Environment and Natural Resources Trust Fund

Research Addendum for Peer Review

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Project Title:	Protecting bacteria from contaminants to preserve water quality
Project number:	033-B

1. Abstract

Humans depend on bacteria to cycle nutrients and carbon. In doing so, bacteria perform critical ecological functions that enable life to exist. Bacteria are also harnessed for use in engineered systems such as wastewater treatment plants and landfills. In fact, it is through the activity of bacteria in engineered systems that engineers protect surface water from excess nitrogen pollution, decompose solid waste, and treat wastewater so that its discharge is cleaner and therefore better supports aquatic life. Unfortunately, the environments where these critical bacteria live are also environments filled with a complex "soup" of chemicals. The chemicals present in personal care products, medicines, and products such as clothing and packaging are eventually found in wastewater, solid waste, and the wastewater-derived biosolids that are applied to agricultural land. These chemicals can negatively affect bacterial function, and can be particularly damaging when present in mixtures. One common class of chemicals that are present throughout the environment is perfluorinated chemicals; based on other research, it is hypothesized herein that perfluorinated chemicals can cause other co-contaminants to be more toxic to bacteria.

The proposed research will study how bacterial function, namely the oxidation of ammonia and the anaerobic degradation of a mixture of carbonaceous compounds, is affected when bacteria are exposed to a mixture of perfluorinated chemicals in the presence and absence of other co-contaminants. This research will help us understand why/when critical bacterial functions such as nitrogen cycling and carbon decomposition are lost as a result of chemical exposure. It will also help us understand which bacteria are more resistant to such harmful affects and why, with the goal of developing engineered methods to protect critical bacterial functions.

2. Background

Humans depend on bacteria to cycle nutrients and carbon. In doing so, bacteria perform critical ecological functions that enable life to exist. We also harness bacteria for use in engineered systems such as wastewater treatment plants and landfills. In fact, it is through the activity of bacteria in engineered systems that we are able to protect Minnesota's surface water from excess nitrogen pollution, decompose solid waste, and treat wastewater so that its discharge is cleaner and therefore better supports fish and aquatic life.

Unfortunately, the environments where these critical bacteria live are also environments filled with a complex "soup" of chemicals. The chemicals present in personal care products, medicines, and the products we buy, such as clothing and packaging, are eventually found in wastewater, solid waste, and the wastewater-derived biosolids that are applied to agricultural land ***(Kolpin et al., 2002; Schwarzenbach et al., 2006; Wigle et al., 2008; Busch et al, 2010; Eggen et al., 2010; Beesoon et al., 2011; Huset et al., 2011)***. These chemicals can negatively affect bacterial function, and can be particularly damaging when present in mixtures ***(e.g., Escher et al., 1996)***. One common class of chemicals that are present throughout the environment in Minnesota is that of perfluorinated chemicals (PFCs).

PFCs comprise a class of chemicals that have been used extensively in polymers and in industrial and domestic applications. As is often the case with high production volume chemicals, they are globally distributed in the environment. PFCs have been found in wastewater treatment effluent ***(Loganathan et al., 2007)***, stormwater ***(Xiao et al., 2011)***, groundwater ***(Moody and Field, 1999)***, surface waters ***(Simcik and Dorweiler, 2005)***, sediments ***(Higgins and Luthy, 2006)***, and biosolids ***(Clarke and Smith, 2011)***. Indeed, although concentrations are typically in the ng/L range in surface waters ***(Saito et al., 2004; Nakayama et al., 2010)***, these compounds will accumulate in sediment and biosolids, with one particularly common PFC, perfluorooctane sulfonate (PFOS, **Figure 1**), detected at concentrations up to 2600 μg/kg in biosolids ***(Higgins et al., 2005; Sinclair et al., 2006; Sepulvado et al., 2011; Sun et al., 2011)***. Even relatively newly manufactured PFCs, such as the 4-carbon perfluorbutane sulfonate (PFBS, **Figure 1**), have been detected in the environment, with Busch *et al*. ***(2010)*** finding PFBS as one of the dominant PFCs in landfill leachate. It is clear that the widespread use of these compounds and their chemical persistence has resulted in their ubiquity in the environment as well as their presence in fish, birds and mammals (including human blood) worldwide ***(e.g., Giesy and Kannan, 2002; Naile et al., 2013).***

