Zhang, L. Identification of Quorum-Quenching N -Acyl Homoserine Lactonases from Bacillus Species Identification of Quorum-Quenching N -Acyl Homoserine Lactonases from Bacillus Species. *Appli. Environ. Microbiol.* **2002**, *68* (4), 1754–1759 DOI: 10.1128/AEM.68.4.1754.

(39) Lin, Y. H.; Xu, J. L.; Hu, J.; Wang, L. H.; Leong Ong, S.; Renton Leadbetter, J.; Zhang, L. H. Acyl-homoserine lactone acylase from Ralstonia strain XJ12B represents a novel and potent class of quorum-quenching enzymes. *Mol. Microbiol.* **2003**, *47* (3), 849–860 DOI: 10.1046/j.1365-2958.2003.03351.x.

Chapter 5: Functional Consequences of PFAS and AFFF Exposure in Anaerobic Communities

Introduction

The use of aqueous film forming foams (AFFF) has resulted in wide-spread and persistent pollution globally. Significant contamination of surface water, groundwater and biota proximate to AFFF use has been reported.[1–4] Areas contaminated with AFFF can contain up to mg/L levels of poly- and perfluoroalkyl substances (PFAS),[5,3] the fluorinated chains of which are resistant to degradation.[3,5] In addition to high levels of PFAS, AFFF also contains solvents, corrosion inhibitors, and hydrocarbon-based surfactants.[e.g., 2] To complicate matters, AFFF formulations are proprietary and have varied over the years of their manufacture, with over 100 per- and polyfluorinated surfactants associated with AFFF use.[6,7]

The PFAS components of AFFF have caused environmental concern because of their effect on biota and persistence in the environment.[8–10] Of particular concern, PFAS have been found to alter the response of organisms to other contaminants present in the environment, so called co-contaminants. Many changes in co-contaminant toxicity in the presence of PFAS have been linked to PFAS-caused changes in cell membrane permeability. PFAS, specifically carboxylates with 7-8 fluorinated carbons and sulfonates with 4-8 fluorinated carbons were shown to increase cell membrane permeability, and as a result, the quorum sensing response in a pure bacterial culture (Chapter 4). Others have shown that cellular uptake and the genotoxicity of cyclophosphamide increased in lung cells exposed to 12 mg/L PFOS.[11] Similarly, the exposure of liver cells to either 50 mg/L PFOS or 41 mg/L PFOA increased the uptake and toxicity of pentachlorophenol.[12] Although these effects were presumably a result of PFAS-induced cell permeability

increases, the toxicity of atrazine and diuron decreased when algal cells were exposed to 10 – 40 mg/L PFOS,[13] complicating a simple extrapolation of the risk associated with mixtures of PFAS and other contaminants.

AFFF and some of its components have also been observed to alter microbial function and transport in ways that cannot easily be attributed to changes in permeability. Simulated AFFF formulations with concentrations of total PFAS of 110 mg/L were shown to inhibit the degradation of trichloroethylene and alter microbial community structure in microcosms enriched for *Dehalococcoides mccartyi* strains, organisms responsible for the dechlorination of chlorinated contaminants.[14,15] In other experiments, **s**imulated AFFF did not change the rate or extent of toluene degradation in a pure culture of bacteria, but did increase biofilm formation and the expression of stress response factors.[16] Finally, PFOA exposure at 100 μg/L increased the transport of a pure bacterial culture in simulated aquifer material.[17] Although AFFF can have an ecological impact, the chemical components responsible for, and reasons for this impact, are not entirely clear. In addition, the complex composition of AFFF makes it particularly difficult to understand which components dominate from a risk perspective.

The research presented herein was based on our previous research on the impact of individual PFAS on microbial membrane permeability and partitioning. That research was expanded to examine the effects of AFFF on microbial function, both in the absence and presence of co-contaminants, and to ascertain why these effects occur (physical-chemical reasons or physiological reasons). We also sought to discern whether these effects could be predicted from the behavior of the dominant PFAS in the AFFF

formulation studied and which 4-9-carbon carboxylated and sulfonated PFAS are likely to cause such effects.

Methods

Chemicals

Six different PFAS were tested to study the effects of fluorinated chain length and functional group. Three different carboxylates and three different sulfonates were tested: perfluorobutanoate (PFBA, CAS 375-22-4), perfluorooctane (PFOA, CAS 335-67-1), and perfluorononanoate (PFNA, CAS 375-95-1) and perfluorobutane sulfonate (PFBS, CAS 375-73-5), perfluorohexane sulfonate (PFHxS, CAS 355-46-4), and perfluorooctane sulfonate (PFOS, CAS 1763-23-1). Other than PFOS, all PFAS were purchased from Sigma Aldrich. PFOS was purchased from Santa Cruz Biotechnology. PFAS were dissolved in methanol and stored in the freezer. Light Water™ AFFF manufactured by 3M (Lot 624 packed in April, 1990) was obtained from a local municipal fire station and stored in a sealed polypropylene tube. The AFFF was analyzed for perfluoroalkyl substances; PFOS and PFHxS were the dominant PFAS present at concentrations of 13 ± 3 and 2.3 ± 0.2 g/L AFFF respectively. Concentrations were measured by diluting AFFF in methanol in triplicate.

Microbial Culture

A complex community containing organisms with both diverse and redundant metabolic functions was desired. Because AFFF has a high organic carbon content[2], environments contaminated with AFFF are expected to be anaerobic, so an anaerobic community was also desired for use in the experiments. An anaerobic digester

community was therefore used to assess effects of AFFF and PFAS on microbial function, as it contains a complex community structure, high biomass levels, and was expected to have members with both highly redundant and diverse metabolic capabilities. Digester seed was obtained from the Empire Wastewater Treatment Plant (Farmington, MN) and was used to inoculate four 1.6-L reactors. Reactors had an equal solids and hydraulic retention time (SRT and HRT, respectively) of three weeks and were fed a 50/50 mixture of thickened waste activated sludge and thickened primary sludge collected monthly from the Empire Wastewater Treatment Plant. Reactors were maintained at a temperature of 37°C. Total gas production and pH were monitored in reactors 1-2 times a week. Reactors were operated for over a year before digester material was removed for experiments. Gas production and pH over this period were 1.65 ± 0.51 L/day and 7.18 ± 0.15, respectively (Appendix C, Figures C.8, C.9).

Experimental Design

***Co-contaminant toxicity*.** Immediately after the digesters were fed, sludge was collected for use in experiments. Sludge was diluted 1:10 with reduced anaerobic mineral media (RAMM,[18]) in an anaerobic glovebag (Coy). RAMM was prepared as described by Shelton and Tiedje[18] then sterilized by boiling under nitrogen gas for over an hour prior to use. pH was adjusted using hydrochloric acid or sodium hydroxide inside the anaerobic glovebag.

Diluted sludge was divided into two portions: one portion was amended to a final concentration of 1 mg/L 2,4-dichlorophenol (DCP) via a methanol stock and the other received the equivalent amount of methanol only, both to a final methanol concentration

of 18.5 mg/L. DCP was chosen as a model co-contaminant because it is expected to partition into cell membranes where it can cause uncoupling,[19,20] similar to the expected partitioning behavior of PFAS. All PFAS-containing treatments contained a final PFAS concentration of 50 mg/L. All treatments were prepared and analyzed in triplicate. PFNA, PFOS, PFOA, and PFHxS were added to empty 100-mL serum bottles via methanol stock and the methanol was allowed to evaporate for at least 48 hrs. The four-carbon PFAS (PFBS and PFBA) were handled slightly differently. These two PFAS were added as a methanol stock directly to serum bottles containing diluted sludge with or without added DCP; again, negative controls for these treatments were prepared identically and therefore contained methanol but no PFBS or PFBA. In experiments with AFFF, neat AFFF was added directly to 100-mL serum bottles such that the final concentration of PFOS and PFHxS were 50 mg/L and 8.8 mg/L, respectively; the equivalent amount of deionized water was added to comparison PFAS-containing treatments and PFAS-free controls. Negative controls for these treatments were again prepared in an identical manner, but without added PFAS or AFFF.

To begin experiments, diluted sludge (90-mL), either with or without added DCP, was added to the serum bottles and capped with butyl rubber caps and aluminum crimps. All treatments were shaken at 150 rpm at 37°C. Methane production was used as an indicator of toxicity and was measured twice daily for approximately 3 days. The pH and volatile solids of the diluted sludge were measured at Day zero. In the experiment with AFFF, the chemical oxygen demand (COD) was measured at Days 0, 3, and 28 (Appendix C, Table C.1).

DCP Degradation. Experiments were set up as described above. During the first five days of the experiment, samples (2 mL) were taken daily from each bottle in the anaerobic glovebag for DCP analysis. After this, DCP was analyzed every 2-3 days in each bottle until the end of the experiment at 25 days, or until the DCP was no longer detected. Methane and biogas samples were measured immediately before DCP samples were taken. COD was monitored in initial diluted sludge and in PFAS/AFFF-amended treatments at Days 0, 3, and 25.

Sorption. The quantity of DCP and PFAS sorption to diluted sludge was also determined. Ten milliliters of sludge diluted by 10 with RAMM made without resazurin or sodium sulfide was placed into glass centrifuge vials on the bench top (aerobic conditions). PFAS, both with and without added DCP (1 mg/L) were added to vials. The PFAS tested included AFFF (at a concentration such that PFOS and PFHxS concentrations were 50 mg/L and 8.8 mg/L, respectively), PFOS (50 mg/L), PFHxS (8.8 mg/L), and PFOS+PFHxS (50 mg/L and 8.8 mg/L, respectively). PFAS-free controls were also prepared and analyzed to determine DCP partitioning in the absence of PFAS. All vials contained equivalent amounts of methanol and water. Vials were sealed with aluminum foil-covered screw caps and mixed using a Glas-Col rotator (18 rpm) at room temperature. After mixing for 24 hr, 1-mL whole-vial samples were taken for DCP analysis. A 4-mL sample was taken for determination of DCP in solid and liquid fractions; fractions were separated via centrifugation at 2,500 rcf for 15 min. Supernatant (1-mL) was sampled and the rest was decanted. Contaminants were extracted from the solids portion as described below.

Analytical Methods

Biogas and methane. Total biogas production was measured by water displacement in a 50-mL glass burette. Methane was measured by gas chromatography via a Hewlett Packard 6890 coupled with a thermal conductivity detector. A 3 m X 3.2 mm X 2.1 mm 45/60 MOL SIEVE 13X Supelco column was used for compound separation. Headspace samples (200-μL) were injected. Briefly, a 250-μL sample was collected from serum vials via gas-tight syringe. Collected gas was brought to ambient pressure by releasing 50-μL into water. The samples were separated at a constant oven temperature (75°C) with a carrier gas (Ultra Pure Grade nitrogen, >99.99%) flow rate of 19.3 mL/min. The inlet was set at 150°C with a total flow of 20 mL/min nitrogen gas and the detector was kept at 210°C with a reference flow of 45 mL/min of nitrogen gas.

DCP. DCP was extracted from samples with hexane. Briefly, 2-mL hexane was added to a previously collected sample and shaken for approximately 60 s. Vials were then settled for 20 min and hexane was drawn off and shaken with 0.5 g sodium sulfate. Samples were then analyzed by a Hewlett Packard 6890 gas chromatograph coupled with a micro electron capture detector and fit with a RESTEK Rtx-1701 column (30 m x 0.25 mm and 0.25 μm film thickness). The GC was operated under the following conditions: carrier gas flow rate, 2 mL/min; oven temperature held at 105°C for 10 min and then ramped to 140°C at a rate of 5°C/min; detector temperature, 350°C; and inlet temperature, 280°C. DCP standards were made with sterilized anaerobic sludge and were prepared identically to samples from vials; therefore, extraction efficiency was accounted for.

***Volatile Solids*.** To measure volatile solids, the mass difference between a sample after drying and after heating overnight in a 550°C furnace was determined. A minimum of six replicates were used to determine the volatile solids concentration in a sample.

Data Analysis

Toxicity. Cumulative methane production was plotted with time and a linear regression was performed on each replicate treatment. The slope was defined as the rate of methane production. The rates for each replicate were averaged for each treatment and compared to the corresponding control. The effect of PFOS was averaged from three separate experiments, each performed in triplicate. Antagonistic interactions were defined as when the predicted combination effect was significantly less than the measured effect and synergistic interactions were defined as when the predicted combination was significantly greater than the measured effect. When predicted and measured effects were statistically equal, the interaction was said to be additive. Predicted methane production in treatments containing both DCP and PFAS were calculated by subtracting the effect of the PFAS and DCP treatments from the corresponding experimental control, as shown below.

Predicted Rate = Rate Control – (Rate Control – Rate PFAS Treatment) – (Rate Control – Rate DCP Treatment)

Degradation. Degradation was modeled by the Gompertz equation[21,22] (below) to compare the lag period prior to the onset of DCP degradation (k). Variable (a), initial DCP concentration, was held constant at 1 mg/L. The decay rate (c) for treatments was obtained from their corresponding experimental controls. Equations were solved using the Solver function in Microsoft Excel to minimize the sum of square differences.

$$f(t) = ae^{-e^{c(t-k)}}$$

where a = initial DCP concentration,
k = time to inflection point, termed lag,
c = fitting parameter associated with degradation rate

Sorption. Partition coefficients (K_d) were defined as the ratio of a compound's concentration in the solid phase (mg/kg) to the compound's concentration in the liquid phase (mg/L), displayed below. Both solid and liquid concentrations were measured directly. The concentration of solids was measured as described above. Outliers were determined using Dixon and Grubb's tests. One outlier in the PFOS treatment was identified and was removed.

$$K_d\,(L{\cdot}kg^{-}) = \frac{C_s\ (mg{\cdot}kg^{-})}{C_w\ (mg{\cdot}L^{-})}$$

where C_s = solids concentration,
C_w = aqueous concentration

Statistical analysis. A two-sided Student t-test with Welch's correction was used to determine whether significant differences existed between treatments. One-sided Student t-tests were performed to access toxicity of singular PFAS treatments. A Spearman correlation was performed to access relationship between the number of

fluorinated carbons and the effect of PFAS on lag (k) in DCP degradation. The *p*-value obtained by Spearman's correlation is reported in the text.

Results & Discussion

AFFF and PFAS toxicity. AFFF and PFAS were toxic to mixed anaerobic cultures and the effect of AFFF on methane production could be estimated by the effect of the dominant PFAS in the AFFF formula, PFOS (Figure 5.1). In addition to g/L concentrations of PFAS, AFFF also contains ingredients such as solvents, corrosion inhibitors, and hydrocarbon-based surfactants.[e.g.,2] Precursors, many of which are unknown, can make up 41-100% of the total PFAS content,[23] the effects of which are also unknown. Nevertheless, our results show that PFOS is likely the driver of AFFF toxicity to this community, with the methane production rate in the PFOS-amended treatments being equivalent to that in the AFFF-amended treatments (p=0.18). Both PFOS- and AFFF-amended treatments were statistically different from the methane production rate in the PFAS-free control treatments ($p < 0.01$ for the both treatments). The presence of PFHxS appeared to play a minor role in the toxicity of AFFF to this mixed anaerobic culture, with the PFOS+PFHxS-amended treatments affecting methane production similarly to the treatments amended with only PFOS (p=0.92). In the literature, PFAS mixtures simulating AFFF enhanced abundance of methane-generating *Archaea* at total PFAS concentrations of 110 mg/L, though methane production was not monitored.[14] Methane production requires multiple microorganisms to work symbiotically and it is possible that be microorganisms other than methane-generating

Archaea were susceptible to PFAS toxicity and caused the inhibition in methane production observed in our work.

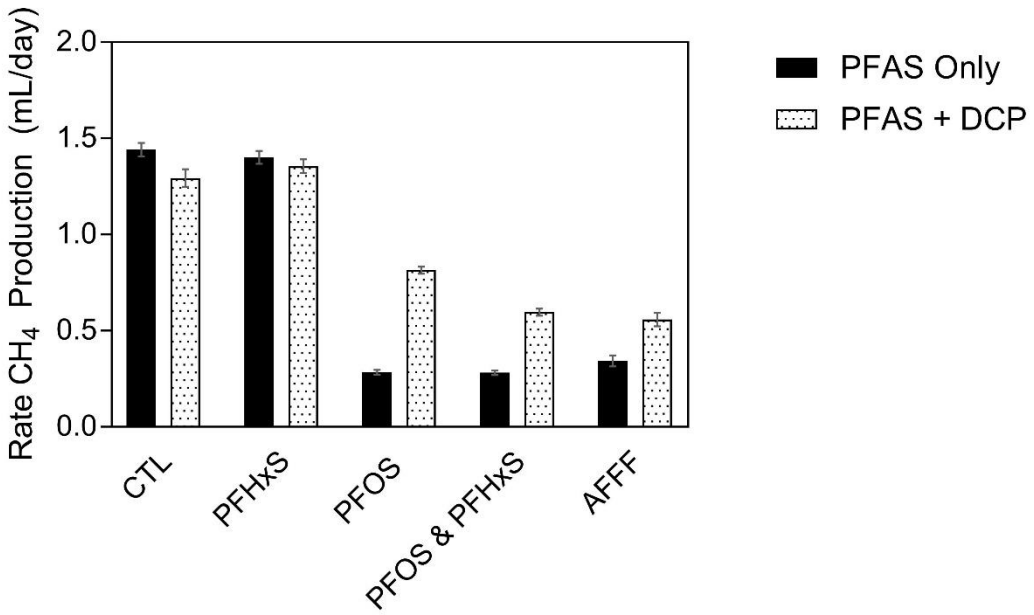


Figure 5.1. Rate of methane production within the first three days of exposure to AFFF or its primary PFAS constituents. Error bars represent the standard error of the mean.

Effects of AFFF and PFAS on co-contaminant toxicity. A co-contaminant was added to AFFF and PFAS treatments to determine if co-contaminant toxicity was altered as a result of PFAS-induced changes to cell membrane properties, such as permeability (Chapter 4). DCP was chosen as a model co-contaminant to test how the addition of two chemicals that accumulate in cell membranes behave. Of interest was whether the combination of PFAS and DCP might enhance uncoupling and cell permeability, and therefore, toxicity, as cited by others,[12,13,20] or whether the two chemicals might compete for available sites within the membrane and moderate observed toxicity. Our results

supported the latter hypothesis. In fact, the amendment of PFOS, PFOS+PFHxS, or AFFF with the co-contaminant DCP were less toxic to the culture than the amendment of PFOS, PFOS+PFHxS, or AFFF alone ($p < 0.01$, $p = 0.06$, $p = 0.01$, respectively). DCP alone had little effect on methane production in PFAS-free controls ($p = 0.12$) (Figure 5.1), (Appendix C, Figure C.6). Furthermore, the effect of AFFF exposure could not be approximated by PFOS exposure (p=0.015) but could be approximated by PFOS+PFHxS exposure (p=0.69). This indicates that as the system becomes more complex, with the presence of both co-contaminants and PFAS, AFFF behavior begins to deviate from that of its dominant PFAS component, PFOS, (Figure 5.1) and consideration of the complex chemical mixture present is needed.

The partitioning of DCP to the solids was measured to test if changes in toxicity could be a result of physical-chemical changes. We saw that the presence of PFOS or PFOS+PFHxS significantly decreased the sorption of DCP to solids when compared to DCP only ($p < 0.01$ for PFOS, p=0.02 for PFOS+PFHxS) (Figure 5.2). These results suggest that PFOS does compete with, and potentially displace, DCP in the cell membrane and also suggests that DCP in turn displaces PFOS. Perhaps this change in sorption is responsible for the moderation of toxicity observed with PFAS+co-contaminant mixtures. Interestingly, while the addition of DCP did decrease the toxicity of AFFF to the mixed anaerobic culture (Figure 5.1), AFFF did not decrease DCP sorption to solids (p=0.75) (Figure 5.2). This again suggests that the many different and unknown non-PFAS compounds, such as salt content, present in AFFF altered DCP sorption in a way that differed from the effect of PFAS alone (Figure 5.2). Additionally,

as noted above, as the system becomes more complex chemically, AFFF behavior begins to deviate from that of its dominant PFAS component. Indeed, in such a complex chemical mixture, components of AFFF could increase solubility of DCP, change interactions between DCP and PFOS outside of the membrane, or alter the interaction of PFOS with the microbial culture in an unpredicted way. More work is needed to understand exactly how DCP moderates PFAS toxicity and whether AFFF moderates co-contaminant sorption into cell surfaces.

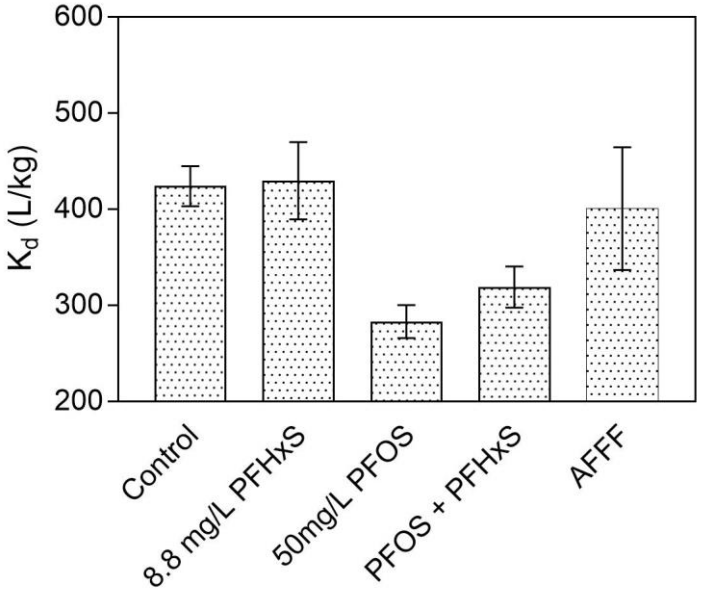


Figure 5.2. Effect of PFAS addition on DCP partitioning. Error bars represent the standard error of the mean.

Other researchers have found mixtures of PFAS and chlorophenols to deviate from toxic additivity and alter partitioning behavior. For instance, both PFOS and PFOA increased cellular uptake of pentachlorophenol and mixtures behaved synergistically towards liver cells.[12] Toxic synergism coupled with increased cellular uptake of PFOS

and pentachlorophenol was also demonstrated in green algae.[13] In contrast, the combination of 2,4,6-trichlorophenol and PFOS was antagonistic towards green algae.[24] Like DCP, both 2,4,6-trichlorophenol and pentachlorophenol are uncouplers, though they have different hydrophobicities and ionization states at experimental pH.[25,26] The effect of PFAS on uncouplers may be complex and the hydrophobicity, ionic state, and individual toxicity of the compounds may play a role.

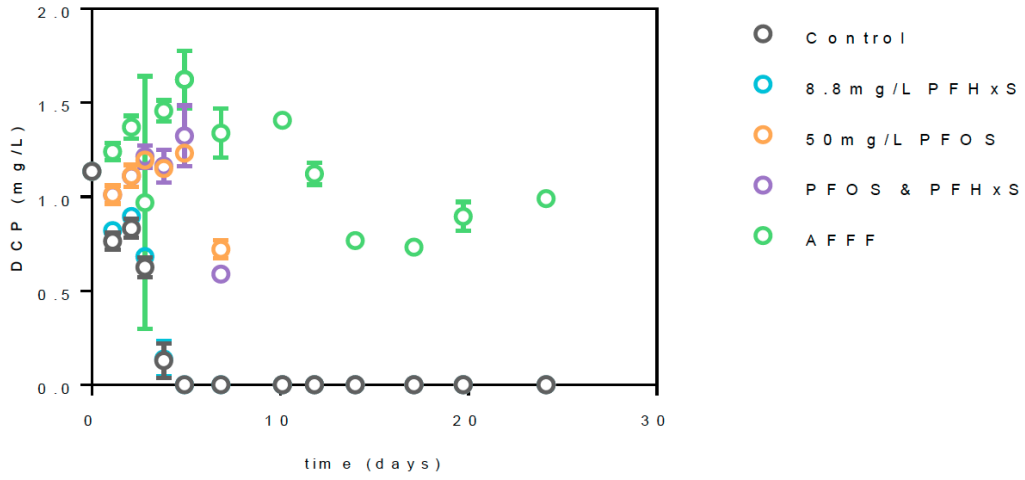


Figure 5.3. Degradation of DCP in the presence of AFFF and its major PFAS constituents. Error bars reflect standard deviation.

