Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2014 Work Plan

Date of Report: January 14, 2014

Date of Next Status Update Report: January 31, 2015

Date of Work Plan Approval:

Project Completion Date: June 30, 2017

Does this submission include an amendment request? _No_

PROJECT TITLE: Methods to Protect Beneficial Bacteria from Contaminants to Preserve Water Quality

Project Manager: Paige J. Novak

Organization: University of Minnesota

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Location: Hennepin, Statewide impact

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$279,000**
	Amount Spent:	**$0**
	Balance:	**$279,000**

Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 03b

Appropriation Language:
$279,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to research how and why bacteria that provide ecological functions humans depend on for water quality are affected by exposure to certain man-made perfluorinated chemicals entering the wastewater treatment system in order to identify methods that can be implemented to protect those bacterial functions from being degraded. This appropriation is available until June 30, 2017, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Methods to Protect Beneficial Bacteria from Contaminants to Preserve Water Quality

II. PROJECT STATEMENT:

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 (PFCs); based on other research, it is hypothesized herein that PFCs 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 PFCs in the presence and absence of other co-contaminants (model contaminants of different structures). 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.

III. PROJECT STATUS UPDATES:

Project Status as of January 31, 2015:

Project Status as of July 31, 2015:

Project Status as of January 31, 2016:

Project Status as of July 31, 2016:

Project Status as of January 31, 2017:

Overall Project Outcomes and Results:

IV. PROJECT ACTIVITIES AND OUTCOMES:

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

Description: Five nitrifying and five methanogenic source cultures will be established. They will be seeded with either activated sludge and fed ammonium and trace nutrients in well-buffered medium or seeded with sludge from a local full-scale anaerobic digester and fed a blend of organic acids, alcohols and glucose (0.18 g COD/L-day) in minimal media. One set of experiments will be performed with strongly flocculating nitrifiers and methanogenic communities cultured as granules so that the physical protection of the biological floc/granule

itself can be assessed. Of the five source reactors, one will be fed perfluorooctane sulfonate (PFOS), one perfluorooctane sulfonamide (FOSA), and one perfluorbutane sulfonate (PFBS), each at approximately 400 µg/kg, 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 retention times before experiments are performed.

Aliquots of the chronically PFC-exposed source cultures will be added to triplicate 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. 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. As with the nitrifiers, replicate experiments will be performed for dispersed and granulated methanogenic cultures. Samples will also be taken to determine whether the melamine and PFCs are outside of, loosely bound to, or inside the cells.

Summary Budget Information for Activity 1:

ENRTF Budget: $ 186,000
Amount Spent: $ 0
Balance: $ 186,000

Activity Completion Date: January 31, 2017

Outcome	Completion Date	Budget
1. In cultures of nitrogen-cycling bacteria, determine which perfluorinated chemicals are most harmful, alone and in mixtures, and at what concentrations	06/30/2015	$46,500
2. In cultures of carbon-cycling bacteria, determine which perfluorinated chemicals are most harmful, alone and in mixtures, and at what concentrations	06/30/2016	$46,500
3. Determine whether certain common bacterial traits (different types and thicknesses of protective “coats”) make bacteria more resistant to this type of toxicity	01/31/2017	$93,000

Activity Status as of January 31, 2015:

Activity Status as of July 31, 2015:

Activity Status as of January 31, 2016:

Activity Status as of July 31, 2016:

Activity Status as of January 31, 2017:

Final Report Summary:

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

Description: We will also investigate the effect of size and hydrophobicity on transport in the presence of PFCs. This will be done by repeating the experiments described for activity 1 for two additional compounds that allow us to investigate a range of similarly structured co-contaminants with different hydrophobicities and sizes: atrazine and hexazinone. Additional experiments will be performed with a diffusion chamber 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, 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.

Summary Budget Information for Activity 2:

ENRTF Budget: $ 93,000
Amount Spent: $ 0
Balance: $ 93,000

Activity Completion Date: March 31, 2017

Outcome	Completion Date	Budget
1. In cultures of nitrogen-cycling bacteria, determine how co-contaminant chemistry affects bacterial function when present in mixtures containing perfluorinated chemicals	03/31/2016	$46,500
2. In cultures of carbon-cycling bacteria, determine how co-contaminant chemistry affects bacterial function when present in mixtures containing perfluorinated chemicals	03/31/2017	$46,500

Activity Status as of January 31, 2015:

Activity Status as of July 31, 2015:

Activity Status as of January 31, 2016:

Activity Status as of July 31, 2016:

Activity Status as of January 31, 2017:

Final Report Summary:

V. DISSEMINATION:

Description: 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 culture bacteria to protect them.

Status as of January 31, 2015:

Status as of July 31, 2015:

Status as of January 31, 2016:

Status as of July 31, 2016:

Status as of January 31, 2017:

Final Report Summary:

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	$ Amount	Explanation
Personnel:	$ 235,500	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.
Equipment/Tools/Supplies:	$43,500	Funds ($12,500, $15,500, and $15,500) are requested for materials, supplies, consumables, analytical costs and repair/upkeep associated with the LC-MS. Required materials include, but are not limited to: pipette tips, glassware, solid phase extraction cartridges for extractions, chemicals for standards and experiments, analytical consumables, analytical fees, solvents, reagents, gloves, digital data storage media, and laboratory notebooks.
TOTAL ENRTF BUDGET:	**$**	The total proposed project amount is $279,000. No indirect costs for the University of Minnesota are included in the budget.