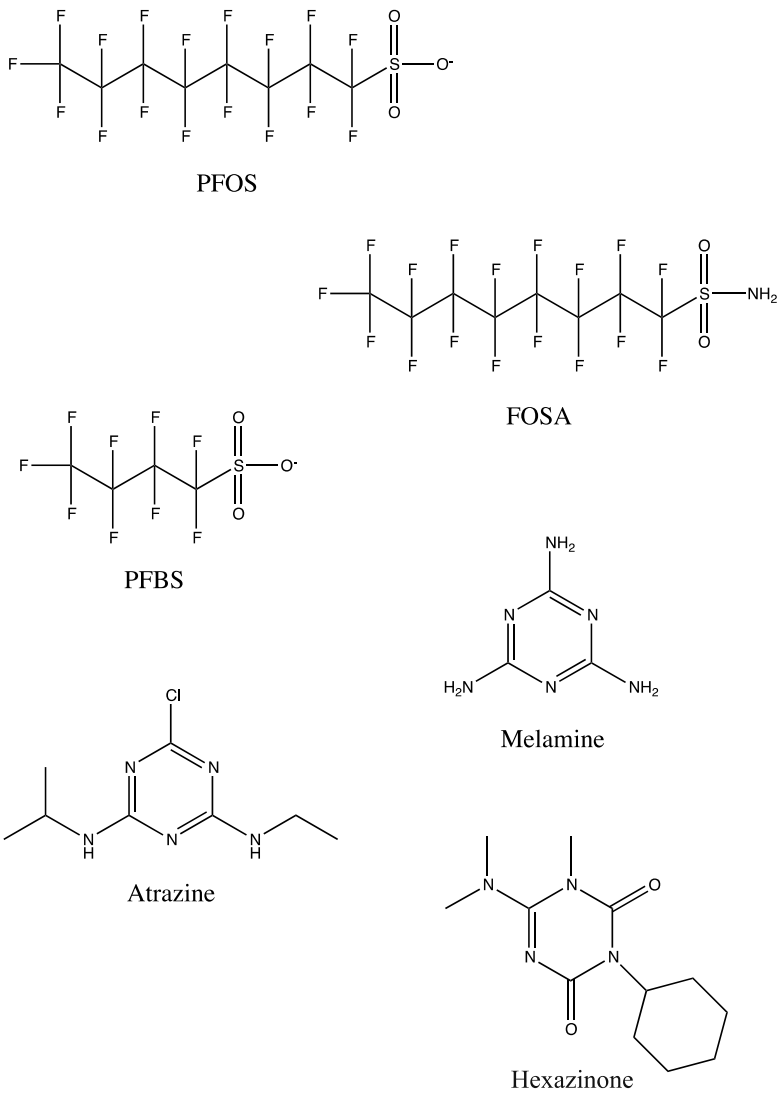

Figure 1. The structures of common perfluorinated compounds (perfluorooctane sulfonate (PFOS), perfluorooctane sulfonamide (FOSA), and perfluorbutane sulfonate (PFBS)) and triazine compounds (melamine, and the environmental contaminants atrazine and hexazinone).

Unfortunately, PFCs have been shown to disrupt biological activity. One mechanism of PFC toxicity that has widespread domain-independent relevance is that of metabolic uncoupling ***(O'Brien and Wallace, 2004; Kubwabo et al., 2005; Beesoon et al., 2011)***. Uncoupling results when the proton motive force across a cell membrane is dissipated, a potentially catastrophic event for cells because the proton motive force is needed to make energy for the cell during respiration. A chemical compound will act as an uncoupler if it can facilitate unregulated proton transport across the cell membrane. One method of uncoupling is where a compound, called a protonophore, directly transports a proton across the membrane. Hydrophobic compounds with a pK_a near 7 are most apt to be protonophoric uncouplers because they can position themselves in the membrane where they can gain or lose a proton ***(Escher et al., 1996)***. Several PFCs, including those with amide groups such as perfluorooctane sulfonamide (FOSA, or PFOSA, **Figure 1**), are known protonophoric uncouplers in mitochondria ***(O'Brien and Wallace, 2004)***. FOSA

was shown to be an extremely potent uncoupler, approximately equivalent in potency to carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone, one of the most potent mitochondrial uncouplers known ***(Starkov and Wallace, 2002)***. The half maximal inhibitory concentration of FOSA (1 μM) ***(O'Brien and Wallace, 2004)*** is one that PFCs can commonly reach in sediments and biosolids (approximately 500 μg/kg) ***(Clarke and Smith, 2011)***. A second mode of action with respect to uncoupling occurs when compounds simply have a high affinity for the membrane, residing there and dismantling membrane function by creating a "leaky" membrane ***(Liu et al., 2009)***. As mentioned above, PFCs have been shown to increase cell membrane permeability and enhance respiratory gas delivery in cell cultures as well as in human lung cells ***(Lowe, 2002)***. PFOS, though only a weak uncoupler, appeared to act in this manner in mitochondria ***(O'Brien and Wallace, 2004)***.