AFFF and PFAS effects on secondary metabolism. AFFF and PFOS also had an impact on the anaerobic community's ability to degrade the co-contaminant, DCP (Figure 5.3). DCP degradation observed in AFFF-amended treatments was not predicted by degradation observed in PFOS- or PFOS+PFHxS-amended treatments. AFFF caused a much greater lag in DCP degradation than any of the other PFAS studied. We suspect that the inhibition of DCP degradation by AFFF could again be a result of the complex chemical mixture present, including a large concentration of organic material (chemical

oxygen demand of AFFF = 670,000 ± 34,000 mg/L), (Appendix C, Table C.1). Compounds in AFFF could have changed interactions between DCP and the microbial culture, been used as a carbon source,[15] causing diauxy (Appendix C, Figure C.2), or altered the microbial community in a way that selected against DCP degradation. It is not known whether DCP would have eventually degraded after the excess carbon had been depleted. Others have seen that the degradation of trichloroethene was inhibited in the presence of PFAS (>66 mg/L) and AFFF formulations (0.3% v/v),[14,15] while PFAS mixtures alone, without the addition of the excess organic material found in AFFF, did not affect the degradation of toluene.[16] The impact of AFFF and PFAS on co-contaminant degradation is likely dependent on the identity of the co-contaminant, whether it is commonly degraded by multiple organisms, and the environmental conditions (e.g. available carbon).

Examination of fluorinated chain length and functional group. While PFOS and PFHxS are the main compounds present in AFFF, other PFAS are commonly found in the environment and formulations of various AFFF products are often changing.[7] Sulfonates and carboxylates of varying fluorinated chain lengths were tested to determine the characteristics of PFAS that could be associated with toxicity to anaerobic microbial cultures. Only PFOS and PFNA, both with eight fluorinated carbons, decreased the rate of methane production compared to PFAS-free treatments (Figure 5.4), although observed decreases where not statistically significant (p= 0.24, p=0.19, respectively). PFOS displayed greater toxicity, however, it was also less consistent. Toxicity is often related to fluorinated chain length and compounds with less than seven fluorinated

carbons are often not associated with biological effects unless present at much higher concentrations than those typically observed in the environment.[27–30] Our results were consistent with this, and overall, methane production seemed to be more adversely affected by the number of fluorinated carbons than the functional group.

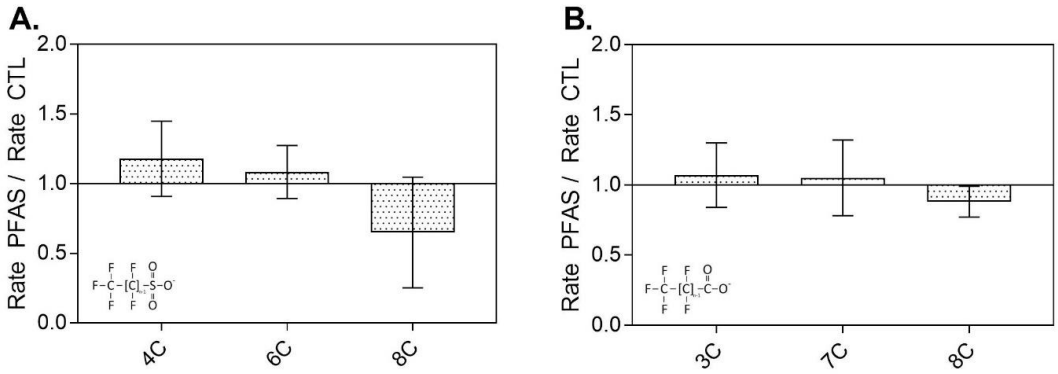


Figure 5.4. Rate of methane production within the first three days of PFAS exposure, normalized to the rate of methane production in the corresponding PFAS-free control. Experiments in which PFOS exposure occurred were repeated three times and the average is presented (e.g., n=9). Treatments are denoted by the number of fluorinated carbons in the amended PFAS; all treatments contained 50 mg/L PFAS. Errors bars represent the standard error of the mean. Panel (A) shows data for the sulfonated PFAS and panel (B) shows data for the carboxylated PFAS.

In PFAS+DCP mixtures, DCP only moderated the effect of PFOS and observed effects were a function of observed PFOS toxicity. Our previous work demonstrated that PFOS had the largest accumulation in model and microbial membranes compared to PFBA, PFOA, PFNA, PFBS, and PFHxS (Chapter 3). As a result of greater membrane deposition, DCP and PFOS may have stronger interactions within cell membranes. Alternatively, PFOS had the largest toxic effect on methane production (Figure 5.4). It is possible that DCP may moderate toxicity of other PFAS at higher PFAS concentrations, concentrations where PFAS are more toxic.

The degradation of DCP was more sensitive to the addition of PFAS than methane production. PFNA caused a slight lag in degradation at 5 mg/L (Appendix C, Figure C.5), yet had no effect on methane production at 5 mg/L. The magnitude of degradation inhibition at a concentration of 50 mg/L was a function of fluorinated chain length, functional group, and concentration (Figure 5.5). Lag, normalized to the control was positively correlated to fluorinated chain length (p=0.02) and at equal concentrations, the effect of PFNA was greater than that of PFOS (p<0.01). A study completed by Weathers *et. al*.[15] demonstrated that mixtures of perfluorinated carboxylates inhibited the degradation of trichloroethene to a greater extent than the mixture of perfluorinated sulfonates, both at concentrations greater than 22 mg/L.[15] Other studies have also demonstrated that the number of fluorinated carbons are correlated to biological effects.[e.g. 27]

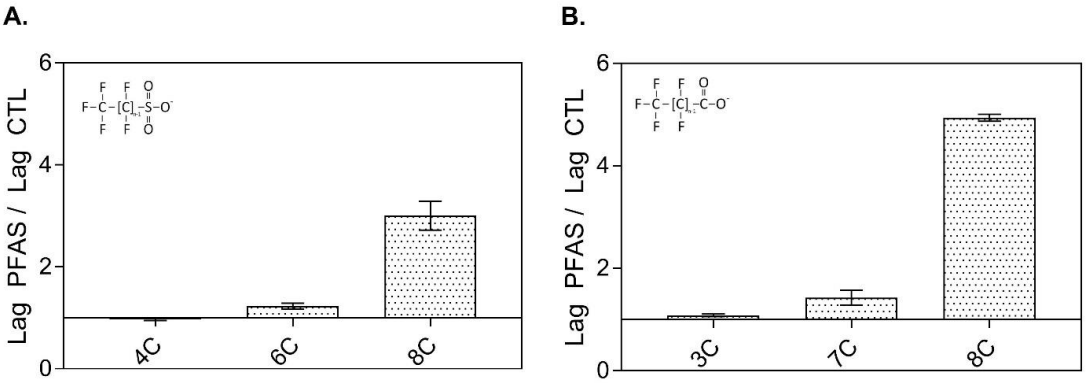


Figure 5.5. Inhibition of DCP degradation by 50mg/L PFAS. Degradation lag modeled by Gompertz equation. Error bars represent standard error of the mean. (A) Sulfonates (B) Carboxylates

Environmental Significance. We observed that PFAS effect the toxicity and degradation of co-contaminants. These effects are likely physical-chemical as well as physiological. The magnitude of the effect of sulfonates versus carboxylates varies, however, it is clear that PFAS with more fluorinated carbons have greater biological effects. Additionally, most functional effects were observed at concentrations of 50 mg/L, indicating that only areas directly affected by fire-training activities are likely to be impacted. Nevertheless, some microbial communities are more sensitive than others and the effect of PFAS will need to be assessed in specific situations if there is a reason for concern.

Acknowledgements

I would like to thank Amy Prok for initially establishing the anaerobic digester cultures and Carlise Sorenson for maintaining them. The work was supported by the Environment and Natural Resources Trust Fund as recommended by the Legislative Citizen Commission on Minnesota Resources.

References

(1) Moody, C. A.; Field, J. A. Determination of Perfluorocarboxylates in Groundwater Impacted by Fire-Fighting Activity. *Environ. Sci. Technol.* **1999**, *33* (16), 2800–2806 DOI: 10.1021/es981355+.

(2) Moody, C. A.; Field, J. A. Perfluorinated Surfactants and the Environmental Implications of Their Use in Fire-Fighting Foams. *Environ. Sci. Technol.* **2000**, *34* (18), 3864–3870 DOI: 10.1021/es991359u.

(3) Arias E, V. a; Mallavarapu, M.; Naidu, R. Identification of the source of PFOS and PFOA contamination at a military air base site. *Environ. Monit. Assess.* **2015**, *187* (1), 4111 DOI: 10.1007/s10661-014-4111-0.

(4) Gewurtz, S. B.; Bhavsar, S. P.; Petro, S.; Mahon, C. G.; Zhao, X.; Morse, D.; Reiner, E. J.; Tittlemier, S. A.; Braekevelt, E.; Drouillard, K. High levels of

perfluoroalkyl acids in sport fish species downstream of a firefighting training facility at Hamilton International Airport, Ontario, Canada. *Environ. Int.* **2014**, *67*, 1–11 DOI: 10.1016/j.envint.2014.02.005.

(5) Moody, C. A.; Field, J. A. Determination of Perfluorocarboxylates in Groundwater Impacted by Fire-Fighting Activity. *Environ. Sci. Technol.* **1999**, *33* (16), 2800–2806 DOI: 10.1021/es981355+.

(6) Agostino, L. A. D.; Mabury, S. A. Identification of Novel Fluorinated Surfactants in Aqueous Film Forming Foams and Commercial Surfactant Concentrates. *Environ. Sci. Technol.* **2014**, *48*, 121–129.

(7) Backe, W. J.; Day, T. C.; Field, J. A. Zwitterionic, cationic, and anionic fluorinated chemicals in aqueous film forming foam formulations and groundwater from U.S. military bases by nonaqueous large-volume injection HPLC-MS/MS. *Environ. Sci. Technol.* **2013**, *47* (10), 5226–5234 DOI: 10.1021/es3034999.

(8) Liou, J. S.-C.; Szostek, B.; DeRito, C. M.; Madsen, E. L. Investigating the biodegradability of perfluorooctanoic acid. *Chemosphere* **2010**, *80* (2), 176–183 DOI: 10.1016/j.chemosphere.2010.03.009.

(9) Vaalgamaa, S.; Vähätalo, A. V; Perkola, N.; Huhtala, S. Photochemical reactivity of perfluorooctanoic acid (PFOA) in conditions representing surface water. *Sci. Total Environ.* **2011**, *409* (16), 3043–3048 DOI: 10.1016/j.scitotenv.2011.04.036.

(10) O'Hagan, D. Understanding organofluorine chemistry. An introduction to the C-F bond. *Chem. Soc. Rev.* **2008**, *37* (2), 308–319 DOI: 10.1039/b711844a.

(11) Jernbro, S.; Rocha, P. S.; Keiter, S.; Skutlarek, D.; Färber, H.; Jones, P. D.; Giesy, J. P.; Hollert, H.; Engwall, M. Perfluorooctane Sulfonate Increases the Genotoxicity of Cyclophosphamide in the Micronucleus Assay with V79 Cells. *Environ. Sci. Pollut. Res. Int.* **2007**, *14* (2), 85–87.

(12) Shan, G.; Ye, M.; Zhu, B.; Zhu, L. Enhanced cytotoxicity of pentachlorophenol by perfluorooctane sulfonate or perfluorooctanoic acid in HepG2 cells. *Chemosphere* **2013**, *93* (9), 2101–2107 DOI: 10.1016/j.chemosphere.2013.07.054.

(13) Liu, W.; Zhang, Y.-B.; Quan, X.; Jin, Y.-H.; Chen, S. Effect of perfluorooctane sulfonate on toxicity and cell uptake of other compounds with different hydrophobicity in green alga. *Chemosphere* **2009**, *75* (3), 405–409 DOI: 10.1016/j.chemosphere.2008.11.084.

(14) Weathers, T. S.; Harding-Marjanovic, K.; Higgins, C. P.; Alvarez-Cohen, L.; Sharp, J. O. Perfluoroalkyl Acids Inhibit Reductive Dechlorination of Trichloroethene by Repressing Dehalococcoides. *Environ. Sci. Technol.* **2016**, *50*, 240–248 DOI: 10.1021/acs.est.5b04854.

(15) Harding-Marjanovic, K. C.; Yi, S.; Weathers, T. S.; Sharp, J. O.; Sedlak, D. L.; Alvarez-Cohen, L. Effects of Aqueous Film-Forming Foams (AFFFs) on Trichloroethene (TCE) Dechlorination by a Dehalococcoides mccartyi -Containing Microbial Community. *Environ. Sci. Technol.* **2016**, *50*, 3352–3361 DOI: 10.1021/acs.est.5b04773.

(16) Weathers, T. S.; Higgins, C. P.; Sharp, J. O. Enhanced Biofilm Production by a Toluene-Degrading Rhodococcus Observed after Exposure to Perfluoroalkyl Acids. *Environ. Sci. Technol.* **2015**, *49*, 5458–5466 DOI: 10.1021/es5060034.

(17) Wu, D.; Tong, M.; Kim, H. Influence of Perfluorooctanoic Acid on the Transport and Deposition Behaviors of Bacteria in Quartz Sand. *Environ. Sci. Technol.* **2016**, *50* (5), 2381–2388 DOI: 10.1021/acs.est.5b05496.

(18) Shelton, D. R.; Tiedje, J. M. General Method for Determining Anaerobic Biodegradation Potential. *Appl. Environ. Microbiol.* **1984**, *47* (4), 850–857.

(19) Chen, G.; Yu, H.; Liu, H.; Xu, D. Response of activated sludge to the presence of 2 , 4-dichlorophenol in a batch culture system. *Process Biochem.* **2006**, *41*, 1758–1763 DOI: 10.1016/j.procbio.2006.03.022.

(20) Song, L.; Wenju, J.; Qiong, T.; Yaozhong, L. Impact of a metabolic uncoupler , 2 , 4-dichlorophenol on minimization of activated sludge production in membrane bioreactor. *Water Sci. && Technol.* **2010**, *62.6*, 1379–1385 DOI: 10.2166/wst.2010.313.

(21) Okpokwasili, G. C.; Nweke, C. O. Microbial growth and substrate utilization kinetics. **2005**, *5* (4).

(22) Gregory Hallett LeFevre. Fate and Degradation of Petroleum Hydrocarbons in Stormwater Bioretention Cells, University of Minnesota, 2012.

(23) Houtz, E. F.; Higgins, C. P.; Field, J. A.; Sedlak, D. L. Persistence of perfluoroalkyl acid precursors in AFFF-impacted groundwater and soil. *Environ. Sci. Technol.* **2013**, *47* (15), 8187–8195 DOI: 10.1021/es4018877.

(24) Boltes, K.; Rosal, R.; García-Calvo, E. Toxicity of mixtures of perfluorooctane sulphonic acid with chlorinated chemicals and lipid regulators. *Chemosphere* **2012**, *86* (1), 24–29 DOI: 10.1016/j.chemosphere.2011.08.041.

(25) Escher, B. I.; Snozzi, M.; Schwarzenbach, R. P. Uptake, speciation, and uncoupling activity of substituted phenols in energy transducing membranes. *Environ. Sci. Technol.* **1996**, *30* (10), 3071–3079 DOI: 10.1021/es960153f.

(26) Escher, B. I.; Hunziker, R.; Schwarzenbach, R. P.; Westall, J. C. Kinetic Model To Describe the Intrinsic Uncoupling Activity of Substituted Phenols in Energy

Transducing Membranes. *Environ. Sci. Technol.* **1999**, *33* (4), 560–570 DOI: 10.1021/es980545h.

(27) Hu, W. yue; Jones, P. D.; DeCoen, W.; King, L.; Fraker, P.; Newsted, J.; Giesy, J. P. Alterations in cell membrane properties caused by perfluorinated compounds. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2003**, *135* (1), 77–88 DOI: 10.1016/S1532-0456(03)00043-7.

(28) Oldham, E. D.; Xie, W.; Farnoud, A. M.; Fiegel, J.; Lehmler, H. Disruption of Phosphatidylcholine Monolayers and Bilayers by Perfluorobutane Sulfonate. *J. Phys. Chem.* **2012**, *116*, 9999–10007.

(29) Lehmler, H. J.; Xie, W.; Bothun, G. D.; Bummer, P. M.; Knutson, B. L. Mixing of perfluorooctanesulfonic acid (PFOS) potassium salt with dipalmitoyl phosphatidylcholine (DPPC). *Colloids Surfaces B Biointerfaces* **2006**, *51* (1), 25–29 DOI: 10.1016/j.colsurfb.2006.05.013.

(30) Ding, G.; Peijnenburg, W. J. G. M. Physicochemical Properties and Aquatic Toxicity of Poly- and Perfluorinated Compounds. *Crit. Rev. Environ. Sci. Technol.* **2013**, *43* (6), 598–678 DOI: 10.1080/10643389.2011.627016.

Chapter 6: Conclusions and Recommendations

Results consistently showed that partitioning and subsequent PFAS-mediated effects (fluidity and permeability changes) were a function of fluorinated chain length and functional group. In all experiments, PFAS with longer chain lengths had greater effects on microbial populations and communities, likely as a result of greater accumulation in membranes. Fluorinated chain length was correlated to greater luminescence, greater inhibition in methane production, and more dramatically delayed DCP degradation. Effects of PFAS with seven or more fluorinated carbons had a greater impact on microbial function and their presence is of greater concern compared to short-chain PFAS. While the effects of PFAS with less than seven fluorinated carbons (short-chain PFAS) were not as pronounced, their concentrations should be monitored as they are increasing in use. It is likely that short-chain PFAS have the same mechanism of action, yet are not as potent. Furthermore, it is unknown how short-chain PFAS behave when they exist in PFAS mixtures. Short-chain PFAS may essentially supplement the effect of long-chain PFAS.

The effects of functional group were less clear and were unique to the particular microbial function. Sulfonates were more accumulative in bacteria and caused greater increases in luminescence. Carboxylates, however, caused a greater lag in the degradation of DCP. Thus, the impact of functional group will likely need to be studied for each microbial function of interest.

Microbial communities were much more tolerant to PFAS than initially thought. PFAS had little to no effect on anaerobic digester communities at levels below 50 mg/L.

The quorum sensing response in the pure culture of *Aliivibrio fischeri* was much more susceptible to PFAS exposure and effects were seen at 10 μg/L. This demonstrates that less redundant microbial communities may be at greater risk. Though on par with average human serum concentrations, in the environment PFAS concentrations of 10 μg/L are higher than average. These concentrations are generally only detected in areas contaminated with related industrial waste or aqueous film forming foams. While more work is needed regarding effects of PFAS on pathogens or the human microbiome, it is probable that unless a location is very contaminated and contains PFAS on the order of mg/L that microbial function will be not be greatly impacted.

Chapter 7: Comprehensive Bibliography

Stasinakis, A. S.; Petalas, A. V; Mamais, D.; Thomaidis, N. S. Application of the OECD 301F respirometric test for the biodegradability assessment of various potential endocrine disrupting chemicals. *Bioresour. Technol.* **2008**, *99* (9), 3458–3467 DOI: 10.1016/j.biortech.2007.08.002.

Moody, C. A.; Field, J. A. Perfluorinated Surfactants and the Environmental Implications of Their Use in Fire-Fighting Foams. *Environ. Sci. Technol.* **2000**, *34* (18), 3864–3870 DOI: 10.1021/es991359u.

EPA. *Emerging Contaminants – Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA) At a Glance*; 2012.

Renner, R. The long and the short of perfluorinated replacements. *Environ. Sci. Technol.* **2006**, *40* (1), 12–13 DOI: 10.1021/es062612a.

Wang, Z.; Cousins, I. T.; Scheringer, M.; Hungerbühler, K. Fluorinated alternatives to long-chain perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFSAs) and their potential precursors. *Environ. Int.* **2013**, *60* (2013), 242–248 DOI: 10.1016/j.envint.2013.08.021.

Chu, S.; Letcher, R. J. In vitro metabolic formation of perfluoroalkyl sulfonamides from copolymer surfactants of pre- and post-2002 scotchgard fabric protector products. *Environ. Sci. Technol.* **2014**, *48* (11), 6184–6191 DOI: 10.1021/es500169x.

Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J. Perfluoroalkyl Acids : A Review of Monitoring and Toxicological Findings. *Toxicol. Sci.* **2007**, *99* (2), 366–394 DOI: 10.1093/toxsci/kfm128.

Busch, J.; Ahrens, L.; Sturm, R.; Ebinghaus, R. Polyfluoroalkyl compounds in landfill leachates. *Environ. Pollut.* **2010**, *158* (5), 1467–1471 DOI: 10.1016/j.envpol.2009.12.031.

Sinclair, E.; Kannan, K. Mass loading and fate of perfluoroalkyl surfactants in wastewater treatment plants. *Environ. Sci. Technol.* **2006**, *40* (5), 1408–1414.

Zareitalabad, P.; Siemens, J.; Hamer, M.; Amelung, W. Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in surface waters, sediments, soils and wastewater - A review on concentrations and distribution coefficients. *Chemosphere* **2013**, *91* (6), 725–732 DOI: 10.1016/j.chemosphere.2013.02.024.

Paul, A. G.; Jones, K. C.; Sweetman, A. J. A First Global Production , Emission , And Environmental Inventory For Perfluorooctane Sulfonate. **2012**, *43* (2), 386–392.

Xiao, F.; Halbach, T. R.; Simcik, M. F.; Gulliver, J. S. Input characterization of perfluoroalkyl substances in wastewater treatment plants: Source discrimination by exploratory data analysis. *Water Res.* **2012**, *46* (9), 3101–3109 DOI: 10.1016/j.watres.2012.03.027.

Konwick, B. J.; Tomy, G. T.; Ismail, N.; Peterson, J. T.; Fauver, R. J.; Higginbotham, D.; Fisk, A. T. Concentrations and patterns of perfluoroalkyl acids in Georgia, USA surface waters near and distant to a major use source. *Environ. Toxicol. Chem.* **2008**, *27* (10), 2011–2018 DOI: 10.1897/07-659.1.

Arias E, V. a; Mallavarapu, M.; Naidu, R. Identification of the source of PFOS and PFOA contamination at a military air base site. *Environ. Monit. Assess.* **2015**, *187* (1), 4111 DOI: 10.1007/s10661-014-4111-0.

de Solla, S. R.; De Silva, a O.; Letcher, R. J. Highly elevated levels of perfluorooctane sulfonate and other perfluorinated acids found in biota and surface water downstream of an international airport, Hamilton, Ontario, Canada. *Environ. Int.* **2012**, *39* (1), 19–26 DOI: 10.1016/j.envint.2011.09.011.

Rayne, S.; Forest, K. Perfluoroalkyl sulfonic and carboxylic acids: a critical review of physicochemical properties, levels and patterns in waters and wastewaters, and treatment methods. *J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng.* **2009**, *44* (12), 1145–1199 DOI: 10.1080/10934520903139811.

Moody, C. A.; Field, J. A. Determination of Perfluorocarboxylates in Groundwater Impacted by Fire-Fighting Activity. *Environ. Sci. Technol.* **1999**, *33* (16), 2800–2806 DOI: 10.1021/es981355+.

Huset, C. a; Barlaz, M. a; Barofsky, D. F.; Field, J. a. Quantitative determination of fluorochemicals in municipal landfill leachates. *Chemosphere* **2011**, *82* (10), 1380–1386 DOI: 10.1016/j.chemosphere.2010.11.072.