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than $5,000: N/A

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 2.79

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: N/A

Other Funds: N/A

VII. PROJECT STRATEGY:

A. Project Partners: The project team consists of the Principal Investigator (PI) Paige Novak (University of Minnesota) and co-PI Dr. Matt Simcik (UMN). Novak is an expert on microbial systems and Simcik is an expert on the analysis of trace chemicals, including perfluorinated chemicals. The PI and co-PI will work together on all aspects of the research.

B. Project Impact and Long-term Strategy: The proposed research fits into a larger research agenda centered at the University of Minnesota that is focused on the problem of Contaminants of Emerging Concern. When taken together, this research and other complementary current and prior research in this area will provide a more complete picture of how to safeguard our environment through engineering different types of treatment systems (systems that encourage the development of protective bacterial "coats" for example). In particular, an emphasis on understanding threats to water quality in Minnesota can help us to then engineer better treatment methods to promote cleaner water and therefore healthier fish and aquatic ecosystems.

C. Spending History: N/A

VIII. ACQUISITION/RESTORATION LIST: N/A

IX. VISUAL ELEMENT or MAP(S): See attached graphic.

X. ACQUISITION/RESTORATION REQUIREMENTS WORKSHEET: N/A

XI. RESEARCH ADDENDUM: See attached Research Addendum

XII. REPORTING REQUIREMENTS:
Periodic work plan status update reports will be submitted no later than January 31, 2015, July 31, 2015, January 31, 2016, July 31, 2016, and January 31, 2017. A final report and associated products will be submitted between June 30 and August 15, 2017.

Environment and Natural Resources Trust Fund

M.L. 2014 Project Budget

Project Title: Methods to Protect Beneficial Bacteria from Contaminants to Preserve Water Quality

Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 03b

Project Manager: Paige J. Novak

Organization: University of Minnesota

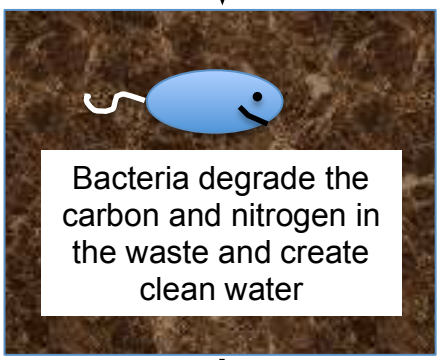
M.L. 2014 ENRTF Appropriation: **$**279,000

Project Length and Completion Date: 3 Years, June 30, 2017

Date of Report: January 14, 2014

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	***Understand how and why perfluorinated chemicals alter bacterial function (nitrogen cycling and carbon decomposition) alone or in mixtures with a co-contaminant***			***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***				
Personnel (Wages and Benefits)	$157,000	$0	$157,000	$78,500	$0	$78,500	$235,500	$235,500
Paige Novak, PI ($39,800 salary, $13,400 fringe, 33.6% fringe rate; total for 3 years; 8% effort). Project supervision, provide guidance on the experimental set-up and microbial culturing)								
Matt Simcik, Co-PI ($26,500 salary, $8,900 fringe, 33.6% fringe rate; total for 3 years; 10% effort). Project supervision, guidance on the analysis methods),								
One Graduate Research Assistant ($75,500 salary, $55,800 fringe (includes healthcare and tuition); total for 3 years; will conduct laboratory experiments, analyze results)								
One Undergraduate Research Assistant ($15,600 salary; 13 weeks (i.e., summer), full time per year for three years; will assist with analysis and laboratory experiments)								
Equipment/Tools/Supplies								
Laboratory supplies including, but not limited to: chemicals for experiments (PFCs and co-contaminants, media constituents), oxygen probes, analysis needs such as standards, gas tanks, needles, septa, consumables such as gloves and solvents.	$26,000		$26,000	$9,000	$0	$9,000	$35,000	$35,000
Diffusion cell construction	$0	$0	$0	$4,000	$0	$4,000	$4,000	$4,000
Equipment repair and maintenance	$3,000	$0	$3,000	$1,500	$0	$1,500	$4,500	$4,500
COLUMN TOTAL	**$186,000**	**$0**	**$186,000**	**$93,000**	**$0**	**$93,000**	**$279,000**	**$279,000**

Waste (containing nitrogen and carbon)



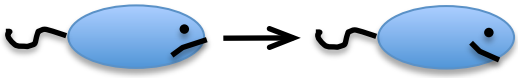
Clean water

The benefit of bacteria

Waste (containing nitrogen and carbon), and manmade chemical mixtures

Dirty water

The problem



Determine why bacteria are harmed by chemical mixtures so that it can be prevented

Our proposed project