Because PFCs impact higher organisms, one might expect that some simpler cells—bacteria and other microorganisms—might also be sensitive to PFCs. Indeed, this was shown to be true, but interestingly, was particularly notable when PFCs were present in mixtures. A recent study found that PFOS was able to alter the toxicity of other hydrophobic compounds to algal cells ***(Liu et al., 2009)***. Other studies found that a ternary combination of PFOS, triclosan, and 2,4,6-trichlorophenol showed greater than additive (*i.e.*, synergistic) toxicity to algal cells ***(Boltes et al., 2012)***. Other surfactants seem to work in a similar manner, with the ternary mixture of the surfactant docusate sodium, triclosan, and 2,4,6-trichlorophenol exhibiting synergistic toxicity to pure cultures of bacteria (*Vibrio fischeri*), algae (*Pseudokirchneriella subcapitata*), and cyanobacteria (an *Anabaena* species) ***(Rosal et al., 2010)***.

The impacts of PFCs may be even more complex, however, with effects depending not only on the structure of the PFC, its concentration, and the presence of co-contaminants, but also on exposure time. Again, studies with other surfactants can provide clues as to what such exposure effects might be. For example, in a recent study an alcohol alkoxylate surfactant was added to mixed methanogenic cultures at 200 mg/L; methane production was initially unaffected, but after approximately 10 days, inhibition occurred ***(Van Ginkel et al., 2007)***. This was thought to be because time was required for the surfactant to partition to the cell membrane where it subsequently uncoupled metabolism ***(Van Ginkel et al., 2007)***.

This suggests that in the environment, where chronic exposure to PFCs occurs, cells may be affected differently than what might be predicted based on shorter-term laboratory studies or single-compounds studies. The widespread presence and persistence of PFCs in a range of environmental matrices coupled to their ability to alter the effect of co-contaminants, likely through their surfactant-like properties, point to a real need for further study. How do these compounds alter toxicity? Are they capable, in mixtures at low concentrations, of impacting a wide range of organisms because of these surfactant-like properties? Is a fundamental change in how scientists, engineers, and policy-makers approach risk assessment needed? These questions are important and lead to the objectives and hypotheses of the proposed research.

3. Hypotheses

Activity 1: Understand how and why perfluorinated chemicals alter bacterial function (nitrogen cycling and carbon decomposition) alone or in mixtures with a co-contaminant.

Hypothesis: PFOS is expected to act as a non-specific surfactant-like uncoupler, FOSA as a potent protonophoric uncoupler, and PFBS as a particularly weak non-specific uncoupler. Upon chronic exposure, each of these compounds will partition into microbial cell membranes, uncoupling metabolism to some extent. FOSA is expected to cause more dramatic uncoupling, with PFOS and PFBS causing little impact when present alone. When a co-contaminant is added, however, its transport into the cell will be enhanced by the PCFs and toxicity will be enhanced. PFOS is expected to cause the greatest increase in co-contaminant toxicity as it non-specifically alters membrane fluidity and will likely increase co-contaminant transport accordingly. These effects will be observed in biofilm cultures as well, though a longer exposure time will be required before uncoupling is observed.

Activity 2: Understand the chemical properties of co-contaminants that make them more harmful to bacterial function (nitrogen cycling and carbon decomposition) in the presence of perfluorinated chemicals.

Hypothesis: Small hydrophobic compounds, such as atrazine, may be expected to transport across cell membranes; therefore, the enhancement of such transport by PFCs will be less dramatic. PFCs will have a much greater effect on the transport of small hydrophilic compounds, such as melamine, however. Very large hydrophilic compounds, such as hexazinone, which is not expected to be transported into the cell, will experience the greatest relative increase in transport in the presence of PFCs.

4. Methodology

Activity 1.

In this research, both a mixed nitrifying culture (typical of wastewater) and a mixed methanogenic culture (typical of digesters and landfills) will be investigated. Chemolithotrophs such as nitrifiers are critical for elemental cycling in the environment (in this case, nitrogen), they play an important role in many engineered systems, and are expected to have a greater sensitivity to contaminants as a result of their low cell yields and high respiration rates. Methanogenic cultures are similar in that they are critical components of the carbon cycle in both natural and engineered systems and are also expected to have a greater sensitivity to contaminants as a result of their lower cell yield. We will also study strongly flocculating nitrifiers and methanogenic communities cultured as granules so that the physical protection of the biological floc/granule itself can be assessed.