Eggen, T.; Moeder, M.; Arukwe, A. Municipal landfill leachates: a significant source for new and emerging pollutants. *Sci. Total Environ.* **2010**, *408* (21), 5147–5157 DOI: 10.1016/j.scitotenv.2010.07.049.

Loos, R.; Wollgast, J.; Huber, T.; Hanke, G. Polar herbicides, pharmaceutical products, perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and nonylphenol and its carboxylates and ethoxylates in surface and tap waters around Lake Maggiore in Northern Italy. *Anal. Bioanal. Chem.* **2007**, *387* (4), 1469–1478 DOI: 10.1007/s00216-006-1036-7.

Kim, S.-K.; Kannan, K. Perfluorinated acids in air, rain, snow, surface runoff, and lakes: relative importance of pathways to contamination of urban lakes. *Environ. Sci. Technol.* **2007**, *41* (24), 8328–8334.

Nakayama, S.; Strynar, M. J.; Helfant, L.; Egeghy, P.; Ye, X.; Lindstrom, A. B. Perfluorinated compounds in the Cape Fear Drainage Basin in North Carolina. *Environ. Sci. Technol.* **2007**, *41*, 5271–5276 DOI: 10.1021/es070792y.

O'Hagan, D. Understanding organofluorine chemistry. An introduction to the C-F bond. *Chem. Soc. Rev.* **2008**, *37* (2), 308–319 DOI: 10.1039/b711844a.

Ochoa-Herrera, V.; Field, J. A.; Luna-Velasco, A.; Sierra-Alvarez, R. Microbial toxicity

and biodegradability of perfluorooctane sulfonate (PFOS) and shorter chain perfluoroalkyl and polyfluoroalkyl substances (PFASs). *Environ. Sci. Process. Impactts* **2016**, *18* DOI: 10.1039/c6em00366d.

Plumlee, M. H.; McNeill, K.; Reinhard, M. Indirect Photolysis of Perfluorochemicals: Hydroxyl Radical-Initiated Oxidation of N-Ethyl Perfluorooctane Sulfonamido Acetate (N-EtFOSAA) and Other Perfluoroalkanesulfonamides. *Environ. Sci. Technol.* **2009**, *43* (20), 3662–3668 DOI: 10.1021/es902634x.

Vaalgamaa, S.; Vähätalo, A. V; Perkola, N.; Huhtala, S. Photochemical reactivity of perfluorooctanoic acid (PFOA) in conditions representing surface water. *Sci. Total Environ.* **2011**, *409* (16), 3043–3048 DOI: 10.1016/j.scitotenv.2011.04.036.

Liou, J. S.-C.; Szostek, B.; DeRito, C. M.; Madsen, E. L. Investigating the biodegradability of perfluorooctanoic acid. *Chemosphere* **2010**, *80* (2), 176–183 DOI: 10.1016/j.chemosphere.2010.03.009.

Nguyen, T. V.; Reinhard, M.; Gin, K. Y.-H. Rate laws and kinetic modeling of N-ethyl perfluorooctane sulfonamidoethanol (N-EtFOSE) transformation by hydroxyl radical in aqueous solution. *Water Res.* **2013**, *47* (7), 2241–2250 DOI: 10.1016/j.watres.2013.01.047.

Dinglasan, M. J. a; Ye, Y.; Edwards, E. a; Mabury, S. a. Fluorotelomer alcohol biodegradation yields poly- and perfluorinated acids. *Environ. Sci. Technol.* **2004**, *38* (10), 2857–2864.

Lange, C. C.; Lundberg, J. K.; Services, P. A.; Division, S. S. The Aerobic Biodegradation of N -EtFOSE Alcohol by the Microbial Activity Present in Municipal Wastewater Treatment Sludge Table of Contents. **2000**.

Benskin, J. P.; Ikonomou, M. G.; Gobas, F. a P. C.; Begley, T. H.; Woudneh, M. B.; Cosgrove, J. R. Biodegradation of N-ethyl perfluorooctane sulfonamido ethanol (EtFOSE) and EtFOSE-based phosphate diester (SAmPAP diester) in marine sediments. *Environ. Sci. Technol.* **2013**, *47* (3), 1381–1389 DOI: 10.1021/es304336r.

Wang, N.; Szostek, B.; Buck, R. C.; Folsom, P. W.; Sulecki, L. M.; Gannon, J. T. 8-2 Fluorotelomer Alcohol Aerobic Soil Biodegradation: Pathways, Metabolites, and Metabolite Yields. *Chemosphere* **2009**, *75* (8), 1089–1096 DOI: 10.1016/j.chemosphere.2009.01.033.

Jensen, A. A.; Leffers, H. Emerging endocrine disrupters: perfluoroalkylated substances. *Int. J. Androl.* **2008**, *31* (2), 161–169 DOI: 10.1111/j.1365-2605.2008.00870.x.

Luebker, D. J.; Hansen, K. J.; Bass, N. M.; Butenhoff, J. L.; Seacat, A. M. Interactions of fluorochemicals with rat liver fatty acid-binding protein. *Toxicology* **2002**, *176* (3), 175–185.

Melzer, D.; Rice, N.; Depledge, M. H.; Henley, W. E.; Galloway, T. S. Association Between Serum Perfluorooctanoic Acid (PFOA) & Thyroid Disease in the U . S .

National Health and Nutrition Examination Survey. *Environ. Health Perspect.* **2010**, *118* (5), 686–692 DOI: 10.1289/ehp.0901584.

Naile, J. E.; Wiseman, S.; Bachtold, K.; Jones, P. D.; Giesy, J. P. Transcriptional effects of perfluorinated compounds in rat hepatoma cells. *Chemosphere* **2012**, *86* (3), 270–277 DOI: 10.1016/j.chemosphere.2011.09.044.

Reistad, T.; Fonnum, F.; Mariussen, E. Perfluoroalkylated compounds induce cell death and formation of reactive oxygen species in cultured cerebellar granule cells. *Toxicol. Lett.* **2013**, *218*, 56–60.

Steenland, K.; Fletcher, T.; Savitz, D. a. Epidemiologic evidence on the health effects of perfluorooctanoic acid (PFOA). *Environ. Health Perspect.* **2010**, *118* (8), 1100–1108 DOI: 10.1289/ehp.0901827.

Weiss, J. M.; Andersson, P. L.; Lamoree, M. H.; Leonards, P. E. G.; Leeuwen, S. P. J. Van; Hamers, T. Competitive Binding of Poly- and Perfluorinated Compounds to the Thyroid Hormone Transport Protein Transthyretin. *Toxicol. Sci.* **2009**, *109* (2), 206–216 DOI: 10.1093/toxsci/kfp055.

Yu, W.; Liu, W.; Jin, Y.; Liu, X.-H.; Wang, F.-Q.; Liu, li; Nakayama, S. F. Prenatal and Postnatal Impact of Perfluorooctane Sulfonate (PFOS) on Rat Development : A Cross-Foster Study on Chemical Burden and Thyroid Hormone System. *Environ. Sci. Technol.* **2009**, *43* (21), 8416–8422.

Geiger, S. D.; Xiao, J.; Ducatman, A.; Frisbee, S.; Innes, K.; Shankar, A. The association between PFOA, PFOS and serum lipid levels in adolescents. *Chemosphere* **2014**, *98*, 78–83 DOI: 10.1016/j.chemosphere.2013.10.005.

Ren, X.-M.; Zhang, Y.-F.; Guo, L.-H.; Qin, Z.-F.; Lv, Q.-Y.; Zhang, L.-Y. Structure-activity relations in binding of perfluoroalkyl compounds to human thyroid hormone T3 receptor. *Arch. Toxicol.* **2015**, *89* (2), 233–242 DOI: 10.1007/s00204-014-1258-y.

Grandjean, P.; Andersen, E. W. Serum Vaccine Antibody Concentrations in Children Exposed to Perfluorinated Compounds. *J. Am. Med. Assoc.* **2012**, *307* (4), 391–397.

Hu, W.; Jones, P. D.; Upham, B. L.; Trosko, J. E.; Lau, C.; Giesy, J. P. Inhibition of gap junctional intercellular communication by perfluorinated compounds in rat liver and dolphin kidney epithelial cell lines in vitro and Sprague-Dawley rats in vivo. *Toxicol. Sci.* **2002**, *68* (2), 429–436.

Fernández-Sanjuan, M.; Faria, M.; Lacorte, S.; Barata, C. Bioaccumulation and effects of perfluorinated compounds (PFCs) in zebra mussels (Dreissena polymorpha). *Environ. Sci. Pollut. Res. Int.* **2013**, *20* (4), 2661–2669 DOI: 10.1007/s11356-012-1158-8.

Nobels, I.; Dardenne, F.; De Coen, W.; Blust, R. Application of a multiple endpoint bacterial reporter assay to evaluate toxicological relevant endpoints of perfluorinated compounds with different functional groups and varying ch ... Toxicology in Vitro functional groups and varying chain length. *Toxicol. Vitr.* **2010**, *24*, 1768–1774 DOI:

10.1016/j.tiv.2010.07.002.

Loos, R.; Carvalho, R.; António, D. C.; Comero, S.; Locoro, G.; Tavazzi, S.; Paracchini, B.; Ghiani, M.; Lettieri, T.; Blaha, L.; et al. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. *Water Res.* **2013**, *47* (17), 6475–6487 DOI: 10.1016/j.watres.2013.08.024.

Ding, G.; Peijnenburg, W. J. G. M. Physicochemical Properties and Aquatic Toxicity of Poly- and Perfluorinated Compounds. *Crit. Rev. Environ. Sci. Technol.* **2013**, *43* (6), 598–678 DOI: 10.1080/10643389.2011.627016.

Rosal, R.; Rodea-Palomares, I.; Boltes, K.; Fernández-Piñas, F.; Leganés, F.; Petre, A. Ecotoxicological assessment of surfactants in the aquatic environment: combined toxicity of docusate sodium with chlorinated pollutants. *Chemosphere* **2010**, *81* (2), 288–293 DOI: 10.1016/j.chemosphere.2010.05.050.

Pasquini, L.; Merlin, C.; Hassenboehler, L.; Munoz, J.-F.; Pons, M.-N.; Gorner, T. Impact of certain household micropollutants on bacterial behavior. Toxicity tests/study of extracellular polymeric substances in sludge. *Sci. Total Environ.* **2013**, *463–464* (August 2016), 355–365 DOI: 10.1016/j.scitotenv.2013.06.018.

Hu, W. yue; Jones, P. D.; DeCoen, W.; King, L.; Fraker, P.; Newsted, J.; Giesy, J. P. Alterations in cell membrane properties caused by perfluorinated compounds. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2003**, *135* (1), 77–88 DOI: 10.1016/S1532-0456(03)00043-7.

Liu, W.; Chen, S.; Quan, X.; Jin, Y. Toxic Effect of Serial Perfluorosulfonic and Perfluorocarboxylic Acids On the Membrane System of a Freshwater Alga Measured by Flow Cytometry. *Environ. Toxicol. Chem.* **2008**, *27* (7), 1597–1604.

Matyszewska, D.; Leitch, J.; Bilewicz, R.; Lipkowski, J. Polarization modulation infrared reflection-absorption spectroscopy studies of the influence of perfluorinated compounds on the properties of a model biological membrane. *Langmuir* **2008**, *24* (14), 7408–7412 DOI: 10.1021/la8008199.

Lehmler, H. J.; Xie, W.; Bothun, G. D.; Bummer, P. M.; Knutson, B. L. Mixing of perfluorooctanesulfonic acid (PFOS) potassium salt with dipalmitoyl phosphatidylcholine (DPPC). *Colloids Surfaces B Biointerfaces* **2006**, *51* (1), 25–29 DOI: 10.1016/j.colsurfb.2006.05.013.

Lehmler, H. J.; Bummer, P. M. Mixing of perfluorinated carboxylic acids with dipalmitoylphosphatidylcholine. *Biochim. Biophys. Acta - Biomembr.* **2004**, *1664* (2), 141–149 DOI: 10.1016/j.bbamem.2004.05.002.

Oldham, E. D.; Xie, W.; Farnoud, A. M.; Fiegel, J.; Lehmler, H. Disruption of Phosphatidylcholine Monolayers and Bilayers by Perfluorobutane Sulfonate. *J. Phys. Chem.* **2012**, *116*, 9999–10007.

Matyszewska, D.; Tappura, K.; Ora, G.; Bilewicz, R. Influence of Perfluorinated

Compounds on the Properties of Model Lipid Membranes. *J. Phys. Chem.* **2007**, *111*, 9908–9918.

Xia, X.; Dai, Z.; Rabearisoa, A. H.; Zhao, P.; Jiang, X. Comparing humic substance and protein compound effects on the bioaccumulation of perfluoroalkyl substances by Daphnia magna in water. *Chemosphere* **2015**, *119*, 978–986 DOI: 10.1016/j.chemosphere.2014.09.034.

Xia, X.; Rabearisoa, A. H.; Jiang, X.; Dai, Z. Bioaccumulation of Perfluoroalkyl Substances by Daphnia magna in Water with Different Types and Concentrations of Protein. *Environ. Sci. Technol.* **2013**, *47*, 10955–10963.

Jones, P. D.; Hu, W.; De Coen, W.; Newsted, J. L.; Giesy, J. P. Binding of perfluorinated fatty acids to serum proteins. *Environ. Toxicol. Chem.* **2003**, *22* (11), 2639–2649.

Eberl, L. N-acyl homoserinelactone-mediated gene regulation in gram-negative bacteria. *Syst. Appl. Microbiol.* **1999**, *22* (4), 493–506 DOI: 10.1016/S0723-2020(99)80001-0.

Waters, C. M.; Bassler, B. L. Quorum sensing: cell-to-cell communication in bacteria. *Annu. Rev. Cell Dev. Biol.* **2005**, *21*, 319–346 DOI: 10.1146/annurev.cellbio.21.012704.131001.

Kaplan, H. B.; Greenberg, E. P. Diffusion of Autoinducer Is Involved in Regilation of the Vibrio fischeri Luminescence System. *J. Bacteriol.* **1985**, *163* (3), 1210–1214.

Shan, G.; Ye, M.; Zhu, B.; Zhu, L. Enhanced cytotoxicity of pentachlorophenol by perfluorooctane sulfonate or perfluorooctanoic acid in HepG2 cells. *Chemosphere* **2013**, *93* (9), 2101–2107 DOI: 10.1016/j.chemosphere.2013.07.054.

Liu, W.; Zhang, Y.-B.; Quan, X.; Jin, Y.-H.; Chen, S. Effect of perfluorooctane sulfonate on toxicity and cell uptake of other compounds with different hydrophobicity in green alga. *Chemosphere* **2009**, *75* (3), 405–409 DOI: 10.1016/j.chemosphere.2008.11.084.

Jernbro, S.; Rocha, P. S.; Keiter, S.; Skutlarek, D.; Färber, H.; Jones, P. D.; Giesy, J. P.; Hollert, H.; Engwall, M. Perfluorooctane Sulfonate Increases the Genotoxicity of Cyclophosphamide in the Micronucleus Assay with V79 Cells. *Environ. Sci. Pollut. Res. Int.* **2007**, *14* (2), 85–87.

Boltes, K.; Rosal, R.; García-Calvo, E. Toxicity of mixtures of perfluorooctane sulphonic acid with chlorinated chemicals and lipid regulators. *Chemosphere* **2012**, *86* (1), 24–29 DOI: 10.1016/j.chemosphere.2011.08.041.

Johnson, R. L.; Anschutz, A. J.; Smolen, J. M.; Simcik, M. F.; Penn, R. L. The Adsorption of Perfluorooctane Sulfonate onto Sand, Clay, and Iron Oxide Surface. *J. Chem. Eng. Data* **2007**, *52*, 1165–1170.

Jing, P.; Rodgers, P. J.; Amemiya, S. High lipophilicity of perfluoroalkyl carboxylate and sulfonate: Implications for their membrane permeability. *J. Am. Chem. Soc.* **2009**, *131* (6), 2290–2296 DOI: 10.1021/ja807961s.

Kim, M.; Li, L. Y.; Grace, J. R.; Yue, C. Selecting reliable physicochemical properties of perfluoroalkyl and polyfluoroalkyl substances (PFASs) based on molecular descriptors. *Environ. Pollut.* **2015**, *196*, 462–472 DOI: 10.1016/j.envpol.2014.11.008.

Madigan, M.; Marktinko, J.; Dunlap, P.; Clark, D. *Brock Biology of Microorganisms*, 13th Editi.; Espinoza, D., Cook, K., Cutt, S., Hutchinson, E., Cogan, D., Marcus, E., Wagner, A., Eds.; Benjamin Cummings: Indianapolis, Indiana, 2006.

Ng, C. a; Hungerbuhler, K. Bioaccumulation of Perfluorinated Alkyl Acids : Observations and Models. *Environ. Sci. Technol.* **2014**, *48*, 4637–4648 DOI: 10.1021/es404008g.

Xie, W.; Ludewig, G.; Wang, K.; Lehmler, H.-J. Model and cell membrane partitioning of perfluorooctanesulfonate is independent of the lipid chain length. *Colloids Surf. B. Biointerfaces* **2010**, *76* (1), 128–136 DOI: 10.1016/j.colsurfb.2009.10.025.

Starkov, a a; Wallace, K. B. Structural determinants of fluorochemical-induced mitochondrial dysfunction. *Toxicol. Sci.* **2002**, *66* (2), 244–252.

Hu, W. yue; Jones, P. D.; DeCoen, W.; King, L.; Fraker, P.; Newsted, J.; Giesy, J. P. Alterations in Cell Membrane Properties Caused by Perfluorinated Compounds. *Comp. Biochem. Physiol. Part C* **2003**, *135*, 77–88.

Levitt, D.; Liss, A. Perflourinated Fatty Acids Alter Merocyanine 540 Dye Binding to Plasma Membranes. *J. Toxicol. Environ. Health* **1987**, *20*, 303–316.

Xu, D.; Li, C.; Chen, H.; Shao, B. Cellular response of freshwater green algae to perfluorooctanoic acid toxicity. *Ecotoxicol. Environ. Saf.* **2013**, *88*, 103–107 DOI: 10.1016/j.ecoenv.2012.10.027.

Houtz, E. F.; Higgins, C. P.; Field, J. A.; Sedlak, D. L. Persistence of perfluoroalkyl acid precursors in AFFF-impacted groundwater and soil. *Environ. Sci. Technol.* **2013**, *47* (15), 8187–8195 DOI: 10.1021/es4018877.

Agostino, L. A. D.; Mabury, S. A. Identification of Novel Fluorinated Surfactants in Aqueous Film Forming Foams and Commercial Surfactant Concentrates. *Environ. Sci. Technol.* **2014**, *48*, 121–129.

Backe, W. J.; Day, T. C.; Field, J. A. Zwitterionic, cationic, and anionic fluorinated chemicals in aqueous film forming foam formulations and groundwater from U.S. military bases by nonaqueous large-volume injection HPLC-MS/MS. *Environ. Sci. Technol.* **2013**, *47* (10), 5226–5234 DOI: 10.1021/es3034999.

Clarke, B. O.; Smith, S. R. Review of "emerging" organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. *Environ. Int.* **2011**, *37* (1), 226–247 DOI: 10.1016/j.envint.2010.06.004.

Weathers, T. S.; Higgins, C. P.; Sharp, J. O. Enhanced Biofilm Production by a Toluene-Degrading Rhodococcus Observed after Exposure to Perfluoroalkyl Acids. *Environ. Sci. Technol.* **2015**, *49*, 5458–5466 DOI: 10.1021/es5060034.

Weathers, T. S.; Harding-Marjanovic, K.; Higgins, C. P.; Alvarez-Cohen, L.; Sharp, J. O. Perfluoroalkyl Acids Inhibit Reductive Dechlorination of Trichloroethene by Repressing Dehalococcoides. *Environ. Sci. Technol.* **2016**, *50*, 240–248 DOI: 10.1021/acs.est.5b04854.

Mcnamara, P. J.; Lapara, T. M.; Novak, P. J. The Effect of Perfluorooctane Sulfonate , Exposure Time , and Chemical Mixtures on Methanogenic Community Structure and Function. *Microbiol. Insights* **2015**, *8* (S2), 1–7 DOI: 10.4137/MBI.S31345.TYPE.

Greenberg, E. P. Quorum sensing in bacteria : the LuxR-LuxI MINIREVIEW Quorum Sensing in Bacteria : the LuxR-LuxI Family of Cell Density-Responsive Transcriptional Regulatorst. *J. Bacteriol.* **1994**, *176* (2), 269–275.

Boyer, M.; Wisniewski-Dyé, F. Cell-cell signalling in bacteria: not simply a matter of quorum. *FEMS Microbiol. Ecol.* **2009**, *70* (1), 1–19 DOI: 10.1111/j.1574-6941.2009.00745.x.

Schultz, M. M.; Barofsky, D. F.; Field, J. a. Fluorinated Alkyl Surfactants. *Environ. Eng. Sci.* **2003**, *20* (5), 487–501 DOI: 10.1089/109287503768335959.

Dong, Y.; Gusti, A. R.; Zhang, Q.; Xu, J.; Zhang, L. Identification of Quorum-Quenching N -Acyl Homoserine Lactonases from Bacillus Species Identification of Quorum-Quenching N -Acyl Homoserine Lactonases from Bacillus Species. *Appli. Environ. Microbiol.* **2002**, *68* (4), 1754–1759 DOI: 10.1128/AEM.68.4.1754.

Harding-Marjanovic, K. C.; Yi, S.; Weathers, T. S.; Sharp, J. O.; Sedlak, D. L.; Alvarez-Cohen, L. Effects of Aqueous Film-Forming Foams (AFFFs) on Trichloroethene (TCE) Dechlorination by a Dehalococcoides mccartyi -Containing Microbial Community. *Environ. Sci. Technol.* **2016**, *50*, 3352–3361 DOI: 10.1021/acs.est.5b04773.

Wu, D.; Tong, M.; Kim, H. Influence of Perfluorooctanoic Acid on the Transport and Deposition Behaviors of Bacteria in Quartz Sand. *Environ. Sci. Technol.* **2016**, *50* (5), 2381–2388 DOI: 10.1021/acs.est.5b05496.

Wang, Z.; Cousins, I. T.; Scheringer, M.; Hungerbuehler, K. Hazard assessment of fluorinated alternatives to long-chain perfluoroalkyl acids (PFAAs) and their precursors: Status quo, ongoing challenges and possible solutions. *Environ. Int.* **2015**, *75* DOI: 10.1016/j.envint.2014.11.013.

Colton, D. M.; Stabb, E. V.; Hagen, S. J. Modeling analysis of signal sensitivity and specificity by Vibrio fischeri LuxR variants. *PLoS One* **2015**, *10* (5), 1–21 DOI: 10.1371/journal.pone.0126474.

Stabb, E. V.; Reich, K. A.; Ruby, E. G. Vibrio fischeri genes hvnA and hvnB encode secreted NAD+-glycohydrolases. *J. Bacteriol.* **2001**, *183* (1), 309–317 DOI: 10.1128/JB.183.1.309-317.2001.

Wargenau, A.; Tufenkji, N. Direct Detection of the Gel-Fluid Phase Transition of a Single Supported Phospholipid Bilayer Using Quartz Crystal Microbalance with

Dissipation Monitoring. *Anal. Chem.* **2014**, *86*, 8017–8020.

Wargenau, A.; Tufenkji, N. Direct Detection of Gel-Fluid Phase Transition of a Single Supported Phospholipid Bilayer Using Quartz Crystal Microbalance with Dissipation Monitoring. *Anal. Chem.* **2014**.

Wilson, W. W.; Wade, M. M.; Holman, S. C.; Champlin, F. R. Status of methods for assessing bacterial cell surface charge properties based on zeta potential measurements. *J. Microbiol. Methods* **2001**, *43*, 153–164.

Rodahl, M.; Kasemo, B. On the measurement of thin liquid overlayers with the quartz-crystal microbalance. *Sensors Actuators A Phys.* **1996**, *54* (1), 448–456 DOI: 10.1109/SENSOR.1995.721939.

Halder, S.; Yadav, K. K.; Sarkar, R.; Mukherjee, S.; Saha, P.; Haldar, S.; Karmakar, S.; Sen, T. Alteration of Zeta potential and membrane permeability in bacteria: a study with cationic agents. *Springerplus* **2015**, *4* (1), 672 DOI: 10.1186/s40064-015-1476-7.

Liu, Y.; Black, M. A.; Caron, L.; Camesano, T. A. Role of Cranberry Juice on Molecular-Scale Surface Characteristics and Adhesion Behavior of Escherichia coli. **2005** DOI: 10.1002/bit.20675.

Habash, M. B.; Mei, H. C. Van Der; Busscher, H. J.; Reid, G. Adsorption of urinary components influences the zeta potential of uropathogen surfaces. **2000**, *19*, 13–17.

Svedhem, S.; Dahlborg, D.; Ekeroth, J.; Kelly, J.; Höök, F.; Gold, J. In situ peptide-nodified supported lipid bilayers for controlled cell attachment. *Langmuir* **2003**, *19* (6), 6730–6736.

Reimhult, E.; Höök, F.; Kasemo, B. Intact vesicle adsorption and supported biomembrane formation from vesicles in solution: Influence of surface chemistry, vesicle size, temperature, and osmotic pressure. *Langmuir* **2003**, *19* (5), 1681–1691 DOI: 10.1021/la0263920.

Reimhult, E.; Larsson, C.; Kasemo, B.; Höök, F. Simultaneous surface plasmon resonance and quartz crystal microbalance with dissipation monitoring measurements of biomolecular adsorption events involving structural transformations and variations in coupled water. *Anal. Chem.* **2004**, *76* (24), 7211–7220 DOI: 10.1021/ac0492970.

Schwarzenbach, R.; Gschwend, P.; Imboden, D. *Environmental Organic Chemistry*; 2003.

Gilli, P.; Pretto, L.; Bertolasi, V.; Gilli, G. Predicting Hydrogen-Bond Strengths from Acid{\textminus}Base Molecular Properties. The p KaSlide Rule: Toward the Solution of a Long-Lasting Problem. *Acc. Chem. Res.* **2009**, *42* (1), 33–44.

Moody, C. A.; Field, J. A. Determination of Perfluorocarboxylates in Groundwater Impacted by Fire-Fighting Activity. *Environ. Sci. Technol.* **1999**, *33* (16), 2800–2806 DOI: 10.1021/es981355+.

Boyer, M.; Wisniewski-Dyé, F. Cell-cell signalling in bacteria: not simply a matter of quorum. *FEMS Microbiol. Ecol.* **2009**, *70* (1), 1–19 DOI: 10.1111/j.1574-6941.2009.00745.x.

Galloway, W. R. J. D.; Hodgkinson, J. T.; Bowden, S. D.; Welch, M.; Spring, D. R. Quorum Sensing in Gram-Negative Bacteria : Small-Molecule Modulation of AHL and AI-2 Quorum Sensing Pathways. *Chem. Rev.* **2011**, *111* (1), 28–67.

38719 Photobacterium Broth.

Maisuria, V. B.; Hosseinidoust, Z.; Tufenkji, N. Polyphenolic Extract from Maple Syrup Potentiates Antibiotic Susceptibility and Reduces Biofilm Formation of Pathogenic Bacteria. No. 514, 1–6.

Kleszczyński, K.; Składanowski, A. C. Mechanism of cytotoxic action of perfluorinated acids. I. alteration in plasma membrane potential and intracellular pH level. *Toxicol. Appl. Pharmacol.* **2009**, *234* (3), 300–305 DOI: 10.1016/j.taap.2008.10.008.

Olsen, G. W.; Burris, J. M.; Ehresman, D. J.; Froehlich, J. W.; Seacat, A. M.; Butenhoff, J. L.; Zobel, L. R. Half-life of serum elimination of perfluorooctanesulfonate,perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. *Environ. Health Perspect.* **2007**, *115* (9), 1298–1305 DOI: 10.1289/ehp.10009.

Kuklenyik, Z.; Reidy, J. A.; Caudill, S. P.; Tully, J. S.; Needham, L. L. Serum Concentrations of 11 Polyfluoroalkyl Compounds in the U.S. Population : Data from the National Health and Nutrition Examination Survey (NHANES) 1999 - 2000. *Environ. Sci. Technol.* **2007**, *41*, 2237–2242.

Calafat, A. M.; Wong, L.-Y.; Kuklenyik, Z.; Reidy, J. a; Needham, L. L. Polyfluoroalkyl chemicals in the U.S. population: data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000. *Environ. Health Perspect.* **2007**, *115* (11), 1596–1602 DOI: 10.1289/ehp.10598.

Xiao, F.; Simcik, M. F.; Halbach, T. R.; Gulliver, J. S. Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in soils and groundwater of a U.S. metropolitan area: Migration and implications for human exposure. *Water Res.* **2015**, *72*, 64–74 DOI: 10.1016/j.watres.2014.09.052.

Lin, Y. H.; Xu, J. L.; Hu, J.; Wang, L. H.; Leong Ong, S.; Renton Leadbetter, J.; Zhang, L. H. Acyl-homoserine lactone acylase from Ralstonia strain XJ12B represents a novel and potent class of quorum-quenching enzymes. *Mol. Microbiol.* **2003**, *47* (3), 849–860 DOI: 10.1046/j.1365-2958.2003.03351.x.

Gewurtz, S. B.; Bhavsar, S. P.; Petro, S.; Mahon, C. G.; Zhao, X.; Morse, D.; Reiner, E. J.; Tittlemier, S. A.; Braekevelt, E.; Drouillard, K. High levels of perfluoroalkyl acids in sport fish species downstream of a firefighting training facility at Hamilton International Airport, Ontario, Canada. *Environ. Int.* **2014**, *67*, 1–11 DOI: 10.1016/j.envint.2014.02.005.

Shelton, D. R.; Tiedje, J. M. General Method for Determining Anaerobic Biodegradation Potential. *Appl. Environ. Microbiol.* **1984**, *47* (4), 850–857.

Chen, G.; Yu, H.; Liu, H.; Xu, D. Response of activated sludge to the presence of 2 , 4-dichlorophenol in a batch culture system. *Process Biochem.* **2006**, *41*, 1758–1763 DOI: 10.1016/j.procbio.2006.03.022.

Song, L.; Wenju, J.; Qiong, T.; Yaozhong, L. Impact of a metabolic uncoupler , 2 , 4-dichlorophenol on minimization of activated sludge production in membrane bioreactor. *Water Sci. && Technol.* **2010**, *62.6*, 1379–1385 DOI: 10.2166/wst.2010.313.

Okpokwasili, G. C.; Nweke, C. O. Microbial growth and substrate utilization kinetics. **2005**, *5* (4).

Gregory Hallett LeFevre. Fate and Degradation of Petroleum Hydrocarbons in Stormwater Bioretention Cells, University of Minnesota, 2012.

Escher, B. I.; Snozzi, M.; Schwarzenbach, R. P. Uptake, speciation, and uncoupling activity of substituted phenols in energy transducing membranes. *Environ. Sci. Technol.* **1996**, *30* (10), 3071–3079 DOI: 10.1021/es960153f.

Escher, B. I.; Hunziker, R.; Schwarzenbach, R. P.; Westall, J. C. Kinetic Model To Describe the Intrinsic Uncoupling Activity of Substituted Phenols in Energy Transducing Membranes. *Environ. Sci. Technol.* **1999**, *33* (4), 560–570 DOI: 10.1021/es980545h.

Rhoads, K. R.; Janssen, E. M. L.; Luthy, R. G.; Criddle, C. S. Aerobic biotransformation and fate of N-ethyl perfluorooctane sulfonamidoethanol (N-EtFOSE) in activated sludge. *Environ. Sci. Technol.* **2008**, *42* (8), 2873–2878.

Escher, B. I.; Schwarzenbach, R. P. Mechanistic studies on baseline toxicity and uncoupling of organic compounds as a basis for modeling effective. **2002**, *64*, 20–35.

Mcnamara, P. J.; Lapara, T. M.; Novak, P. J. The Impacts of Triclosan on Anaerobic Community Structures, Function, and Antimicrobial Resistance. *Environ. Sci. Technol.* **2014**, *48* (13), 7393–7400.

WHO's first global report on antibiotic resistance reveals serious, worldwide threat to public health.

Fleischhacker, N. T. Phytoestrogen distribution and degradation in natural and engineered systems, University of Minnesota, 2012.

Avanti Polar Lipids, Inc. avantilipids.com (accessed Sep 10, 2017).

Appendix

Appendix A: Supporting Information for Chapter 3

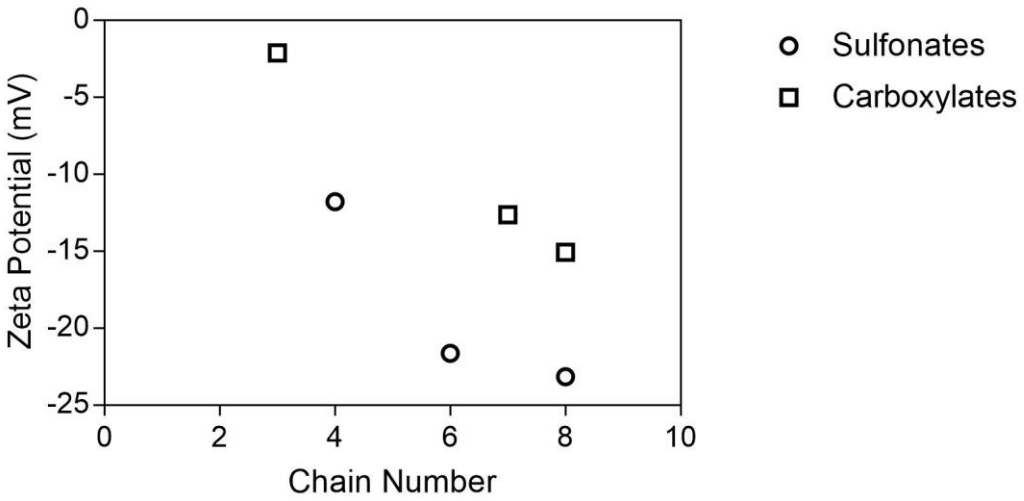


Figure A.1. Changes in zeta potential of liposomes after exposure to 50 mg/L of PFAS of varying fluorinated chain length and functional group.

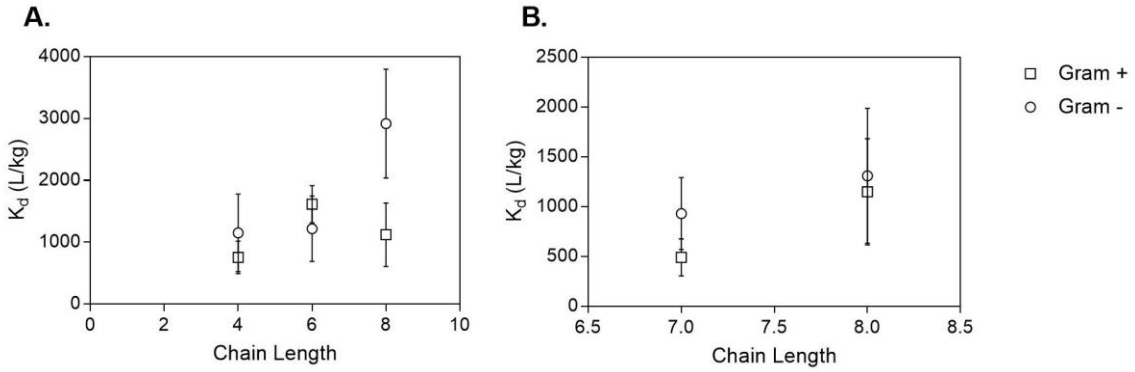


Figure A.2. Partitioning of (A) sulfonate PFAS and (B) carboxylate PFAS to Gram positive and Gram negative bacteria.

Table A.1. Mass labeled standards used in PFAS analysis purchased from Wellington Laboratories

PFAS	Internal Standard	m/z	Concentration (µg/L)
PFBS	Sodium perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonate	302	266
PFHxS	Sodium perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonate	402	267
PFOS	Sodium perfluoro-[$^{13}C_8$]octanesulfonate	507	265.1
PFBA	Perfluoro-n-[2,3,4-$^{13}C_3$]butanoic acid	216	286
PFOA	Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid	417	286
PFNA	Perfluoro-n-[$^{13}C_9$]nonanoic acid	468	286

Table A.2. Analytical standards used during PFAS analysis. All were purchased from Wellington Laboratories.

PFAS	m/z	Concentration (µg/L)
PFBS	299	333
PFHxS	399	333
PFOS	499	382
PFBA	213	400
PFOA	413	333
PFNA	463	333

Figure A.3. Phase transitions in bilayers exposed to 10.1, 1, 5, and 50 mg/L of PFOS. Grey lines show transition in methanol-control and the colored lines show transition in PFAS-exposed bilayer. Red arrows indicate phase transition peaks in bilayers exposed to PFAS.

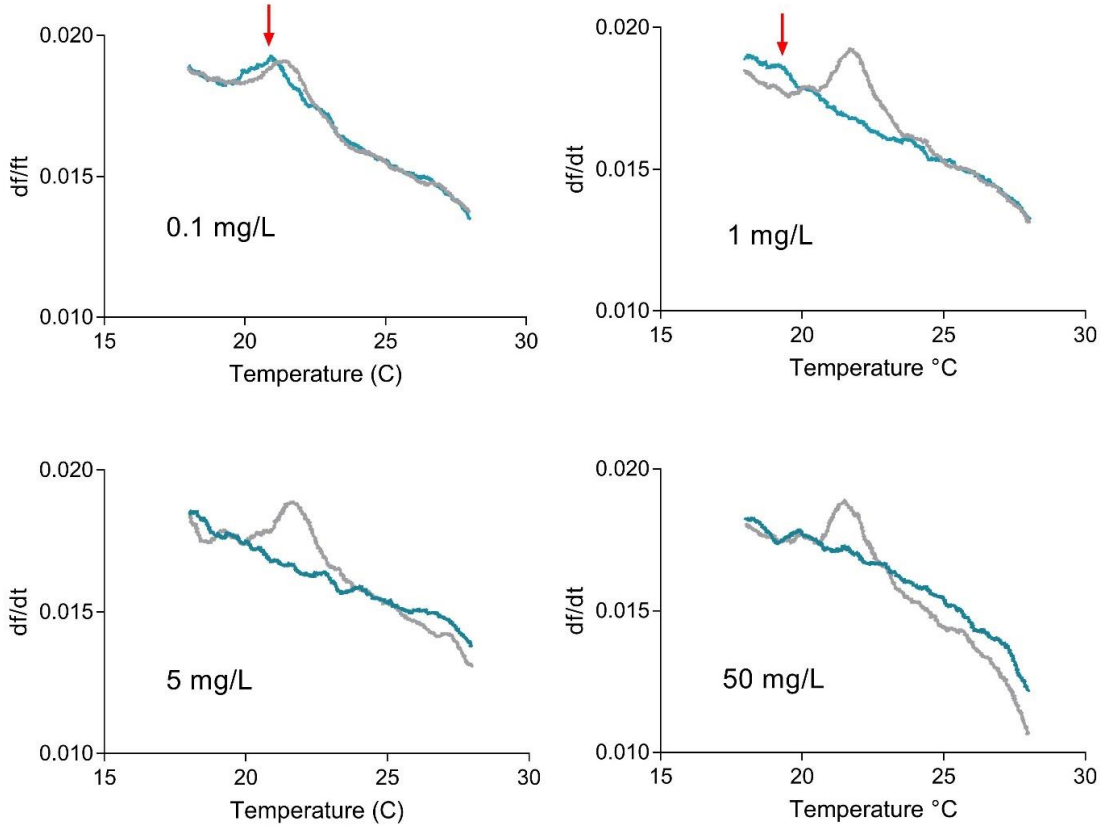


Figure A.4. Phase transitions in bilayers exposed to 10.1, 1, 5, and 50 mg/L of PFOS. Grey lines show transition in methanol-control and the colored lines show transition in PFAS-exposed bilayer. Red arrows indicate phase transition peaks in bilayers exposed to PFAS.

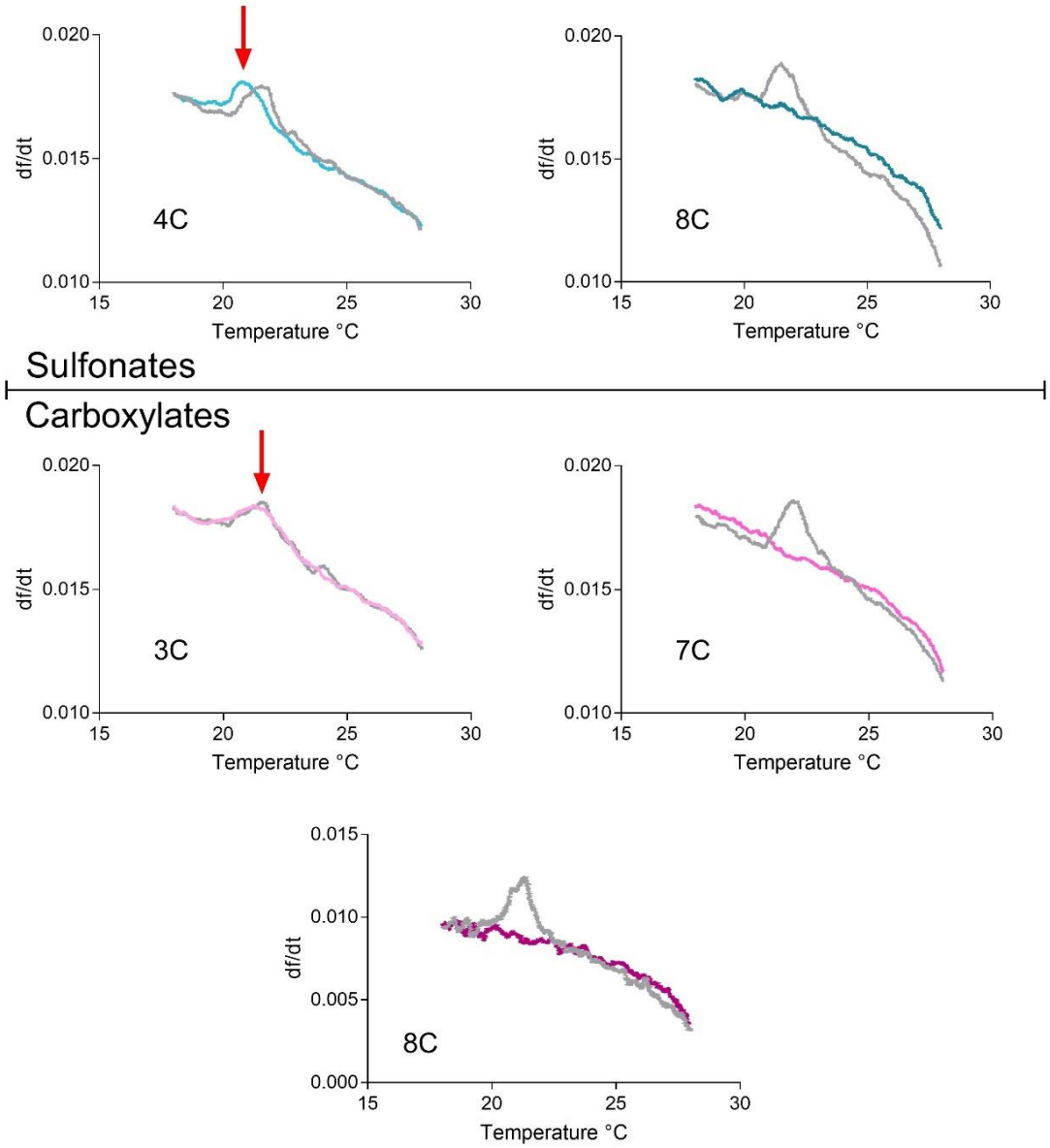
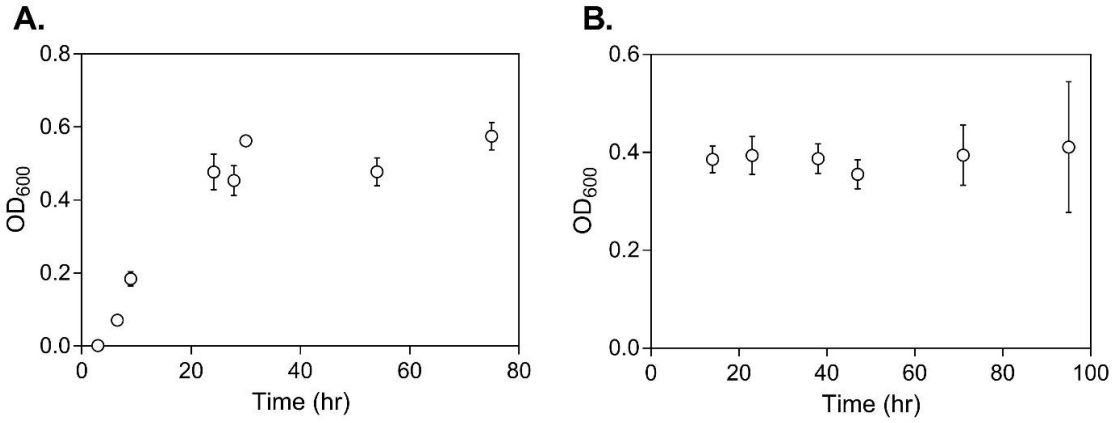
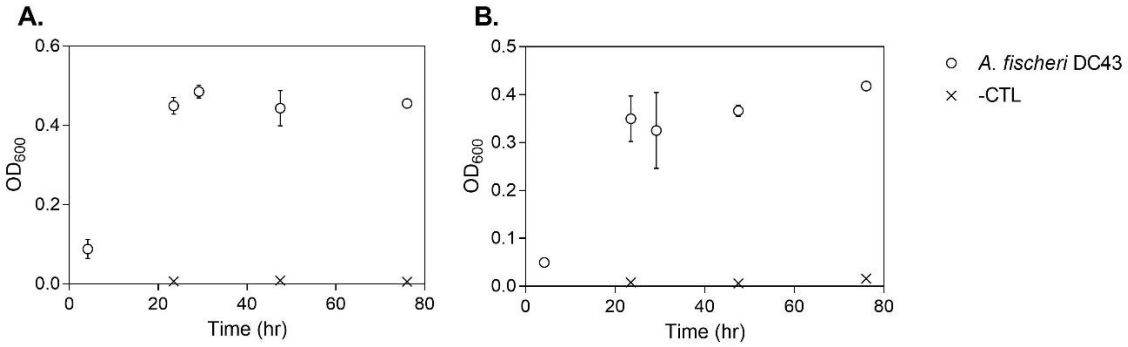


Figure A.5. Growth of *Aliivibrio fischeri* DC43 (A) and *Staphylococcus epidermis* (B).



Appendix B. Supporting Information for Chapter 4

Figure B.1. Growth curve of *Aliivibrio fischeri* DC43. Experiments were completed in triplicate and error bars are standard deviation. (A) *A. fischeri* DC43 grown in 1-mL of photobacterium broth and shaken at 150 rpm. (B) *A. fischeri* DC43 was grown in 6-mL of photobacterium broth and shaken at 150 rpm.



Appendix C: Supporting Information for Chapter 5

Table C.1. Initial experimental conditions in serum vials used to measure methane production and 2,4-dichlorophenol degradation.