Five 3-L nitrifying source cultures will be established. They will be seeded with activated sludge from a local wastewater treatment plant (Metropolitan Plant, St. Paul, MN) and fed ammonium (500 mg/L as ammonium sulfate, approximately 7.6 mM NH_4^+) and trace nutrients in well-buffered (pH 7.8) medium. The solids residence time (SRT) will be 15 days. In one set of experiments nitrifiers will be vigorously mixed and additional Ca^{2+} (approximately 4 mM as $CaCl_2$) will be added to encourage flocculation. Five bench-scale 3-L methanogenic source cultures will also be established. These cultures will be seeded with sludge from a local full-scale anaerobic digester. These source reactors will be fed a synthetic blend of organic acids (20% acetate, 10% propionic acid, 9% butyric acid, and 3% valeric acid), alcohols (40% ethanol, 4% isopropanol, 2% methanol) and glucose (12%) in minimal media ***(Shelton and Tiedje, 1984)***. The organic loading rate will be approximately 0.18 g COD/L-day and the SRT will be 18 days. As with the nitrifiers, a set of experiments will be performed with granulated anaerobic cultures generated via higher organic loading (1.8 g/L-day) and vigorous mixing. We are currently culturing both nitrifiers and mixed methanogenic cultures in our laboratory in this manner.

Of the five source reactors, one will be fed PFOS, one FOSA, and one PFBS, each at approximately 400 μg/kg (expected to be below the half maximal inhibitory concentration of FOSA, but a concentration likely to be observed in soils and biosolids), and a fourth will be fed PFOS at 2000 μg/kg. One reactor will be maintained in the absence of PFCs. Reactors that are chronically-exposed to the PFCs will be maintained for a period of three SRTs before experiments are performed.

Initial experiments will be performed with melamine **(Figure 1)** as the co-contaminant. Melamine contains a triazine ring, which is a structure commonly found in other environmental contaminants, such as atrazine **(Figure 1)**, making it a good model compound.

Aliquots (100 mL) of the chronically PFC-exposed source cultures will be added to triplicate batch reactors to which either (1) nothing or (2) melamine will be added. Aliquots of the unexposed source cultures (i.e. no chronic PFOS, FOSA, or PFBS exposure) will also be added to triplicate batch reactors to which the following is added: (1) no chemical contaminants, (2) single compounds (PFOS, FOSA, PFBS, or melamine), or (3) mixtures of each of the PFCs and melamine. PFCs will be added at concentrations of approximately 400 μg/kg. Melamine will be added at a concentration that is approximately 10% of the dose that results in a significant decrease in respiration (determined separately). Nitrifying reactors will be fed with oxygen and ammonia and the biomass-normalized rate of oxygen use and ammonia consumption will be determined for each batch reactor. Oxygen will be measured via electrodes linked to data loggers and ammonia will be measured via an ion-specific electrode. Biomass will be measured as total DNA (to enable comparison to biofilm systems) and optical density (for ease). Replicate experiments will be performed for dispersed and biofilm-forming nitrifying cultures. Methanogenic reactors will be fed the mixture of organic acids, alcohols, and glucose fed to the source reactors and the biomass-normalized rate of methane production will be determined for each batch reactor. Methane will be measured via gas

chromatography. As with the nitrifiers, replicate experiments will be performed for dispersed and granulated methanogenic cultures. Statistical significance will be determined with results from replicate reactors.

Reactor samples will be taken with time and analyzed to determine the concentration of melamine and PFCs outside and inside the cells. Samples will be filtered through a 0.2 μm filter. The filter will be resuspended in sterile phosphate-buffered saline ("buffer"), vortexed aggressively, and filtered a second time. The pooled filtrate will be concentrated via solid phase extraction and analyzed using LC-MS/MS to determine the concentration of melamine ***(Wang et al., 2012)*** and PFCs ***(Xiao et al., 2011; Xiao et al., 2012)*** outside of, or loosely bound to, the cells. After washing the filter with additional sterile buffer, the filter will be resuspended in methanol, sonicated, concentrated via rotary evaporation, and analyzed by LC-MS/MS to determine the concentration of melamine and PFCs inside the cells.