Date	PFAS Tested	pH	Volatile Solids (g/L)	Initial DCP (mg/L)
2-29-2016	50 mg/L PFOS 50 mg/L PFOA 50 mg/L PFBS 50 mg/L PFBA	7.6	2.37	1.15
10-17-2016	50 mg/L PFOS 50 mg/L PFOA 50 mg/L PFNA	7.18	2.58	0.69
12-05-2016	50 mg/L PFBA 50 mg/L PFBS 50 mg/L PFHxS 50 mg/L AFFF	7.34	2.00	0.86
12-12-2016	50 mg/L PFHxS 50 mg/L PFNA 50 mg/L AFFF	7.01	2.19	0.87
12-29-2016	AFFF as 50 mg/L PFOS 50 mg/L PFOS 8.8 mg/L PFHxS PFOS + PFHxS	7.01	1.96	1.14
02-27-2017	25 mg/L PFOS 40 mg/L PFOS 50 mg/L PFOS 60 mg/L PFOS	7.51	1.58	0.74

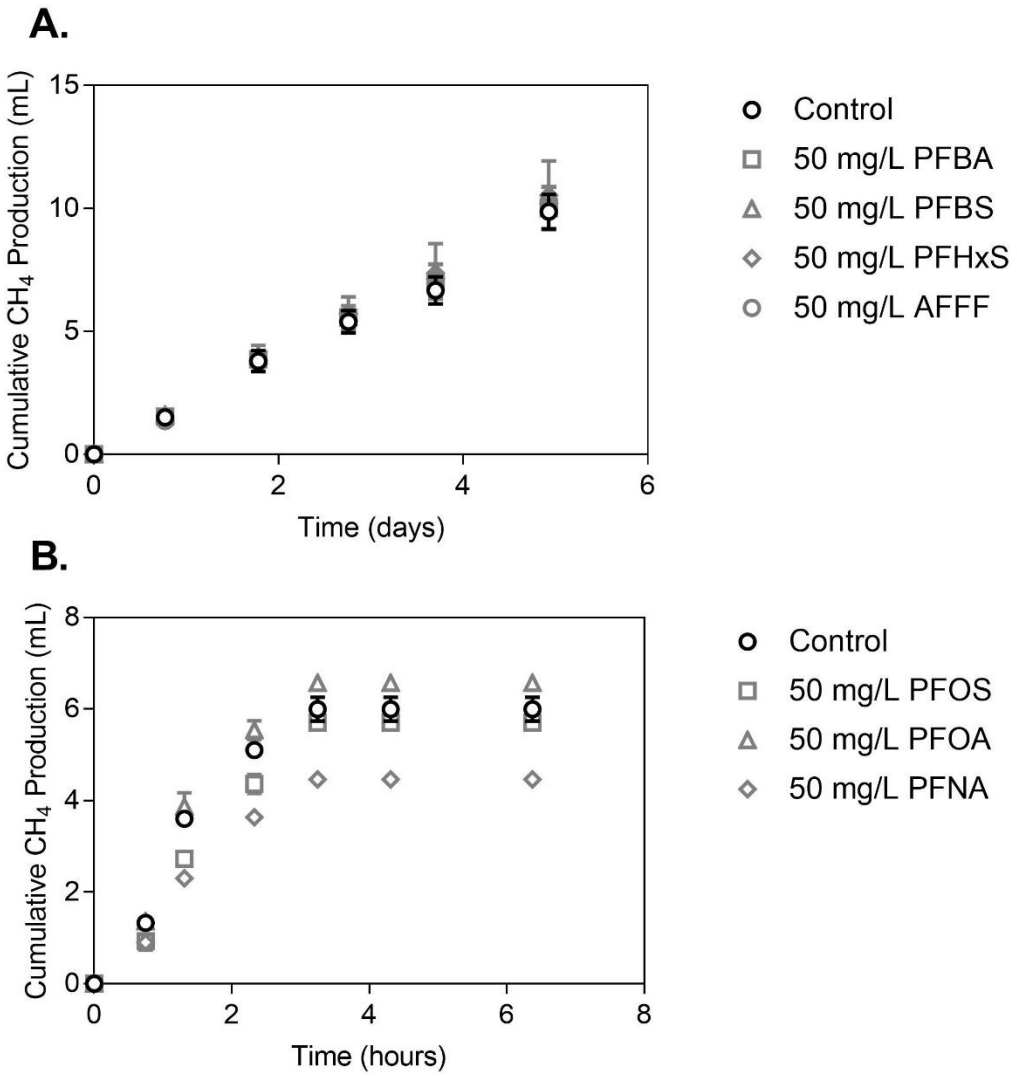


Figure C.1. Cumulative Methane Production in 2,4-dichlorophenol (DCP) degradation experiments ran on 12-5-2016 (A) and 10-17-2016 (B). All treatments contain 1 mg/L DCP.

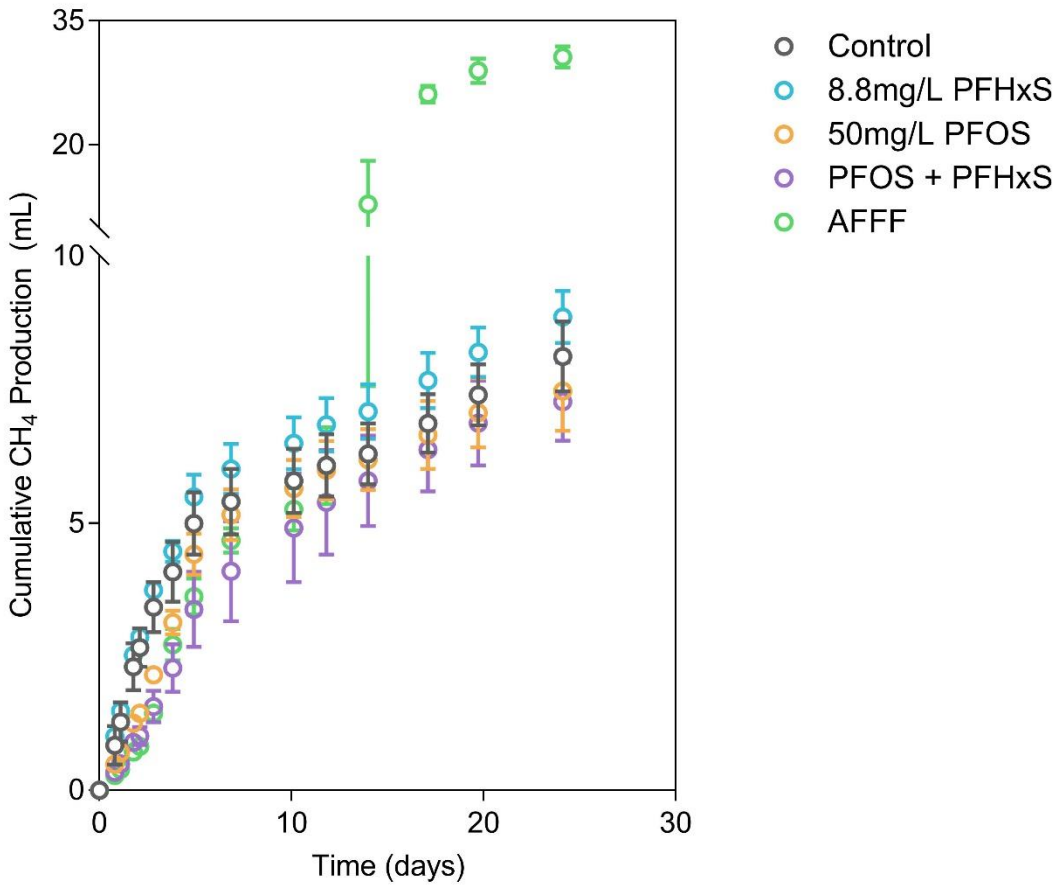


Figure C.2. Methane production in serum vials used to determine the effect of AFFF and its major PFAS constituents on the degradation of 2,4-dichlorophenol (DCP). Experiment performed on 12-29-2016.

Table C.1. Chemical oxygen demand (COD) in serum vials used to test the effect of AFFF and its major PFAS constituents on methane production and degradation of 2,4-dichlorophenol (DCP). The t=0 measurement was from the bulk mixture of reduced anaerobic mineral media (RAMM) and digester communities, The t=3 measurements were taken from vials that did not contain DCP. The t=25 measurements were taken from vials that contained DCP. COD was measured using a HACH kit.

	Day		
	0	**3**	**25**
Control	391.5	319.0	203.3
AFFF as 50 mg/L PFOS	-	2,681.3	1,339.6
PFOS + PFHxS	391.5	585.3	216.7
50 mg/L PFOS	391.5	583.3	226.0
8.8 mg/L PFHxS	391.5	321.3	207.3

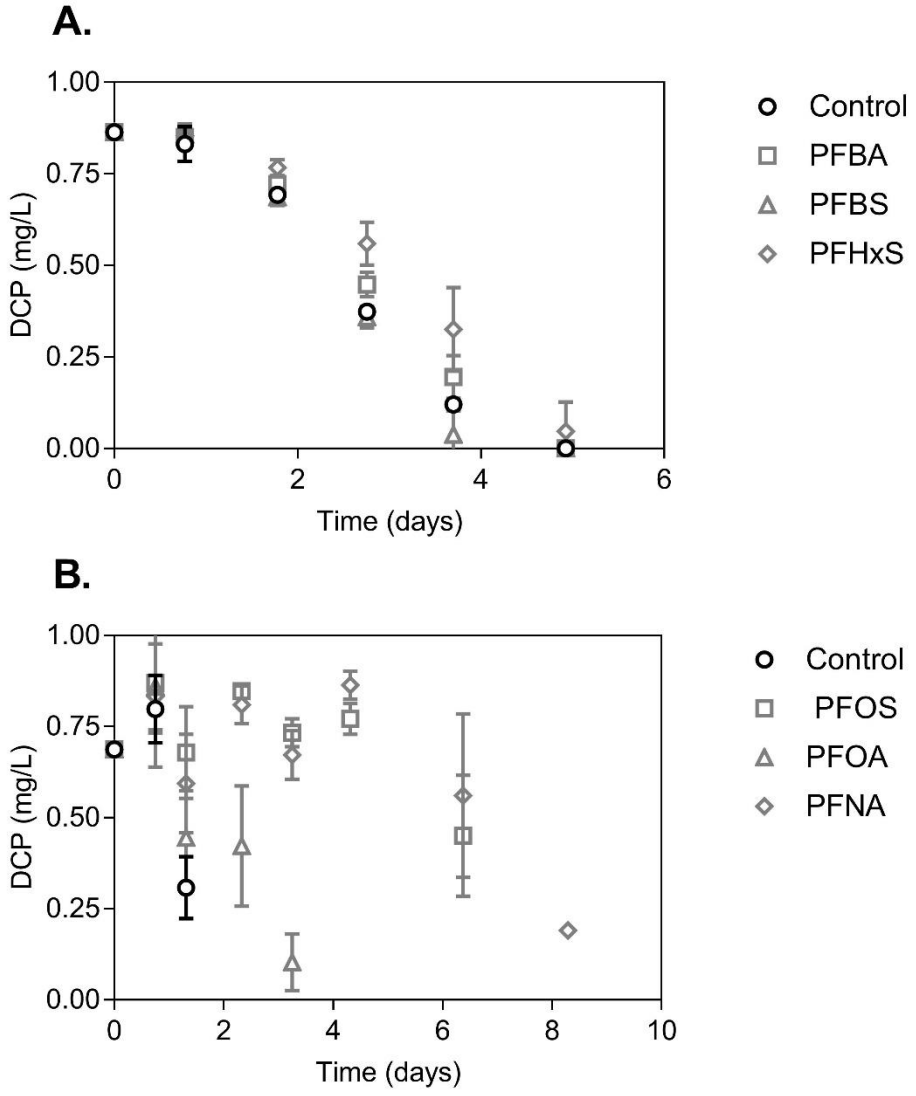


Figure C.3. Degradation of 2,4-dichlorophenol (DCP) in a no PFAS addition control and in the presence of 50 mg/L of varying PFAS. The data presented here was used to determine the lag time in the Gompertz model. (A) Experiment performed with 50mg/L PFBA, PFBS, and PFHxS on 12-05-2016. (B) Experiment performed with 50 mg/L PFOS, PFOA, PFNA experiment on 10-17-2016.

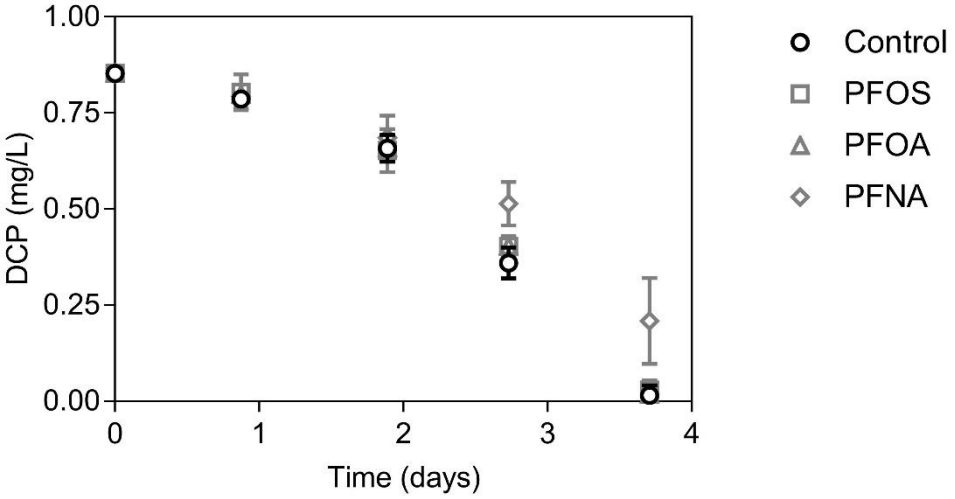


Figure C.4. Degradation of 2,4-dichlorophenol (DCP) in a PFAS free control and the presence of 5 mg/L of either PFOS, PFOA, or PFNA. The presence of 5 mg/L PFNA causes a slight inhibition of DCP degradation. Experiment was performed on 9-26-2016.

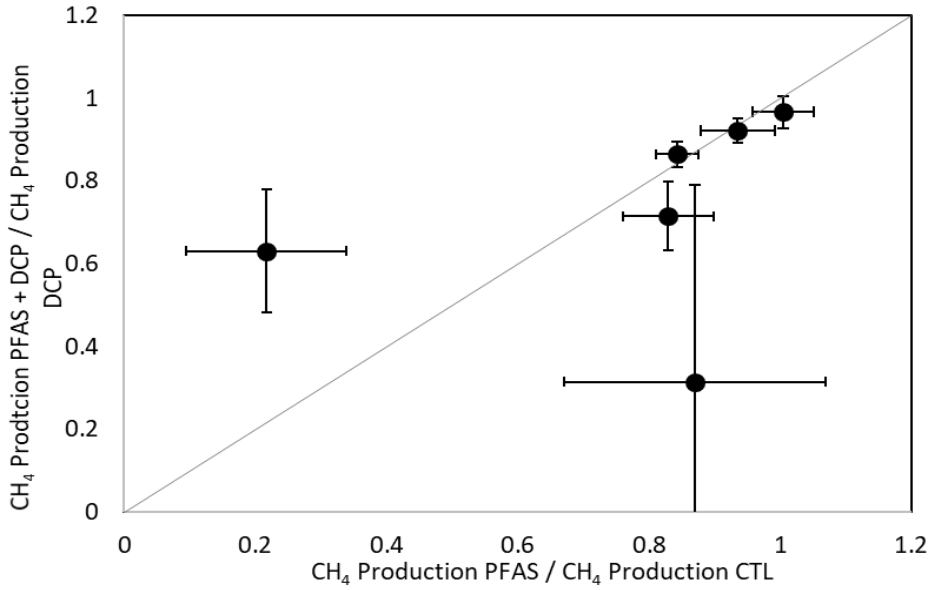


Figure C.5. Changes in co-contaminant toxicity are related to observed PFOS toxicity. In this case, all anaerobic digester communities were exposed to either no contaminants, 50 mg/L PFOS, or the combination of 50 mg/L PFOS and 1 mg/L DCP. The solid line shows additive toxicity as DCP never had a significant effect on methane production. Points that fall above the line demonstrate an antagonistic relationship and points that fall below the line represent a synergistic relationship. Observed toxicity was variable between experiments and hence so was effects on toxicity changes in the presence of 2,4-dichlorophenol.

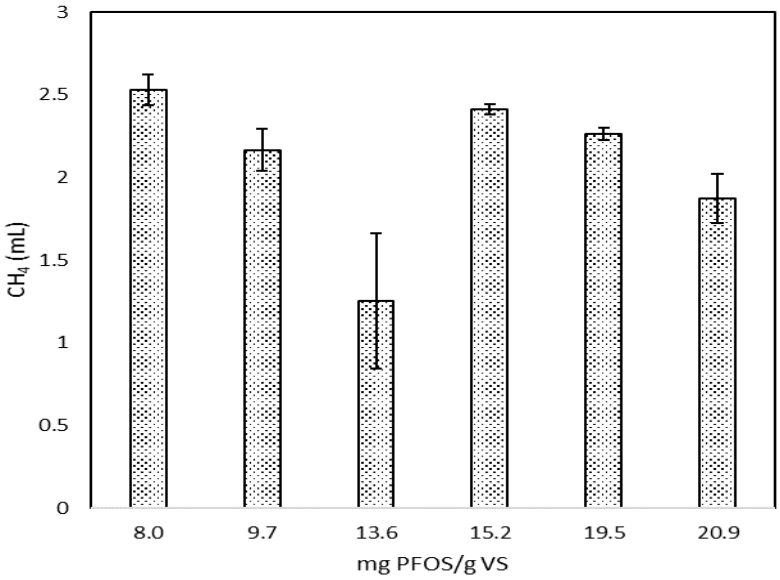


Figure C.6. Cumulative methane production after approximately 2.75 days in treatments targeted to contain 50 mg/L PFOS and 1 mg/L 2,4-dichlorophenol (DCP). The interaction between DCP and PFOS does not appear to be related to PFOS concentration.

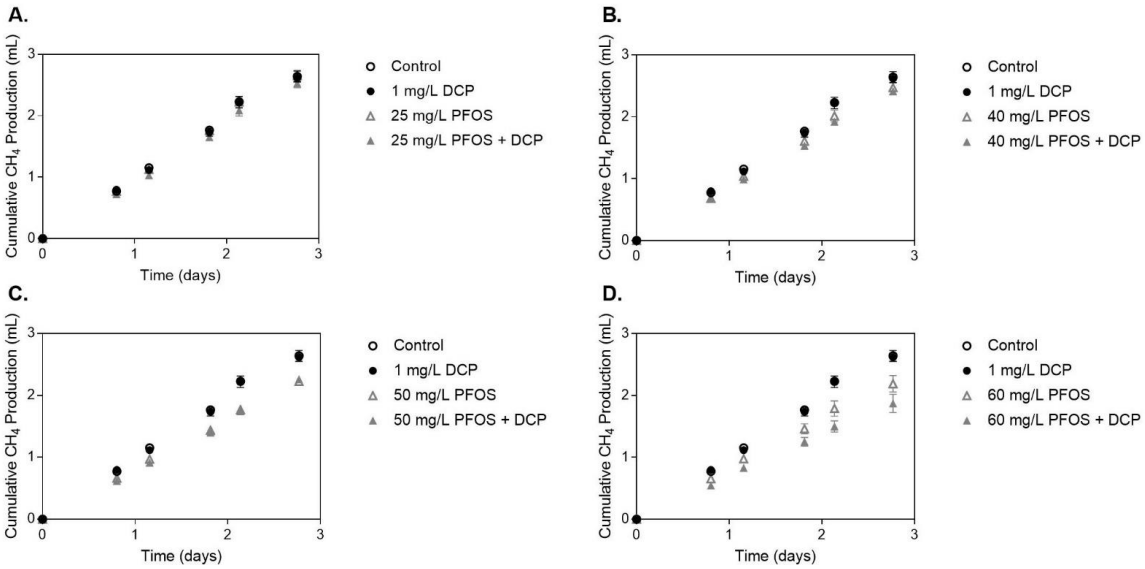


Figure C.7. Cumulative methane production from experiment started on 2-27-2016. In this experiment varying concentrations of PFOS were tested to determine if communities were sensitive to PFOS dose in terms of toxicity or co-contaminant toxicity.

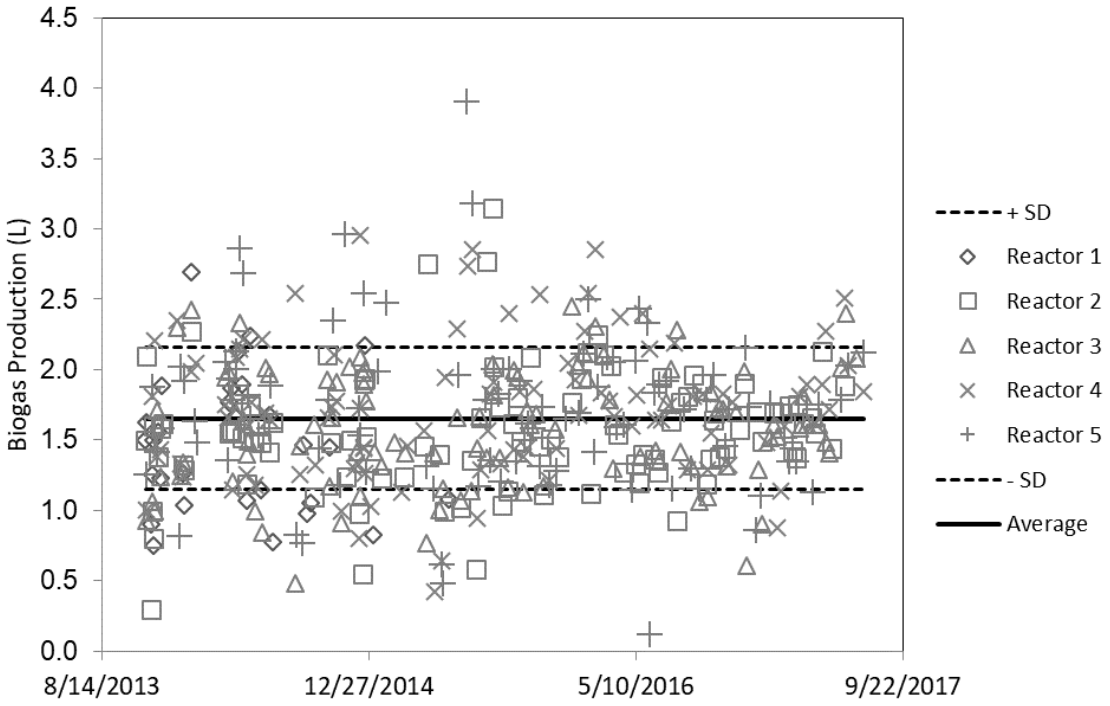


Figure C.8. Biogas production from source reactors. All reactors are identical, however, reactor 6 is exposed to 2,000 µg/L of PFOS.

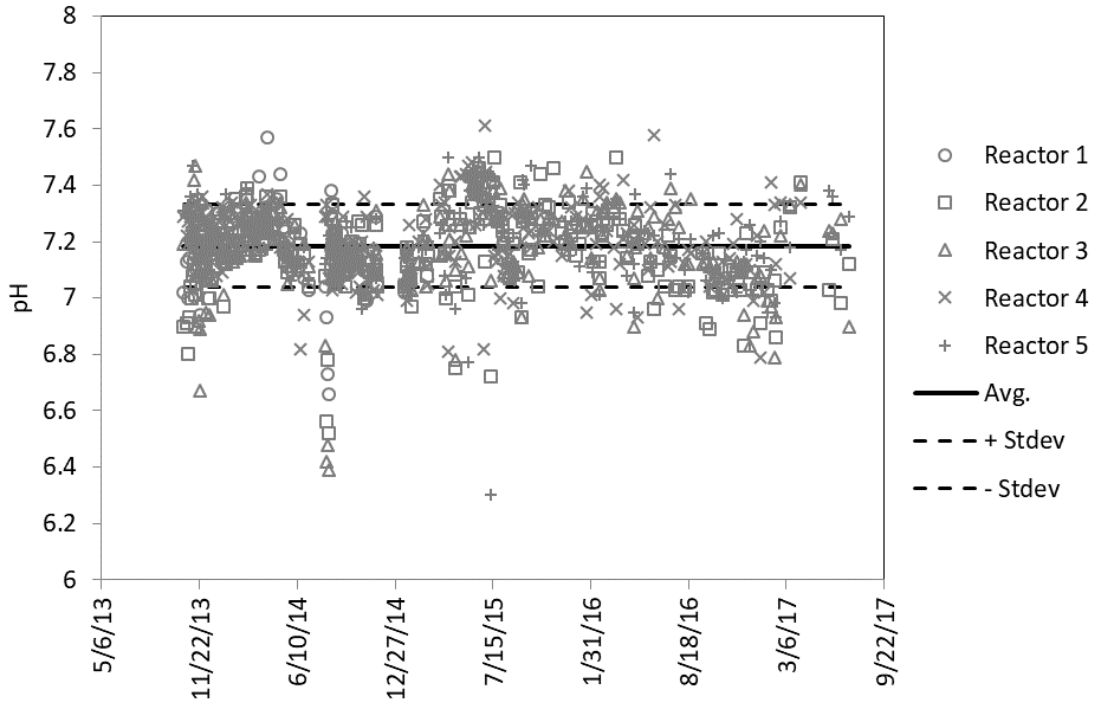


Figure C.9. pH in source reactors. All reactors are identical, however, reactor 6 is exposed to 2,000 µg/kg of PFOS.

Appendix D: Toxicity of Perfluoroalkyl Substances and Co-Contaminants to Anaerobic Digesters.

Motivation

Microbial communities in anaerobic digesters are exposed to elevated levels of perfluoroalkyl substances (PFAS) in addition to other contaminants, so called co-contaminants.[1] Literature presents evidence that PFAS alter membrane structure and may also increase the toxicity of co-contaminants.[2–4] This synergistic effect, however, appears to be dependent on the dose and chemical characteristics of the co-contaminants, though this has not been clarified.[5] Furthermore, the vast majority of the research performed to date has been on eukaryotic cells; little is known about the effect of PFAS and co-contaminants on prokaryotic cells.

This study hoped to determine the effect of PFAS on anaerobic digester function and clarify co-contaminant characteristics that could cause co-contaminants to have synergistic toxicity on digesters. Methane production (cumulative and production rate) was used to assess community function. Methane production is the result of a symbiotic relationship between many different microbial populations and the loss of any population could inhibit methane production. Thus, methane production is a sensitive indicator of anaerobic community function and a good indicator of toxicity. Different co-contaminants were chosen to pin point characteristics (size, ionization state, hydrophobicity) that could cause synergism and a potential upset in an anaerobic digester community.

Methods

Chemicals. PFAS that were tested include perfluorobutane (PFBA), perfluorooctane (PFOA), perfluorononanoic acid (PFNA), perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS). PFOA and PFOS were chosen as they are commonly detected in the environment, are degradation products of more complex PFAS, and have been more widely studied in the literature compared to other PFAS.[6–8] As a result of the push to phase out 8C PFAS, environmental levels of PFBA, PFHxS and PFBS are increasing.[9,10] Additionally, the use of the 4C and 6C compounds in these experiments will enable exploration of the impact of fluorinated chain length on microbial function. PFNA was chosen to compare to PFOS and allow testing the impact of different functional groups. PFNA and PFOS have the same number of fluorinated carbons. Sodium dodecyl sulfate (SDS) was used as a positive control for a membrane permeabilizing substance in one experiment. Multiple co-contaminants were investigated and chosen based on size, hydrophobicity, and charge (Table D.1). PFAS and co-contaminants were purchased as described previously in Chapter 5.

Table D.1. Characteristics and abbreviations of co-contaminants used in this study.

		K_{ow}	pK_a	Molecular Weight
2,4-dichlorophenol	**DCP**	3.06	7.85	163
2,4,6-trichlorophenol	**TCP**	3.69	6.23	197.45
pentachlorophenol	**PCP**	5.12	4.7	266.34
2,4-dichloroaniline	**DCA**	2.78	2	162.02
2,4-dinitrophenol	**DNP**	1.67	4.09	184.11

Microbial Culture. Anaerobic digester communities were used to investigate toxicity of chemical mixtures containing PFAS. Anaerobic digester communities for the experiments were cultured in four 1.6-L reactors with conditions were identical to those described in Chapter 5.

Experimental Set-Up. More details on experimental set-up and analytical analysis are provided in Chapter 5. Briefly, Microbial communities were collected and diluted in reduced anaerobic mineral media (RAMM)[11] as described previously in Chapter 5. Preliminary test results showed that PFAS have no detectable effect on co-contaminant toxicity at levels below 50 mg/L, thus PFAS was added at a concentration of 50 mg/L. Treatments were always investigated in triplicate and treatments including (1) control, (2) co-contaminant only, (3) PFAS only, and (4) combination of PFAS and co-contaminant. Initial samples were collected for the analysis of pH and volatile solids. Methane was monitored via gas volume measurement and gas chromatographic analysis (Chapter 5) once or twice daily until the rate of gas production in the control treatments begin to decrease. Toxicity of the chemical mixtures was assumed proportional to methane production (i.e. any decrease in methane production relative to a control was assumed to be a result of toxicity).

Results

Results are shown in Figures D.1-D.6. In some cases, PFOS and PFOA had a slight negative impact on methane production. The conditions under which PFOS and PFOA had a negative effect were not clear, though it is likely that 50 mg/L may represent a toxic tipping point. Inconsistent toxicity was also observed for PCP (Figure D.2.). 1mg/L TCP

and 10 mg/L DCP increased methane production, likely as a result of uncoupling effects.[12]

In most cases, the toxicity of PFAS + co-contaminant could be explained with additivity where the resulting combination toxicity is explained by the sum of individual toxicities. Deviances from additivity were observed in three instances. The combinations of 1mg/L PCP+PFOS and 1mg/L PCP+PFOA had antagonistic tendencies while the combination of 1mg/L DNP+PFOS had synergistic tendencies. The antagonist relationship between PFAS and PCP was unexpected as synergism has been reported in other studies.[2,3] Small neutral compounds can diffuse through cell membranes[13] and it is likely that effects on their diffusion will be more easily observed. Additionally, negatively charged PFAS may repel negatively charged compounds. This could explain antagonism observed in some cases. Although DCA is also neutral at experimental pH, there may be a different type of interaction with PFOS as a result of the amino group (PFAS have been shown to bind protein). Additionally, chlorophenols have been shown to cause metabolic uncoupling in bacteria.[14] PFAS may be able to affect the toxicity of the phenols as a result of their specific mode of toxicity (both effect cell membranes). This could be another reason why the toxicity of DCA is not effected.

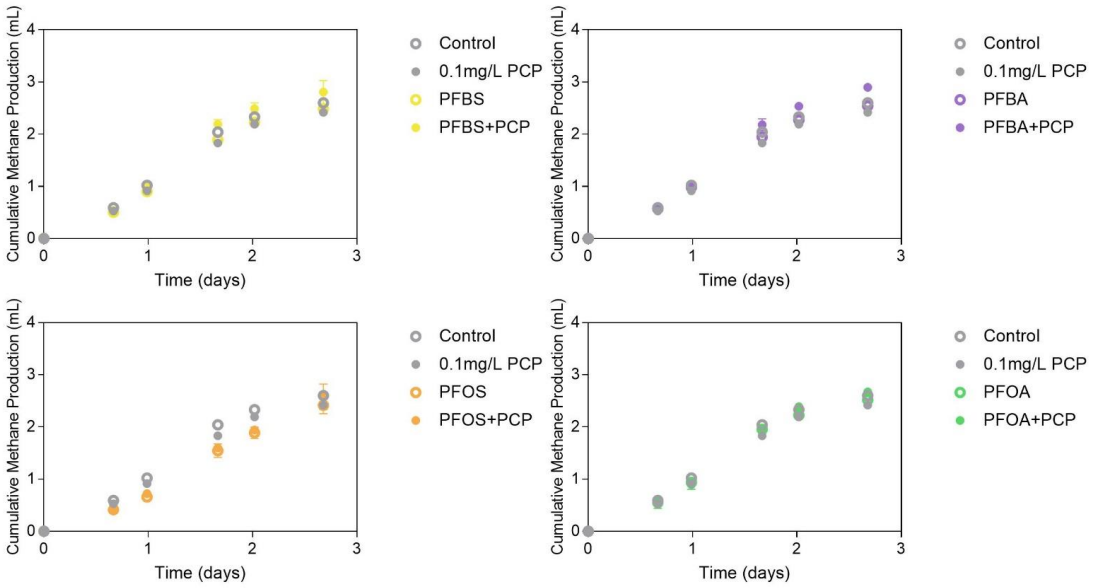


Figure D.1. Cumulative methane production in anaerobic communities exposed to 0.1 mg/L pentachlorophenol and 50 mg/L PFBS, PFBA, PFOS, and PFOA. No effects were observed in regards to methane production.

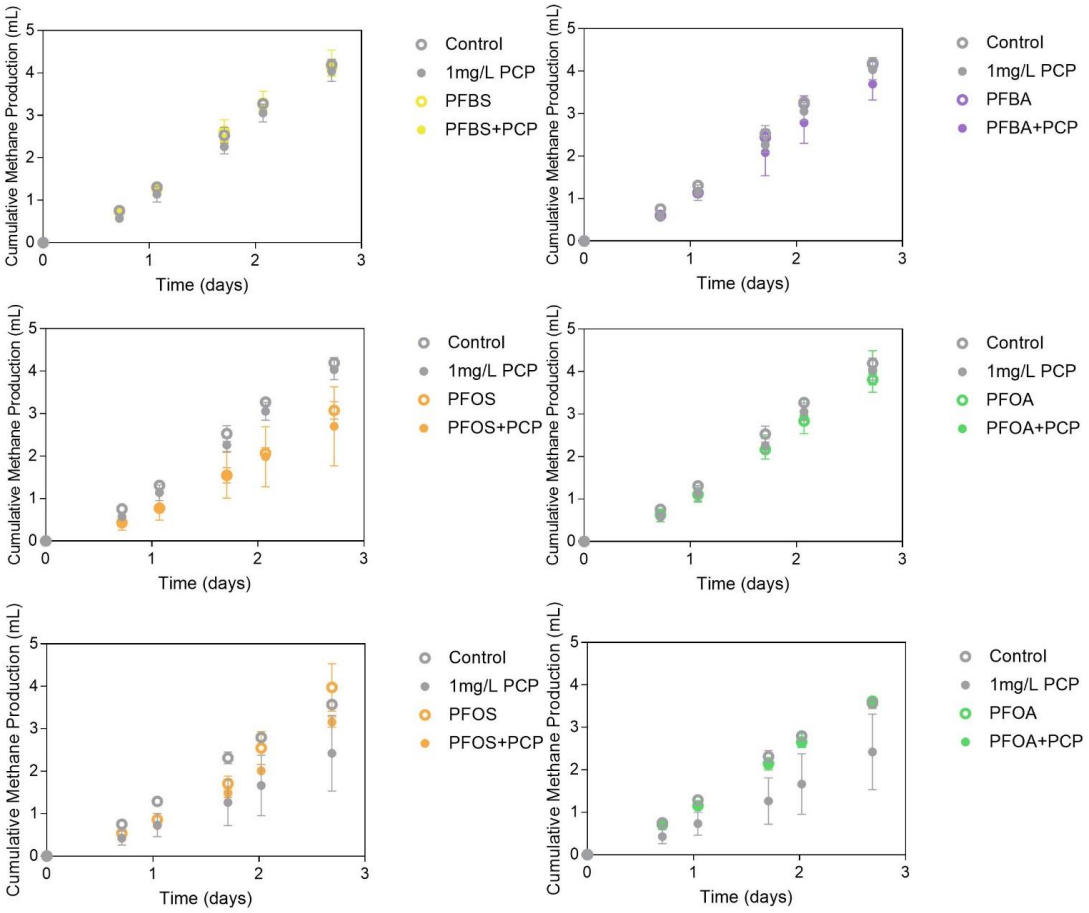


Figure D.2. Cumulative methane production in anaerobic communities exposed to 1 mg/L pentachlorophenol and 50 mg/L PFBS, PFBA, PFOS, and PFOA. Experiments using PFOA and PFOS were repeated twice. In one of the two experiments completed with PFOS/PFOA and PCP, contaminants appear to behave antagonistically. In this case PCP appears toxic while PCP + PFOA/PFOS do not.

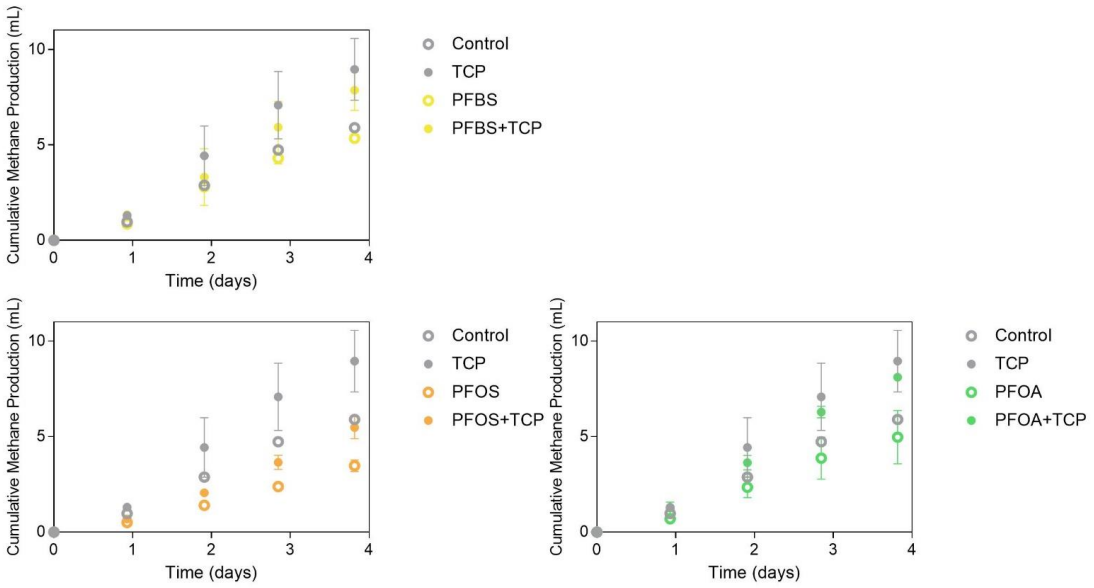


Figure D.3. Cumulative methane production in anaerobic communities exposed to 1 mg/L 2,4,6 trichlorophenol and 50 mg/L PFBS, PFOS, and PFOA. In this experiment, PFOS and TCP inhibited methane production. Combination effects were deemed to be additive.

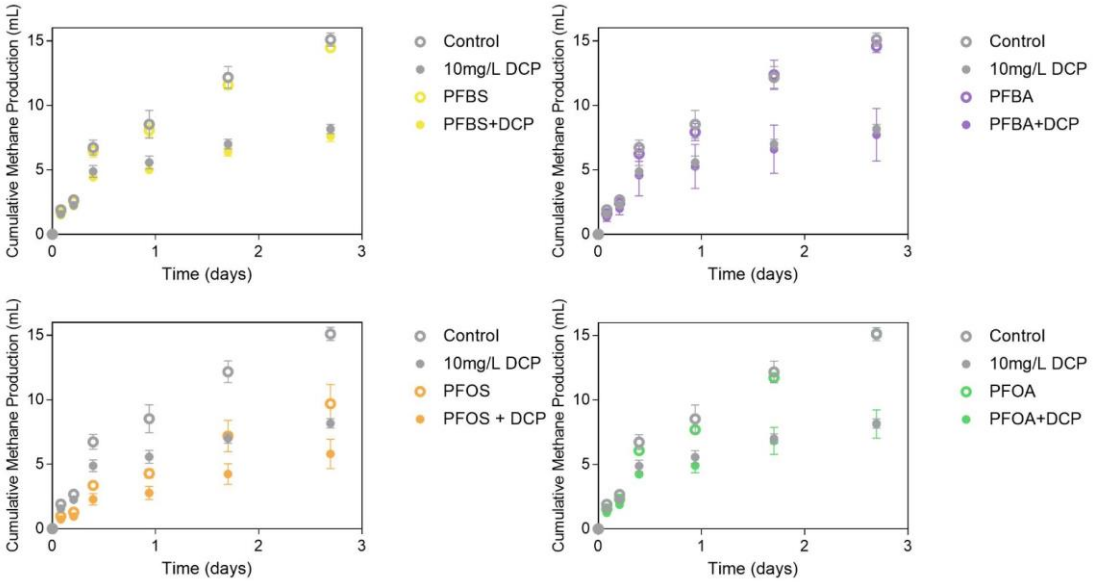


Figure D.4. Cumulative methane production in anaerobic communities exposed to 10 mg/L 2,4-dichlorophenol and 50 mg/L of PFBS, PFBA, PFOS, and PFOA.

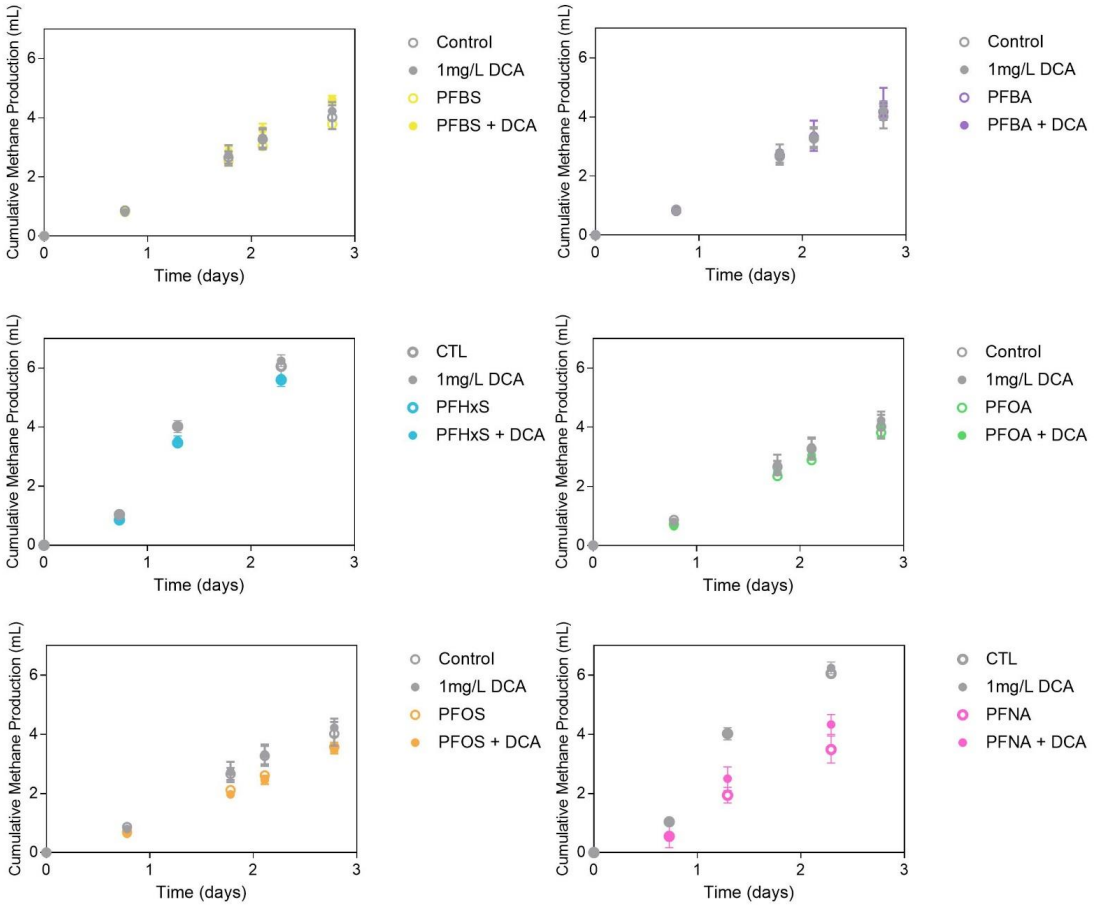


Figure D.5. Cumulative methane production in anaerobic communities exposed to 1 mg/L 2,4-dichloroaniline and 50 mg/L PFBS, PFBA, PFHxS, PFOA, PFOS, and PFNA. PFOS and PFNA slightly inhibited methane production. Additionally, the combination of DCA and PFNA appears to be antagonistic.

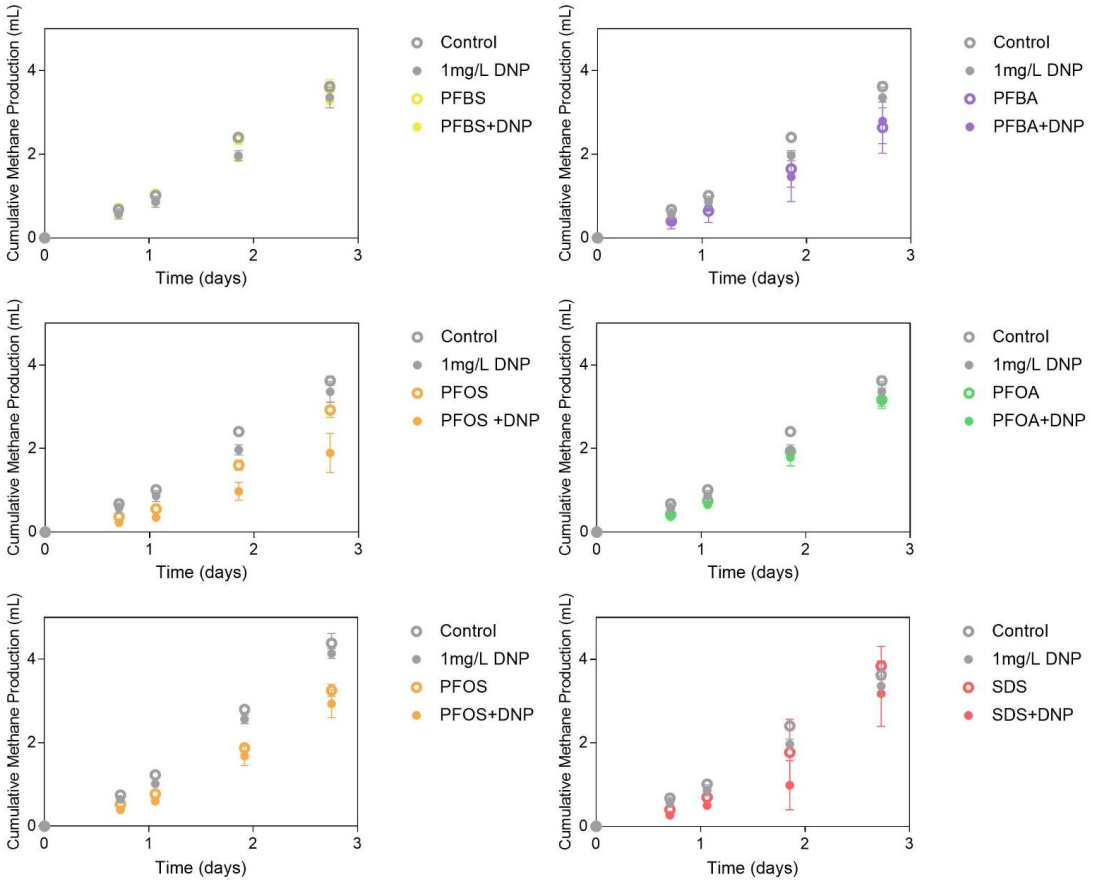


Figure D.6. Cumulative methane production in anaerobic communities exposed to 1 mg/L 2,4-dinitrophenol and 50 mg/L PFBS, PFBA, PFOA, PFOS, and SDS (as a positive control). Experiments with PFOS were repeated twice. In one, the combination of PFOS and DNP is synergistic. At a time point close to 2 days, the positive control also appears to have a synergistic relationship.

References

(1) Loos, R.; Carvalho, R.; António, D. C.; Comero, S.; Locoro, G.; Tavazzi, S.; Paracchini, B.; Ghiani, M.; Lettieri, T.; Blaha, L.; et al. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. *Water Res.* **2013**, *47* (17), 6475–6487 DOI: 10.1016/j.watres.2013.08.024.