From this data it can be determined whether biomass-normalized respiration rates are altered in an additive, synergistic (greater than additive), or antagonistic (less than additive) manner when cultures are incubated with contaminant mixtures. It will also be determined whether the presence of an extracellular coating in the flocculated nitrifiers or the granulated methanogenic culture protects the organisms from the effects of chemical mixtures. **It is hypothesized** that in previously unexposed reactors, FOSA will result in some protonophoric uncoupling, causing a faster consumption of oxygen or more rapid production of methane, but PFOS and PFBS will have little obvious impact. In reactors to which only melamine is added, little impact on respiration is anticipated. In reactors exposed to mixtures of PFCs and melamine, enhanced toxicity is expected (seen as a decrease in respiration), particularly in PFOS-exposed reactors at both the high and the low PFOS concentrations. Mixture effects, potentially causing the complete cessation of respiration, are expected to be greatest in the batch reactors started with material to which there is chronic PFC exposure, again, particularly in those chronically exposed to high levels of PFOS. The flocculated nitrifiers and granulated methanogenic cultures are expected to be resistant to the effects of PFCs when present at 400 μg/kg, but will still be susceptible to mixture effects in the reactors chronically exposed to high concentrations (2000 μg/kg) of PFOS.

In addition, from the chemical fractionation data the enhancement, or lack thereof, of melamine transport into cells as a result of chronic PFC exposure will be determined. It is hypothesized that in chronically-exposed cultures, PFOS will lead to an increase in melamine transport into the cells, whereas PFBS and FOSA will only result in slight increases in melamine uptake. This is thought to be because, although a strong uncoupler, FOSA acts as a protonophoric uncoupler, resulting in uncoupling without much of a change in the membrane permeability. PFBS, as a result of its small size, is expected to be only a very weak uncoupler, not partitioning substantially into cell membranes. PFOS is expected to act as a non-specific uncoupler, transporting into the cell membranes and changing permeability and cell uptake of co-contaminants. This is expected to be evident in all of the PFOS-exposed reactors, but particularly so in the reactors to which there is high chronic PFOS exposure.

Activity 2.

We know that molecular size and hydrophobicity affect the transport of compounds into the cell cytoplasm. In general, small hydrophobic compounds transport quite readily, small hydrophilic compounds less so, and large hydrophilic compounds, much less. Therefore, we propose to investigate the effect of size and hydrophobicity on transport in the presence of PFCs. This will be done by repeating the experiments described above (except for the imaging experiments) for two additional compounds: atrazine and hexazinone **(Figure 1)**. Like melamine, atrazine and hexazinone are s-triazines of environmental significance, but vary greatly in size and hydrophobicity **(Table 1)**. The use of these compounds in this research provides a small hydrophilic compound (melamine), a small hydrophobic compound (atrazine), and a large hydrophilic compound (hexazinone) with which to test our hypothesis that not only do PFCs change the transport of co-contaminants, but that the magnitude of this change can be predicted based on the chemistry of the co-contaminant.

Table 1. Molar volume and solubility of test compounds

Compound	Molar Volume (mL/mole)	Aqueous Solubility (M)
melamine	80	0.0257
atrazine	182	0.0003
hexazinone	435	0.1308

Exposure experiments will follow the outline of Activity 1. The only exception being that if no toxicity is observed with hexazinone, given its propensity not to bioaccumulate, cells will be exposed at 10% of the solubility of hexazinone rather than at 10% the concentration that causes a decrease in respiration. Extraction of batch reactor samples will occur as described above, but the extracts will be split for separate analysis of the triazines and PFCs. PFCs will be analyzed by LC-MS/MS as described above, but atrazine and hexazinone will be analyzed by gas chromatography/mass spectrometry (GC/MS) similar to the method used for diazinon in Raynor *et al*. ***(2010)***.

The increase in transport with the presence of PFCs will be determined as a % increase:

$$\%Increase = \frac{{C_{cellnoPFC}}/{C_{aqnoPFC}} - {C_{cell+PFC}}/{C_{aq+PFC}}}{{C_{cell+PFC}}/{C_{aq+PFC}}} \times 100$$

These increases will be compared among the three s-triazines and the three PFCs to determine the effect of size and hydrophobicity on PFC-aided transport. Furthermore, the three PFCs will be compared to determine their ability to affect the transport of each of these co-contaminants.

Finally, additional experiments will be performed with a diffusion chamber **(Figure 2)** to measure the transport of H^+ or co-contaminants across a model artificial cell membrane. The diffusion chamber will be created such that the upstream chamber has a large volume in which the concentrations of various dissolved species are held relatively constant and the downstream chamber is very small, allowing for the rapid concentration of species as they diffuse from the upstream chamber into the downstream chamber. The two

chambers will be separated by a plate containing a small (1 mm diameter) hole. An artificial cell membrane can be created in the hole with phospholipids (ref), much like a soap bubble, resulting in a system in which chemicals can be placed in only the upstream chamber (H^+ or melamine for example) and their diffusion across the artificial membrane to the downstream chamber can be monitored. Experiments will be performed with no membrane present (only a hole), with membranes containing phospholipids only (modeling a healthy unaffected cell membrane), and with membranes containing both phospholipids and various quantities of the three PFCs present (modeling a chronically PFC-exposed cell membrane). In this manner the transport of materials across a model cell membrane can actually be measured and used to corroborate the results observed above.