(2) Shan, G.; Ye, M.; Zhu, B.; Zhu, L. Enhanced cytotoxicity of pentachlorophenol by perfluorooctane sulfonate or perfluorooctanoic acid in HepG2 cells. *Chemosphere* **2013**, *93* (9), 2101–2107 DOI: 10.1016/j.chemosphere.2013.07.054.

(3) Liu, W.; Zhang, Y.-B.; Quan, X.; Jin, Y.-H.; Chen, S. Effect of perfluorooctane sulfonate on toxicity and cell uptake of other compounds with different hydrophobicity in green alga. *Chemosphere* **2009**, *75* (3), 405–409 DOI: 10.1016/j.chemosphere.2008.11.084.

(4) Jernbro, S.; Rocha, P. S.; Keiter, S.; Skutlarek, D.; Färber, H.; Jones, P. D.; Giesy, J. P.; Hollert, H.; Engwall, M. Perfluorooctane Sulfonate Increases the Genotoxicity of Cyclophosphamide in the Micronucleus Assay with V79 Cells. *Environ. Sci. Pollut. Res. Int.* **2007**, *14* (2), 85–87.

(5) Boltes, K.; Rosal, R.; García-Calvo, E. Toxicity of mixtures of perfluorooctane sulphonic acid with chlorinated chemicals and lipid regulators. *Chemosphere* **2012**, *86* (1), 24–29 DOI: 10.1016/j.chemosphere.2011.08.041.

(6) Sinclair, E.; Kannan, K. Mass loading and fate of perfluoroalkyl surfactants in wastewater treatment plants. *Environ. Sci. Technol.* **2006**, *40* (5), 1408–1414.

(7) Zareitalabad, P.; Siemens, J.; Hamer, M.; Amelung, W. Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in surface waters, sediments, soils and wastewater - A review on concentrations and distribution coefficients. *Chemosphere* **2013**, *91* (6), 725–732 DOI: 10.1016/j.chemosphere.2013.02.024.

(8) Rhoads, K. R.; Janssen, E. M. L.; Luthy, R. G.; Criddle, C. S. Aerobic biotransformation and fate of N-ethyl perfluorooctane sulfonamidoethanol (N-EtFOSE) in activated sludge. *Environ. Sci. Technol.* **2008**, *42* (8), 2873–2878.

(9) Wang, Z.; Cousins, I. T.; Scheringer, M.; Hungerbühler, K. Fluorinated alternatives to long-chain perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFSAs) and their potential precursors. *Environ. Int.* **2013**, *60* (2013), 242–248 DOI: 10.1016/j.envint.2013.08.021.

(10) Renner, R. The long and the short of perfluorinated replacements. *Environ. Sci. Technol.* **2006**, *40* (1), 12–13 DOI: 10.1021/es062612a.

(11) Shelton, D. R.; Tiedje, J. M. General Method for Determining Anaerobic

Biodegradation Potential. *Appl. Environ. Microbiol.* **1984**, *47* (4), 850–857.

(12) Escher, B. I.; Snozzi, M.; Schwarzenbach, R. P. Uptake, speciation, and uncoupling activity of substituted phenols in energy transducing membranes. *Environ. Sci. Technol.* **1996**, *30* (10), 3071–3079 DOI: 10.1021/es960153f.

(13) Madigan, M.; Marktinko, J.; Dunlap, P.; Clark, D. *Brock Biology of Microorganisms*, 13th Editi.; Espinoza, D., Cook, K., Cutt, S., Hutchinson, E., Cogan, D., Marcus, E., Wagner, A., Eds.; Benjamin Cummings: Indianapolis, Indiana, 2006.

(14) Escher, B. I.; Schwarzenbach, R. P. Mechanistic studies on baseline toxicity and uncoupling of organic compounds as a basis for modeling effective. **2002**, *64*, 20–35.

Appendix E: The Impact of Perfluorooctane Sulfonate and Triclosan on Anaerobic Digester Function and Antibiotic Resistance

Motivation

Perfluorooctane sulfonate (PFOS) has been demonstrated to alter membrane permeability and thus alter the toxicity of co-contaminants. In eukaryotic cell cultures, exposure to PFOS has resulted in an increase in uptake and toxicity of pentachlorophenol and cyclophosphamide.[1,2] Changes in toxicity and uptake are variable and in an algae culture, PFOS was shown to increase permeability of pentachlorophenol, but decrease the permeability of atrazine and diuron.[3] It is unknown if PFOS exposure causes similar changes in co-contaminant toxicity and permeability in prokaryotes, though they are often exposed to higher concentrations of PFOS in environments such as wastewater treatment plants where we depend on them to treat waste. Evidence for altered toxicity was in fact observed in a model anaerobic digester community; prolonged exposure to PFOS increased community susceptibility to upsets caused with triclosan (TCS) addition.[4] Though, it was never verified if increased susceptibility was a result of increased permeability or the observed community shift.[4]

In addition to effecting microbial function, some co-contaminants, have been found to increase the abundance of antibiotic resistance genes (ARGs).[5] Antimicrobial resistance has been classified by the World Health Organization as a '…major threat to public health.'[6] For example, exposure to TCS has been found to increase ARG abundance in simulated anaerobic digester communities.[5] It is unknown how the presence of co-contaminant mixtures will effect ARGs. It is possible that ARGs will increase to

combat leakier membranes and a potentially greater influx of co-contaminants, such as TCS.

To protect microbial functions that we depend on as well as human health, it is important to understand how the presence of contaminants effect microbial function. In particular, we are concerned with microbial function in anaerobic digesters in wastewater plants. This study focused on the effect of PFOS on the toxicity of TCS as well as the abundance of ARGs in anaerobic digesters.

Methods

Chemicals

PFOS as potassium salt and TCS were obtained from Sigma-Aldrich.

Microbial Community

An anaerobic digester community was studied. The community is previously described in Chapter 5. One of the digesters was pre-exposed to PFOS. This digester was continually dosed with 2,000 μg/L of PFOS via methanol stock. Methanol added to this reactor was much less than 0.025% by volume. After dosing began, three retention times had passed before material was taken from this digester.

Experimental Set-Up

Anaerobic digesters were fed approximately one hour prior to collecting anaerobic material. Material was then transferred to an anaerobic chamber and mixed with phosphate buffered saline (PBS, pH 7.4, 8 g/L NaCl, 0.2 g/L KCl, 1.42 g/L Na_2HPO_4 0.245 g/L KH_2PO_4). The digester material and PBS were combined at a ratio of 5 parts digester material to 2 parts PBS and added to serum vials. The mass of the serum vials

was recorded before and after the addition of the digester mixture. Each vial received 70 mL of the mixture. Four treatments were tested: (1) methanol-only control, (2) 100 mg TCS /kg volatile solid, (3) 10 mg PFOS/kg volatile solid, and (4) 100 mg TCS/kg volatile solid + 10 mg PFOS/kg volatile solid. PFOS and TCS were added to vials via methanol stock and equivalent amounts of methanol were added to each reactor. In the vials containing PFOS-exposed material, additional PFOS was added to supplement concentration. Vials were shaken and 13 mL of sample was taken for analysis of volatile solids, ARGs, and contaminant concentrations. Vials were sealed with crimp caps, incubated at 37 °C, and shaken at 150 rpm. Methane production and total gas were measured as described previously in Chapter 5. Volatile solids and DNA were sampled approximately weekly. After methane production plateaued, serum vials were fed with a mixture of equal amounts thickened waste activated sludge and thickened primary sludge diluted in PBS in a ratio of 5 parts food to 2 parts PBS. Serum vials were fed with a volume that represented 35% of volume in serum vial at the time of feeding. After feeding methanol, PFOS, and TCS were dosed to avoid contaminant dilution.

Analytical Methods

***Methane Production*.** Methane production was measured daily during the first week of the experiment. As the experiment progressed, less data points were taken. Total gas and methane production were measures as described in Chapter 5.

***Volatile Solids*.** Volatile solids were measured as described in Chapter 5. In this instance, a 0.9 mL volume was analyzed. Volatile solids samples were collected after

sampling total gas and methane production. All samples were collected in the anaerobic chamber. The mass of the vials was recorded every time a sample was removed.

ARG Analysis. Samples were taken for ARG analysis at the same time as they were taken for volatile solids analysis. Briefly a 100 μL sample was collected in a sterile micro-centrifuge tube. The tube was massed before and after sample addition to accurately measure the mass that was collected. DNA was extracted and analyzed for copies of *16S*, *intI1*, and *mexB*. More details on the methodology can be found in McNamara et. al 2014.[5] The ARGs *intI1* and *mexB* were previously found to be effected by the presence of TCS and respectively encode for acquisition of ARGs and an efflux pump.

Data Analysis

Methane production was normalized by the mass of volatile solids in each vial. This was determined by multiplying the mass of digester material in the reactor by the percentage of volatile solids. For gas measurements where volatile solids were not subsequently measured, the weighted average was taken of samples taken at surrounding time points.

Results

There were no significant differences in treatments containing 100 mg TCS/kg volatile solids, 10 mg PFOS/kg volatile solids, or their combination from the control (Figure E.1.). This was true for vials that were inoculated with anaerobic digester effluent that had been previously exposed to 2 mg PFOS /kg volatile solid as well as anaerobic digester effluent that was unexposed. This was unexpected as a previous study showed a dramatic increase in methane production and rapid subsequent drop in anaerobic reactors

that had been chronically exposed to 60 or 800 μg PFOS/kg volatile solid and then received disturbances of in the form of the addition of 20-225 mg TCS/kg volatile solid.[4] The microbial communities in our study are likely more complex and redundant as they are fed a mixture that was collected from a waste water treatment plant compared to synthetic wastewater**.** While insignificant, it does appear that the rate of methane production in the treatment containing TCS and PFOS is less than the control while the cumulative volume of methane production is greater (Figure E.1.). This is only true for the experiment with sludge that had been previously unexposed to PFOS. The lack of effect in reactors previously exposed to PFOS could be a result of a community shift towards a community resistant to effects of PFOS. A community shift after chronic PFOS exposure was also observed in the McNamara 2015 study.[4]

Additionally, exposure to TCS, PFOS, or their combination caused no significant increase in the presence of antibiotic resistance genes (Figure E.2, E.3). In fact, in both experiments (unexposed and exposed), the concentration of the genes *ini*1 and *mex*B decreased, most likely because microbial communities were not continuously fed. It is postulated that the decrease was a result of bacteria attempting to decrease energy expenditure. While statistically insignificant, the treatments containing both PFOS and TCS did have very slightly higher copies of *inti1* and *mexB* than other treatments in most comparisons.

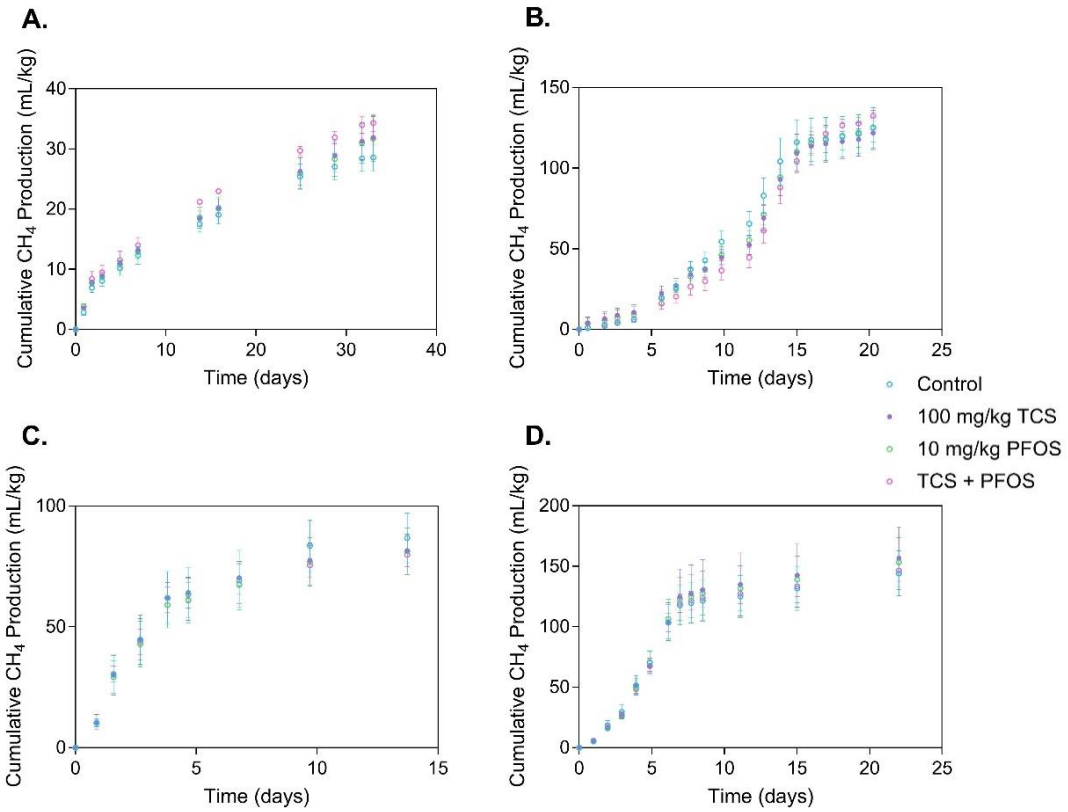


Figure E.1. Cumulative methane production in experiments amended with TCS and PFOS. (A) First feed and previously unexposed to PFOS (B) Second feed and previously unexposed to PFOS (C) First feed and previously exposed to PFOS (D) Second feed and previously exposed to PFOS.

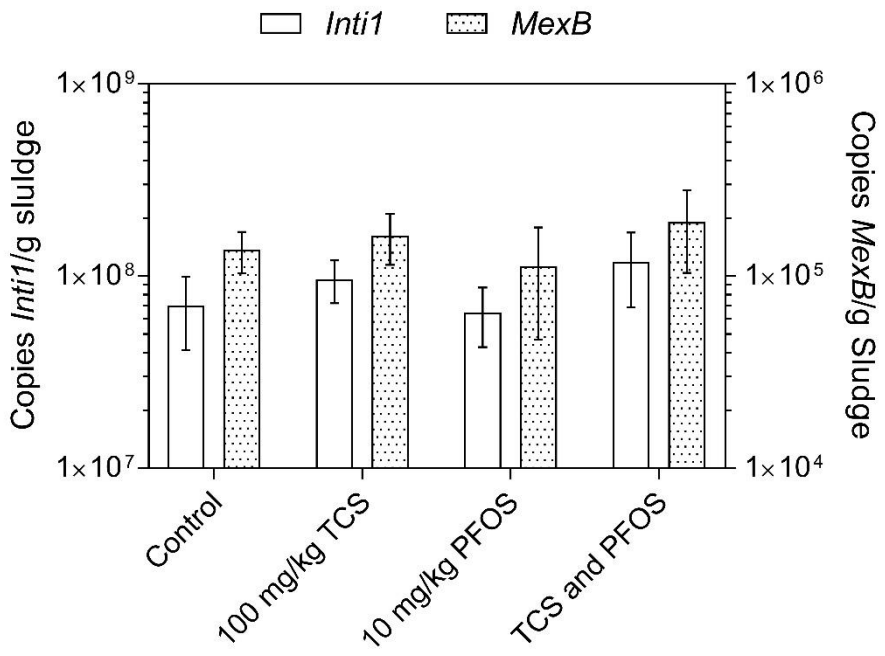


Figure E.2. Concentration of ARGs, *inti1* and *mexB,* in experiments conducted with communities previously unexposed to PFOS. DNA analyzed after approximately 62 days.

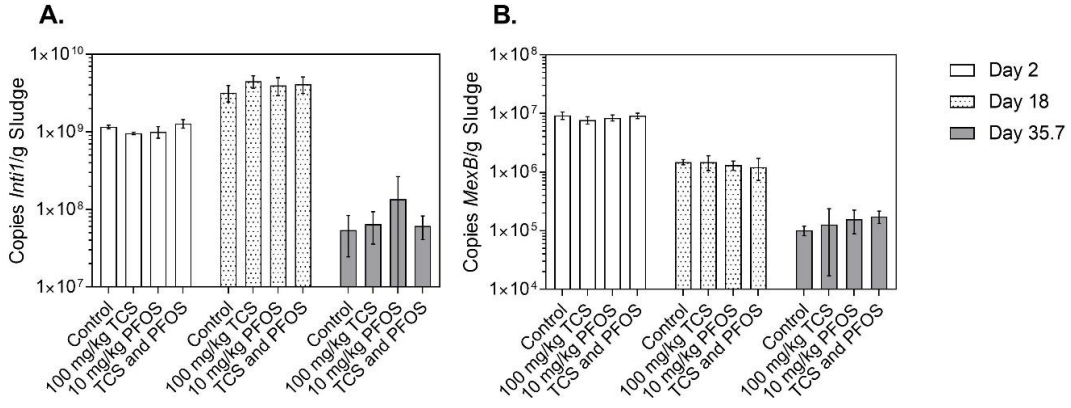


Figure E.3. Concentration of ARGs (A) *inti1* and (B) *mexB* in experiments conducted with communities previously exposed to PFOS.

References

(1) Jernbro, S.; Rocha, P. S.; Keiter, S.; Skutlarek, D.; Färber, H.; Jones, P. D.; Giesy, J. P.; Hollert, H.; Engwall, M. Perfluorooctane Sulfonate Increases the Genotoxicity of Cyclophosphamide in the Micronucleus Assay with V79 Cells. *Environ. Sci. Pollut. Res. Int.* **2007**, *14* (2), 85–87.

(2) Shan, G.; Ye, M.; Zhu, B.; Zhu, L. Enhanced cytotoxicity of pentachlorophenol by perfluorooctane sulfonate or perfluorooctanoic acid in HepG2 cells. *Chemosphere* **2013**, *93* (9), 2101–2107 DOI: 10.1016/j.chemosphere.2013.07.054.

(3) Liu, W.; Zhang, Y.-B.; Quan, X.; Jin, Y.-H.; Chen, S. Effect of perfluorooctane sulfonate on toxicity and cell uptake of other compounds with different hydrophobicity in green alga. *Chemosphere* **2009**, *75* (3), 405–409 DOI: 10.1016/j.chemosphere.2008.11.084.

(4) Mcnamara, P. J.; Lapara, T. M.; Novak, P. J. The Effect of Perfluorooctane Sulfonate , Exposure Time , and Chemical Mixtures on Methanogenic Community Structure and Function. *Microbiol. Insights* **2015**, *8* (S2), 1–7 DOI: 10.4137/MBI.S31345.TYPE.

(5) Mcnamara, P. J.; Lapara, T. M.; Novak, P. J. The Impacts of Triclosan on Anaerobic Community Structures, Function, and Antimicrobial Resistance. *Environ. Sci. Technol.* **2014**, *48* (13), 7393–7400.

(6) WHO's first global report on antibiotic resistance reveals serious, worldwide threat to public health.

Appendix F: Effect of Perfluoroalkyl Substances on Nitrifiers and Co-Contaminants

Motivation

Perfluoroalkyl substances (PFAS) are manmade compounds resistant to degradation. They are used in a variety of industrial and consumer products and are thus, ubiquitous in the environment. PFAS can be found in wastewater treatment at levels up to μg/L,[1,2] yet their effect on the microbial communities is not well studied. Exposure to two PFAS, perfluorooctane sulfonate (PFOS) and perfluorooctane (PFOA) has been shown to increase the formation of extracellular polymeric substances in activated sludge flocs at levels as low as 100 ng/L, however, the degradation of ammonium and organic carbon was not affected. The mechanism of action was not determined and more work is needed to clarify other unexpected effects PFAs could have on bacteria.

A potential and unexpected adverse effect is increased co-contaminant toxicity after exposure to PFAS. PFAS have been shown to increase cell membrane permeability, increasing toxicity to other contaminants, so-called co-contaminants. In fact, cellular uptake and the genotoxicity of cyclophosphamide increased in lung cells exposed to 12 mg/L PFOS.[3] Similarly, the exposure of liver cells to either 50 mg/L PFOS or 41 mg/L PFOA increased the uptake and toxicity of pentachlorophenol.[4 5] It has not been studied if permeability changes could also occur in bacteria, however, one study demonstrated that chronic exposure of anaerobic digester communities to PFOS caused the communities to be more susceptible to triclosan (TCS).[6] This study did not determine if increased susceptibility was a result of permeability changes or another factor, such as community change.

The aim of this study is to access the impact of PFAS on a nitrifier community enriched from a wastewater treatment plant. This culture was chosen because as chemotrophs, nitrifers may be more sensitive to contaminants[7] and nitrogen cycling is ecologically important. Additionally, PFOS was used as a model PFAS because it is frequently detected in environmental samples[8,9] and has been associated with increased membrane permeability and other adverse biologic affects.

Methods

Culture

A culture enriched with nitrifiers was obtained from a previous student.[10] The culture was maintained as a source culture and was operated under conditions similar to those outlined in Fleischhacker 2012.[10] The volume of the source culture was 4 L and was fed nutrient mixture (continuously) and a buffer (once every 6 hr). A PFOS exposed culture was operated in the same manner except that nutrient feed also contained PFOS at a concentration such that the bacteria would receive a dose of 100 ng/L PFOS.

Experimental Set-Up

***Long term PFOS exposure*.** A second source culture was started with biomass from the original source culture but exposed to 100 ng/L. Respiration rate was monitored before and after PFOS addition. All glassware used in respiration experiments was baked at 550°C for at least 1 hr. Source PFOS culture was mixed as biomass was removed and biomass was only used if pH in reactors was above 7. Each 250 mL glass flask received 250 mL of biomass as well as glass beads for mixing. Ammonia was added through a 1 mL addition of a 27.5 g/L $(NH_4)_2SO_4$ stock solution and flasks were aerated using house

air. The flasks were sealed with a rubber caps fit to hold dissolved oxygen probes. Flasks were shaken via shaker table and shaking speed was adjusted so that all biomass was well-mixed. Dissolved oxygen was recorded every 10-s. Experiments were stopped when DO fell below 2 mg/L, flasks were reaerated using house air and experiments were run a total of three times. Two 50-mL samples were taken for suspended volatile solids analysis.

***Co-contaminant toxicity*.** Respiration rate was determined for cultures exposed to PFAS and co-contaminants. PFOS and perfluorooctanesulfonamide (FOSA) were used. PFOS is frequently detected in the environment and FOSA has been shown to be a more potent uncoupler than PFOS.[11] Experiments were always performed with treatments consisting of a chemical-free control, co-contaminant only, PFAS only, and co-contaminant + PFAS mixture. PFAS were added via methanol stock to empty flasks and methanol was evaporated overnight. Co-contaminants were added were added via methanol stock and equal amounts of methanol were added to all treatments. Flasks were allowed to mix for 2 hr to allow contaminants dissolve. To start the experiment, flasks were aerated and experiments proceeded as previously described with the exception that once DO fell below 2 mg/L experiments were ended.

Experiments were also performed with biomass from the PFOS source culture. In this case, we were interested in the difference between how short vs. chronic exposure to PFOS effected how bacteria responded to co-contaminants. Thus, experiments were performed with treatments including short term exposure to 100 ng/L PFOS, short term

PFOS exposure + co-contaminant, chronic exposure to 100 ng/L PFOS, and chronic exposure to 100 ng/L PFOS + co-contaminant.

***Combination of triclosan and PFOS*.** Because TCS and PFOS had previously shown to act synergistically,[6] experiments were performed to more thoroughly examine any combination effects. First, 500 mL of biomass was removed from the source cultures. The pH was adjusted to a value between 7.7 and 7.8. TCS and PFOS were previously added to 250 mL glass flasks via methanol stock. Methanol was allowed to evaporate and 50 mL of biomass was added. Flasks were stirred overnight to allow PFOS and TCS to dissolve and for remaining ammonium to transform. Nitrifying growth media[10] was prepared and filtered with a 0.22 μm Durapore Millipore filter. The pH of the nitrifying media was adjusted between 7.7 and 7.8. Aerated media was added to flasks so that there would be no headspace. A 1.5 mL and a 1 mL sample was taken for DNA and ammonia analysis respectively. The flasks were capped and DO was recorded every 10-s. After DO fell below 2 mg/L a 5-mL sample was taken for DNA analysis.