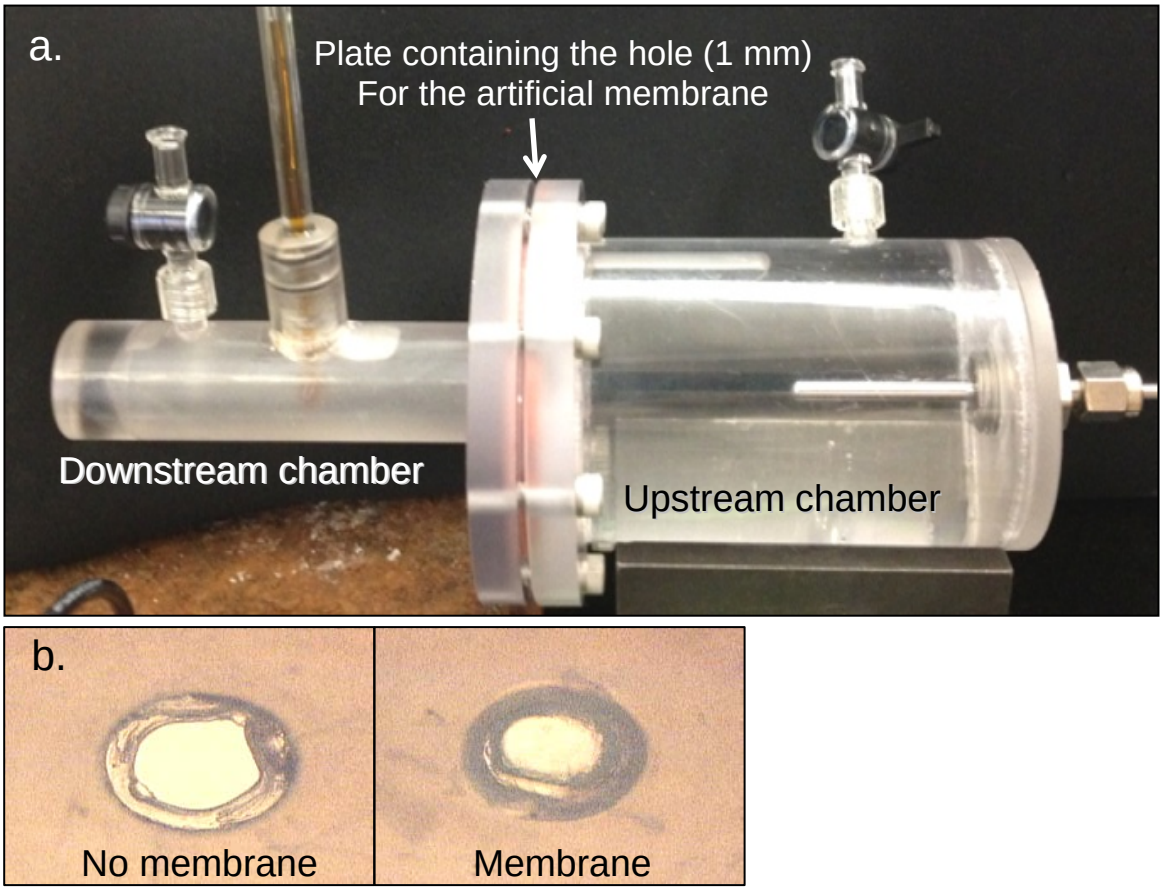

Figure 2. Photograph of (a) the diffusion cell experimental set-up and (b) the hole in the separation plate.

5. Results and Deliverables

In this project we expect to gain an understanding of 1) how several PFCs with different chemistries affect the metabolism of nitrifying organisms and methanogenic organisms in the presence and absence of a co-contaminant, 2) how responses differ in systems subject to chronic PFC exposure, and 3) how responses differ as a function of co-contaminant chemistry (hydrophobicity and size). For one very common PFC, the effect of concentration (400 μg/kg versus 2000 μg/kg) will also be investigated. Through

experiments with a model system (diffusion cells separated by a model cell membrane), the actual effect of PFCs on the transport of co-contaminants and H+ can be measured to verify mechanism. The deliverables based on all of this information will be the diffusion coefficients for various co-contaminants through model membranes with and without PFCs incorporated into their structure and the percent decrease or increase in the metabolism of nitrifying organisms and methanogens in the presence of PFCs and/or co-contaminants (including for chronic exposure and higher concentrations). In addition, experiments associated with Activity 1 and Activity 2 will also be performed with flocculated or granulated bacteria. Because these bacteria will have a protective extracellular coating, the degree to which this coating alters the change in metabolism of these organisms in the presence of PCFs and/or co-contaminants will also be determined. This is the final deliverable associated with this task. Taken together, these deliverables will enable us to make predictions as to when different microbial functions in critical engineered systems (wastewater treatment plants, anaerobic digesters, landfills) are likely to decline as a result of exposure to PFCs and/or co-contaminants and what measures (increases in loading and mixing or seeding with particular organisms for example) are likely to protect the microbial communities and safeguard their function.

6. Timetable

This project is a three-year project, beginning in July, 2014. The timetable for completion of the described project follows in table format, divided into 3-month (quarter) increments.

	Quarter											
Tasks	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Source cultures established	X	X										
Culture exposure experiments with melamine		X	X	X	X	X	X	X				
Culture exposure experiments with atrazine and hexazinone					X	X	X	X	X	X	X	
Sample collection, fractionation, and analysis			X	X	X	X	X	X	X	X	X	
Diffusion Cell Experiments					X	X	X	X	X	X	X	
Prepare manuscripts for the dissemination of results (oral dissemination at local and national conferences or meetings will occur throughout the project)											X	X

7. Budget

The budget is as outlined on the previously submitted proposal (see Attachment A). A budget justification is provided below.

Personnel

Over the course of the 3-year project, support for one graduate student for three years, undergraduate support for the summers, and support for the two PIs is budgeted. The PI (Novak) will require 4 weeks of salary a year and the Co-PI (Simcik) will require 10% salary per year. Fringe benefits for the PIs at UMN are set at 33.6% by the University of Minnesota. The PIs will be responsible for project oversight, guidance of the graduate student, data interpretation and analysis, and report preparation and submission. One graduate student research assistant will devote 100% of their research time to the project over the 3-year project. Fringe benefits for the graduate student include tuition, health insurance, and summer FICA. Undergraduate support is also budgeted to assist the graduate student with experimental set-up, reactor maintenance, and sample processing for analysis.

Materials and Supplies

Funds ($12,500, $15,500, and $15,500) are requested for materials, supplies, consumables, analytical costs and repair/upkeep associated with the LC-MS, and image analysis/imaging center costs. Required materials include, but are not limited to: pipette tips, glassware, solid phase extraction cartridges for extractions, chemicals for standards and experiments, analytical consumables, analytical fees, solvents, reagents, gloves, digital data storage media, and laboratory notebooks.

Total amount proposed

The total proposed project amount is $279,000. No indirect costs for the University of Minnesota are included in the budget.

8. Credentials

Paige J. Novak

Professor, Environmental Engineering, Department of Civil Engineering and Resident Fellow of the Institute on the Environment, University of Minnesota

B.S., Chemical Engineering, 1992, The University of Virginia, Charlottesville, VA.
M.S., Environmental Engineering, 1994, The University of Iowa, Iowa City, IA.
Ph.D., Environmental Engineering, 1997, The University of Iowa, Iowa City, IA.

Research
Research interests are in the areas of hazardous substance biodegradation, anaerobic biological processes, and the occurrence and fate of estrogenic compounds. Current research focuses on the enhanced transformation of chlorinated compounds in the presence of anaerobic organisms and the treatment of plant-based estrogens in industrial wastewater. Dr. Novak was the 2007 recipient of the Paul L. Busch Award (Water Environment Research Foundation), the 2013 Bill Boyle Educator of the Year Award (Central States Water Environment Association), and the 2011 Samuel Arnold Greeley Award (American Society of Civil Engineers).

Selected Publications (51 total)
McNamara, P. J., LaPara, T. M., Novak, P. J. 2013. The impacts of triclosan on methanogenic community structure, function, and antimicrobial resistance. *Environmental Science and Technology*, submitted.

Krzmarzick, M. J., Miller, H. R., Yan, T., Novak, P. J. 2013. Novel *Firmicutes* group implicated in the dechlorination of two chlorinated xanthones, analogues of natural organochlorines. *Applied and Environmental Microbiology*, in press.

Rearick, D. C., Fleischhacker, N. T., Kelly, M. M., Arnold, W. A., Novak, P. J., Schoenfuss, H. L. 2013. Phytoestrogens in the Environment: I. Occurrence and Exposure Effects on Fathead Minnows. *Environmental Toxicology and Chemistry*, in press.