Triclosan and PFOS impact on ammonia transformation. All glassware was ashed at 550 °C before use. Two liters were drawn from source reactors (original source and PFOS source). Bacteria were aerated overnight to remove residual ammonia. PFOS and TCS were added to 500 mL glass flasks via methanol stock and methanol was allowed to evaporate. Chronically exposed cultures were still amended with PFOS. One hundred mL of growth media was then added and flasks were stirred overnight to allow PFOS and triclosan to dissolve. Final concentration of ammonia was targeted at 51.43mg/L as NH4 or 40 mg/L NH4-N.

The next morning, residual ammonia was measured to ensure that it had been degraded. Four hundred mL of bacteria culture was then added to each flask. Flasks were aerated and stirred via magnetic stir plates. Oxygen concentrations were occasionally measured to make sure they remained greater than 4 mg/L. Samples for ammonia concentration were taken during the first six hours. Ammonia concentrations were determined by using a HACH kit. After 6 hrs, flasks aeration and mixing of flasks continues to increase exposure time to contaminants. The procedure was repeated after 3 and 6 days of exposure.

Triclosan and PFOS effect on antibiotic resistance. Nitrifiers were exposed to PFOS (low, 1 mg/L and high, 100 mg/L) and TCS (1 mg/L) overnight to determine if the proportion of bacteria containing ARGs would increase. It was hypothesized that bacteria may contain more ARGs to counteract increase in permeability resulting from PFOS. Source culture was collected and aliquoted into 5-mL treatments repeated in quintuplicate. PFOS and TCS were added via methanol stock, however, methanol was evaporated before cultures were added. Initial samples (1 mL) were taken for DNA analysis, as well as samples (1.5 mL) the following morning.

Analytical Methods

Volatile Solids. Suspended volatile solids was measured using Whatman® Glass microfiber filters, Grade GF/A. Prior to use, filters were washed with 100 mL of deionized water and baked at 550 °C overnight. Biomass was filtered and filters were baked at 105 °C overnight and mass was recorded. Filters were then baked at 550 °C for an hour and mass was again recorded.

Antibiotic resistance genes. DNA was extracted and *mexB*, *inti1*, and 16s was quantified as described by Appendix E.

Data Analysis

Respiration rate. Respiration rate is defined as the change in dissolved oxygen concentration over time. Linear regression was performed to determine this value. Respiration rate was normalized by volatile solids concentration in each flask.

For experiments run in the presence of co-contaminants, the volatile solids normalized respiration rate was normalized to the respiration rate of the experimental control. In the case of the experiments performed with chronically exposed PFOS, the respiration rates in the PFOS + co-contaminant treatments were normalized to the PFOS only treatment. Results are presented as percentages: a value greater than 100% corresponds to a rate faster than the control whereas a value less than 100% corresponds to a rate slower than the control.

Antibiotic resistance genes. *MexB* and *inti1* genes were normalized to total DNA via 16s. Confidence intervals (α=0.05) were calculated via Student t test.

Results and Discussion

Long term PFOS exposure. Chronic exposure to 100 ng/L PFOS did not affect respiration rate in this nitrifier culture (Figure F.1). Respiration rate was monitored for close to 50 days (>3 solid retention times) following continuous PFOS exposure. While respiration rate was somewhat variable, there was no increasing or decreasing trend. PFOS was not expected to effect metabolism at a concentration this low. In a previous study, no effect on the degradation of organic carbon or ammonium was seen in activated

sludge exposed to 100 ng/L PFOS.[5] Additionally, PFOS has only been shown to be toxic to microorganisms at a concentration on the order of mg/L.[12]

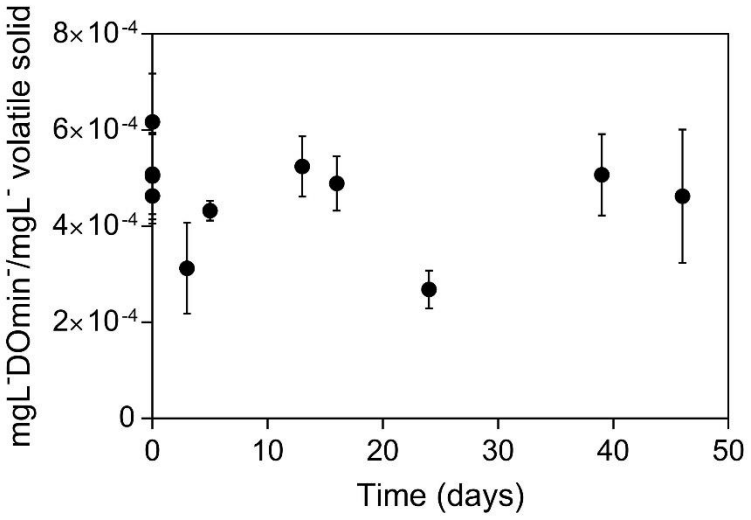


Figure F.1. Respiration rate in nitrifier source culture after receiving continuous 100 ng/L PFOS dose. Respiration rates plotted at t=0 were performed before the reactor received the PFOS dose. Error bars show standard deviation.

Co-contaminant toxicity. PFAS had little effect on respiration of this nitrifier community. PFOS showed no increase in respiration rate (uncoupling) or decrease (toxicity), however, FOSA appeared to slightly increase respiration rate (Figure F.2.B). The increase is not significantly significant, however the slight increase in respiration rate is likely a result of uncoupling ability. Additionally, toxicity was not observed in nitrifiers exposed to the co-contaminants diuron and TCS.

Mixtures of PFAS + co-contaminants did show slight changes in co-contaminant toxicity, though they were not statistically significant. While FOSA increased respiration rate, the combination of FOSA + 100 ug/L diuron had a respiration rate similar to the control (Figure F.2.B). Thus, the presence of diuron could be moderating the effect of

FOSA. Though, the lack of effect could also be a result of additional variability resulting from the presence of another co-contaminant. Additionally, while PFOS had no effect on respiration rate, the combination of 100 ng/L PFOS + 100 μg/L diuron decreased respiration rate (Figure F.2.A). This could be a result of increased permeability as diuron alone did not alter respiration rate. This is unexpected because when an algae culture was exposed to diuron + PFOS, diuron was less toxic in the presence of PFOS (antagonistic).[13] Our results depict a synergistic relationship. In our experiments, synergism was not always demonstrated between diuron and PFOS. Figure F.2.C shows that the ratio of respiration rate between short term PFOS + diuron and short term PFOS was not different than 1. Had synergistic toxicity been observed, the ratio should be less than 1. Lastly, it appears that long chronic exposure to 100 ng/L PFOS did not cause bacteria to respond differently to co-contaminants (diuron and TCS) than bacteria that only received short term exposure (Figures F.2.C, F.2.D).

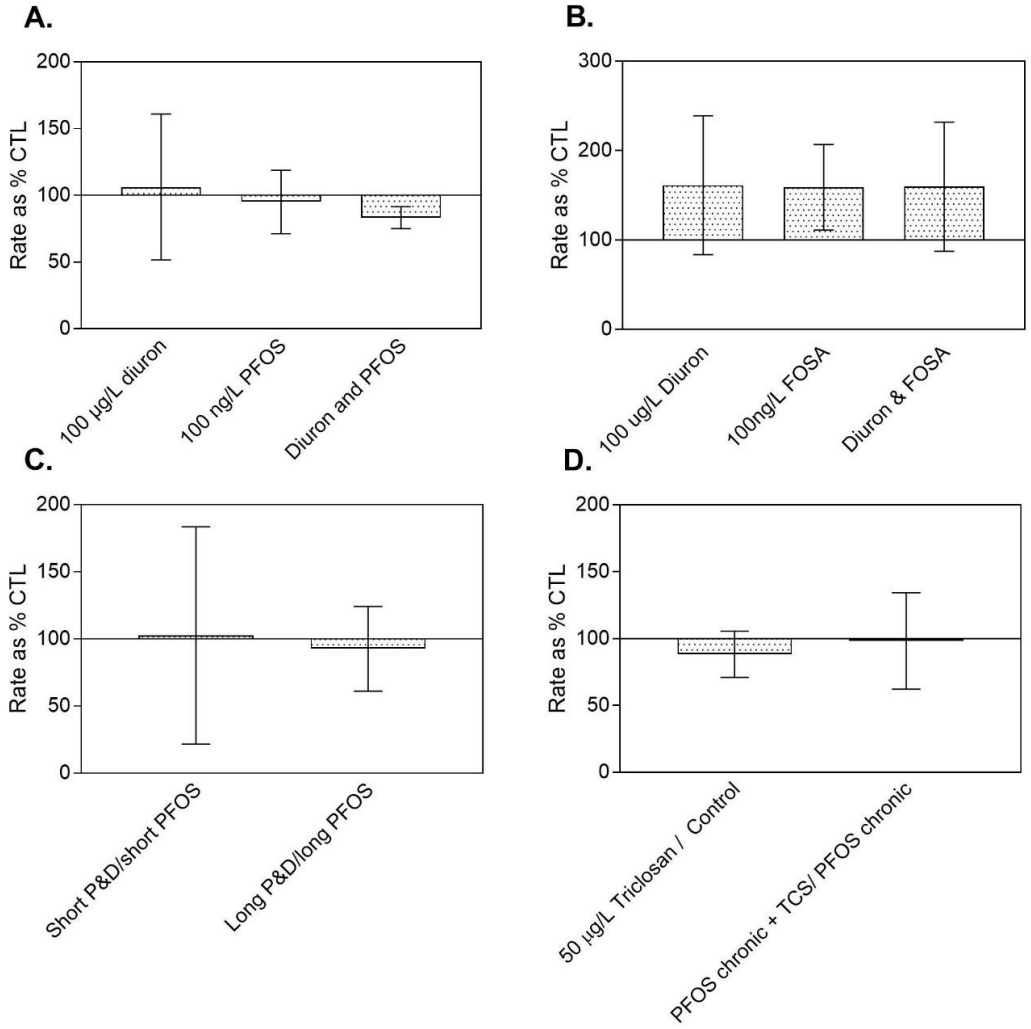


Figure F.2. The effect of PFOS, FOSA, and co-contaminants diuron and TCS on nitrifier respiration rate. Error bars show standard deviation. (A) shows the effect in nitrifiers that have not previously been exposed to PFOS. These bacteria were exposed to 100 ng/L PFOS and 100 µg/L diuron. (B) shows the effect in nitrifiers that have not previously been exposed to PFOS. These bacteria were exposed to 100 ng/L FOSA and 100 µg/L diuron. (C) shows the comparison of diuron (100 µg/L) toxicity to bacteria that are only exposed to 100 ng/L PFOS for a short time vs those that are chronically exposed to 100 ng/L PFOS. (D) shows the comparison of TCS (50 µg/L) toxicity to bacteria that are only exposed to 100 ng/L PFOS for a short time vs those that are chronically exposed to 100 ng/L PFOS.

Combination effects of PFOS and triclosan. TCS was not toxic to nitrifiers at concentrations up to 1 mg/L (Figure F.3). Some toxicity was observed (decreased respiration rate) in PFOS-exposed cultures at a concentration of 3 mg/L (Figure F.3.B). There may be a difference at a TCS concentration of 3 mg/L, though non-exposed cultures were not treated with 3 mg/L TCS and the observed decrease in the PFOS exposed cultures is very slight. This makes it unlikely that any observed difference would be significant.

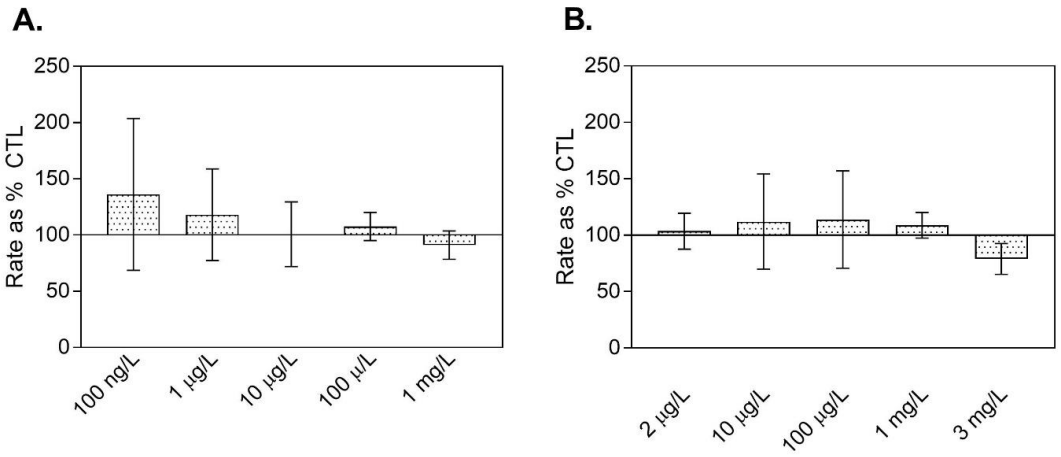


Figure F.3. Effect of varying triclosan doses on respiration rate in (A) nitrifiers not exposed to PFOS and (B) nitrifiers chronically exposed to 100 ng/L PFOS.

Effect of PFOS and triclosan on ammonia transformation. Time appears to have a bigger impact on degradation of ammonia than exposure to PFOS or co-contaminants (Figure F.4). Ammonia seems to be transformed much slower at day 6 than the initial time points, however, bacteria that are chronically exposed to 100 ng/L PFOS appear to be more susceptible than unexposed bacteria. The rates at day 3 and 6 for

chronically exposed bacteria seem less overall than the rates for unexposed bacteria. Hence, the communities chronically exposed to 100 ng/L PFOS may be altered in such a way that they are more susceptible to non-ideal/starvation conditions. Experiments were not replicated so it is difficult to make definitive statements.

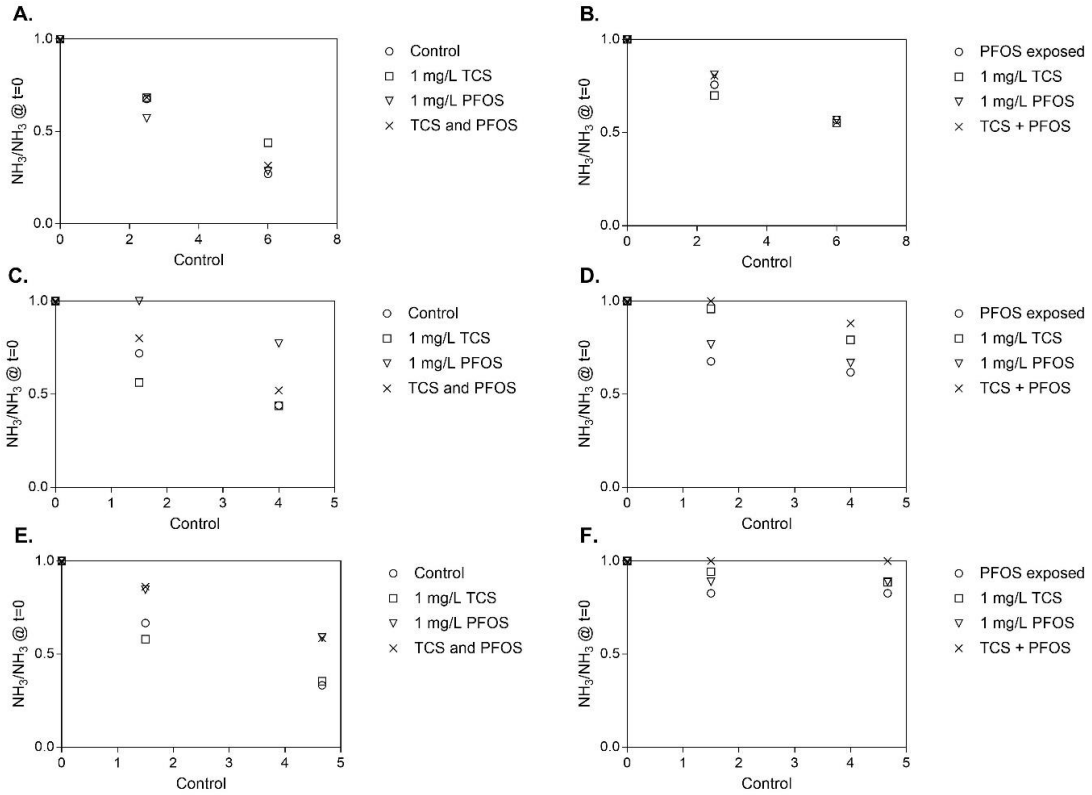


Figure F.4. Ammonia removal in nitrifying communities. Ammonia concentrations are normalized to initial ammonia concentration in each flask. (A) Previously unexposed nitrifiers at day 1. (B) Previously exposed nitrifiers at day 1. (C) Previously unexposed nitrifiers after 3 days. (D) Previously exposed nitrifiers after 3 days. (E) Previously unexposed nitrifiers after 6 days. (F) Previously exposed nirtifiers after 6 days.

Triclosan and PFOS effect on antibiotic resistance. The presence of PFOS (1-mg/L, 100 mg/L) and TCS (1 mg/L) also did not have a great effect on antibiotic resistance genes (Figure F.5). First, *inti1* and *mexB* abundance is very low, as expected because bacteria were not previously exposed to contaminants or antibiotics. The proportion of *inti1* genes appeared to be greater in PFOS + TCS treatments, however, *Inti1* proportion also was greater in the control and observed increases in the two treatments was extremely variable (comparatively high standard deviation). It is likely that these small increases are random.

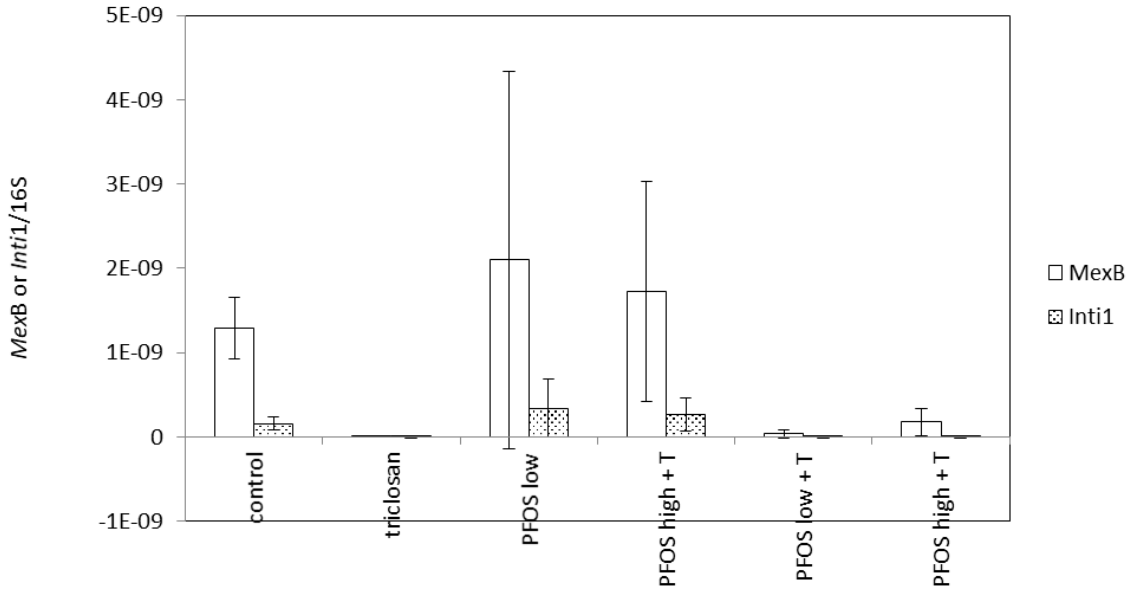


Figure F.5. Antibiotic resistance genes in a nitrifying culture after overnight exposure to PFOS and triclosan. Resitance genes are normalized to 16s.

References

(1) Sinclair, E.; Kannan, K. Mass loading and fate of perfluoroalkyl surfactants in wastewater treatment plants. *Environ. Sci. Technol.* **2006**, *40* (5), 1408–1414.

(2) Loos, R.; Wollgast, J.; Huber, T.; Hanke, G. Polar herbicides, pharmaceutical products, perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and nonylphenol and its carboxylates and ethoxylates in surface and tap waters around Lake Maggiore in Northern Italy. *Anal. Bioanal. Chem.* **2007**, *387* (4), 1469–1478 DOI: 10.1007/s00216-006-1036-7.

(3) Jernbro, S.; Rocha, P. S.; Keiter, S.; Skutlarek, D.; Färber, H.; Jones, P. D.; Giesy, J. P.; Hollert, H.; Engwall, M. Perfluorooctane Sulfonate Increases the Genotoxicity of Cyclophosphamide in the Micronucleus Assay with V79 Cells. *Environ. Sci. Pollut. Res. Int.* **2007**, *14* (2), 85–87.

(4) Shan, G.; Ye, M.; Zhu, B.; Zhu, L. Enhanced cytotoxicity of pentachlorophenol by perfluorooctane sulfonate or perfluorooctanoic acid in HepG2 cells. *Chemosphere* **2013**, *93* (9), 2101–2107 DOI: 10.1016/j.chemosphere.2013.07.054.

(5) Pasquini, L.; Merlin, C.; Hassenboehler, L.; Munoz, J.-F.; Pons, M.-N.; Gorner, T. Impact of certain household micropollutants on bacterial behavior. Toxicity tests/study of extracellular polymeric substances in sludge. *Sci. Total Environ.* **2013**, *463–464* (August 2016), 355–365 DOI: 10.1016/j.scitotenv.2013.06.018.

(6) Mcnamara, P. J.; Lapara, T. M.; Novak, P. J. The Effect of Perfluorooctane Sulfonate , Exposure Time , and Chemical Mixtures on Methanogenic Community Structure and Function. *Microbiol. Insights* **2015**, *8* (S2), 1–7 DOI: 10.4137/MBI.S31345.TYPE.

(7) Madigan, M.; Marktinko, J.; Dunlap, P.; Clark, D. *Brock Biology of Microorganisms*, 13th Editi.; Espinoza, D., Cook, K., Cutt, S., Hutchinson, E., Cogan, D., Marcus, E., Wagner, A., Eds.; Benjamin Cummings: Indianapolis, Indiana, 2006.

(8) Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J. Perfluoroalkyl Acids : A Review of Monitoring and Toxicological Findings. *Toxicol. Sci.* **2007**, *99* (2), 366–394 DOI: 10.1093/toxsci/kfm128.

(9) Rayne, S.; Forest, K. Perfluoroalkyl sulfonic and carboxylic acids: a critical review of physicochemical properties, levels and patterns in waters and wastewaters, and treatment methods. *J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng.* **2009**, *44* (12), 1145–1199 DOI: 10.1080/10934520903139811.

(10) Fleischhacker, N. T. Phytoestrogen distribution and degradation in natural and engineered systems, University of Minnesota, 2012.

(11) Starkov, a a; Wallace, K. B. Structural determinants of fluorochemical-induced mitochondrial dysfunction. *Toxicol. Sci.* **2002**, *66* (2), 244–252.

(12) Ding, G.; Peijnenburg, W. J. G. M. Physicochemical Properties and Aquatic Toxicity of Poly- and Perfluorinated Compounds. *Crit. Rev. Environ. Sci. Technol.* **2013**, *43* (6), 598–678 DOI: 10.1080/10643389.2011.627016.

(13) Liu, W.; Zhang, Y.-B.; Quan, X.; Jin, Y.-H.; Chen, S. Effect of perfluorooctane sulfonate on toxicity and cell uptake of other compounds with different hydrophobicity in green alga. *Chemosphere* **2009**, *75* (3), 405–409 DOI: 10.1016/j.chemosphere.2008.11.084.