Kelly, M. M., Fleischhacker, N. T., Rearick, D. C., Arnold, W. A., Schoenfuss, H. L., Novak, P. J. 2013. Phytoestrogens in the Environment: II. Microbiological Degradation of Phytoestrogens and the Response of Fathead Minnows to Degradate Exposure. *Environmental Toxicology and Chemistry*, in press.

Tan, D. T., Arnold, W. A., Novak, P. J. 2013. Impact of Organic Carbon on the Biodegradation of Estrone in Mixed Culture Systems. *Environmental Science and Technology*, in press.

Matt Simcik

Associate Professor, Environmental Health Sciences, School of Public Health, University of Minnesota

B.S., Chemistry, 1992, Michigan State University
M.S., Civil Engineering, 1994, University of Minnesota
Ph.D., Environmental Sciences, 1998, Rutgers, The State University of New Jersey

Research
Dr. Simcik is an expert in environmental chemistry. He will direct the analysis of perfluorinated compounds (PFCs) and co-contaminant analysis. He has been analyzing trace organic contaminants in various environmental media for 20 years, including PFCs for the past 10 years.

Selected Publications
Feng Xiao, Thomas R. Halbach, Matt F. Simcik, John S. Gulliver. Input characterization of perfluoroalkyl substances in wastewater treatment plants: Source discrimination by exploratory data analysis. Water Research 46(9), 3101-3109, 2012.

Feng Xiao, Matt F. Simcik, John S. Gulliver. Partitioning Characteristics of Perfluorooctane Sulfonate BetweenWater and Foods. Archives of Environmental Contamination and Toxicology 62(1), 42-48, 2012.

Feng Xiao, Matt F. Simcik, John S. Gulliver. Perfluoroalkyl acids in urban stormwater runoff: Influence of land use. Water Research 46(20), 6601-6608, 2012.

Peter C. Raynor, Andrea Barteková, J. Girard Griggs, Matt F. Simcik and John L. Adgate. Airborne Diazinon Concentrations During and After Outdoor Spray Application. Journal of Occupational and Environmental Hygiene 7(9):506-515 2010.

9. Dissemination and Use

The target audience for results from this research will be professionals in the area of wastewater treatment, landfill management, and industry. Specific targets will be environmental engineers and scientists in academia, industry, state agencies such as the MDA and MPCA, and environmental consultants. Results will be disseminated through scholarly publications in peer-reviewed journals such as *Environmental Science and Technology*. Results from the research project will also be presented at regional conferences such as the *Minnesota Water* conference. Results will be used to target what compounds are most problematic to microbial function, when, where, and how to best to treat industrial wastewater streams that contain high concentrations of phytoestrogens so that aquatic organisms are adequately protected.

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2014 Detailed Project Budget

Project Title: Protecting bacteria from contaminants to preserve water quality

IV. TOTAL ENRTF REQUEST BUDGET 3 years

BUDGET ITEM	AMOUNT
Personnel: Novak (PI, 8% time per year for three years, salary 75% of cost, fringe benefits 25% of cost). Project supervision, provide guidance on the experimental set-up and microbial culturing.	$ 53,000
Personnel: Simcik (PI, 10% time per year for three years, salary 75% of cost, fringe benefits 25% of cost). Project supervision, guidance on the analysis methods.	$ 35,500
Personnel: Graduate student (50% time per year for three years, 57% salary, 33% tuition, 10% fringe benefits). Conducting laboratory experiments, performing analysis, and imaging studies.	$ 131,500
Personnel: Undergraduate student (13 weeks (i.e., summer), full time per year for three years). Assisting with analysis and laboratory experiments.	$ 15,500
Equipment/Tools/Supplies: Laboratory supplies including, but not limited to: chemicals for experiments (PFCs and co-contaminants), bacterial cultures, oxygen probes, analysis needs such as standards, gas tanks, needles, septa, supplies for bacterial imaging (fluorescent antibodies, chemicals), consumables such as gloves and solvents ($8,500/yr). Additional funds budgeted for equipment repair and maintenance ($6,000), and imaging and image analysis ($10,000).	$ 43,500
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 279,000

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ Being Applied to Project During Project Period: none	$ -	
Other State $ Being Applied to Project During Project Period: none	$ -	
In-kind Services During Project Period: Novak and Simcik will provide unpaid time to the project (including 1% cost-share each). Because the project is overhead-free, laboratory space, electricity, and other overhead costs are provided in kind. The University of Minnesota overhead rate is 52%.	$ 116,000	
Remaining $ from Current ENRTF Appropriation (if applicable): no prior projects directly related to proposed project	$ -	
Funding History: Preliminary research in this area has been supported by a seed grant from the University of Minnesota (The Office of the Vice President for Research at the University of	$ 33,366	