Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2016 Work Plan

Date of Report: February 8, 2017

Date of Next Status Update Report: July 1, 2017

Date of Work Plan Approval: June 7, 2016

Project Completion Date: June 30, 2019

Does this submission include an amendment request? No

PROJECT TITLE: Wastewater Treatment Process Improvements

Project Manager: Timothy M. LaPara

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Location: Statewide

Total ENRTF Project Budget:	**ENRTF Appropriation:**	**$398,000**
	Amount Spent:	**$53,869**
	Balance:	**$344,131**

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 04k

Appropriation Language:

$398,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to characterize and quantify the nutrient-removing microorganisms used for municipal wastewater treatment, in order to improve the process used to reduce total nitrogen discharge. This appropriation is available until June 30, 2019, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Wastewater treatment process control improvements

II. PROJECT STATEMENT:

In the near future (5-10 years), new regulations are expected on Minnesota's municipal wastewater discharges for total nitrogen (ammonia, nitrite, and nitrate), which is needed to prevent the eutrophication of the Gulf of Mexico. The goal of this project is to understand the composition of the microbial communities used for municipal wastewater treatment and to provide baseline information of the quantities of nutrient-removing microorganisms used for wastewater treatment in the State of Minnesota. The benefit of this research will be a useful set of tools that can be used to better control wastewater treatment operations.

All wastewater treatment facilities in Minnesota are currently regulated with respect to the release of biodegradable organic compounds (known as "BOD" – biochemical oxygen demand). These regulations ensure that the harmful impacts of wastewater are avoided, particularly the consumption of oxygen in the receiving water body (oxygen is needed for fish and other aquatic fauna and flora to thrive). Presently, many wastewater treatment facilities are also seasonally regulated for ammonia (due to fish toxicity) and continuously regulated for phosphorus, which contributes to the eutrophication (the excessive growth of algae) in lakes.

The most cost-effective way to treat municipal wastewater is to utilize microorganisms to metabolize pollutants of concern. Wastewater treatment facilities use microorganisms to remove the nutrients (BOD, phosphorus, nitrogen) of concern from the wastewater. From an engineering and operational perspective, it is relatively straight-forward to achieve either BOD and phosphorus removal or BOD and total nitrogen removal, but it is **much more difficult problem** to achieve BOD, total nitrogen, and phosphorus removal because all three processes occur only over a very narrow range of operating conditions. Future wastewater treatment operations, therefore, will likely require better process control; the research performed in this project will delineate the tools needed to provide this better process control.

Surprisingly, wastewater treatment bioreactors are currently operated with very little knowledge of the microorganisms that provide the treatment. The scientific reason for the lack of monitoring has been the inability of microbiologists to culture organisms from environmental samples. Over the past 5-10 years, however, microbiologists have developed next-generation DNA sequencing technology to generate 50,000+ sequences per sample to address the question of "*who is there*?" In addition, quantitative polymerase chain reaction (qPCR) can be used to determine the precise quantities of specific organisms, such as those responsible for phosphorus and nitrogen removal, allowing us to address "*how many of them are there*?"

Of particular importance, qPCR techniques are now relatively affordable (less than $30,000 for all of the needed instrumentation – on par with other lab techniques) and practical, requiring no special skills beyond those possessed by typical laboratory technicians. The final activity will be to disseminate the research results to Minnesota's wastewater treatment plant managers and operators (and providing training, if requested), with the goal of using these new technologies for better wastewater treatment process control.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of January 1, 2017:

The project has thus far focused on collecting wastewater bioreactor samples from wastewater treatment facilities throughout Minnesota. Genomic DNA has been extracted and purified from the majority of these samples and the quantities of nutrient-removing organisms have been quantified. Activated sludge bioreactors have thus far contained 10^9 bacteria per milliliter, of which 10^8 are capable of denitrification (i.e., contain a *nosZ* gene) and 10^6 are bacteria capable of ammonia-oxidization (i.e., contain a bacterial *amoA* gene). In addition,

about 10^6 ammonia-oxidizing archaea (i.e., contain an archaeal *amoA* gene) were detected at one facility, but were not detectable at other facilities.

Project Status as of July 1, 2017:

Project Status as of January 1, 2018:

Project Status as of July 1, 2018:

Project Status as of January 1, 2019:

Overall Project Outcomes and Results:

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Characterize Minnesota's wastewater treatment microbiome.

Description:

The goal of this activity is to provide a general characterization of microorganisms in Minnesota's biological wastewater treatment facilities. This information will be critically useful to corroborate the organism-specific quantifications performed in Activity 2. This work is needed because the organism-specific quantifications are based on well-described model organisms, but these organisms might not be the pertinent nutrient-removing organisms in Minnesota's wastewater treatment facilities. This Activity will also allow us to compare the bacterial community composition of wastewater bioreactors throughout the State, determining if there are correlations between bacterial community composition and treatment facility performance, size, design, and other factors.

Samples will be collected from the bioreactors treating wastewater and wastewater sludges at selected wastewater treatment facilities on a weekly basis. We will specifically collect samples from the wastewater treatment facilities both in St. Cloud and in Brainerd, because these two facilities were recently upgraded to perform simultaneous removal of BOD, nitrogen, and phosphorus. We will also collect samples from wastewater treatment facilities in Little Falls, Duluth, St. Peter, and Mankato; these facilities do not currently perform simultaneous BOD, nitrogen, and phosphorus removal. We also intend to get samples from as many as 50 wastewater treatment facilities throughout the State. Our intention is to collect samples weekly from each of their bioreactors (many facilities have a single bioreactor, others have multiple bioreactors). We anticipate collecting ~5,000 samples (50 facilities $\times$ 1 sample per week per bioreactor $\times$ 2 bioreactors per facility $\times$ 100 weeks = 5000) for this Activity.

Assessment of the bacterial community composition will be achieved by using next-generation DNA sequencing will be performed using an Illumina MiSeq analyzer at the University of Minnesota Genomics Center. We will then use software that is available at the Minnesota Supercomputing Institute to statistically analyze the data and correlate our data to process performance (which is routinely collected from each facility). The goal is to obtain 50,000-100,000 DNA sequences per sample, which should allow us to characterize the bacterial community composition of these bioreactors in considerable detail.

Summary Budget Information for Activity 1:

ENRTF Budget: $ 194,000
Amount Spent: $ 43,095
Balance: $ 150,905

Outcome	Completion Date

1. *Sample collection and Genomic DNA extractions (5,000 samples)*	December 31, 2017
2. *Next-Generation DNA sequencing (20 Illumina MiSeq runs)*	December 31, 2018
3. *Data Analysis at the Minnesota Supercomputing Institute*	April 30, 2019

Activity Status as of January 1, 2017:

To date, relatively little progress has been made on this Activity. The accomplishments, so far, have included sample collection and DNA extraction/purification from numerous wastewater treatment facilities. These DNA samples have been stored and will be used for Illumina sequencing in subsequent project periods.

Activity Status as of July 1, 2017:

Activity Status as of January 1, 2018:

Activity Status as of July 1, 2018:

Activity Status as of January 1, 2019:

Final Report Summary:

ACTIVITY 2: Quantify nutrient-removing microbial populations in wastewater bioreactors.

Description:

In this activity, we will quantify the presence of specific microbial populations that are known to perform nitrogen and phosphorus removal. These organisms will include all bacteria, ammonia-oxidizing bacteria, ammonia-oxidizing Archaea, nitrite-oxidizing bacteria, three different types of denitrifying bacteria, and phosphate-accumulating organisms. That is, we will quantify the presence of as many as 8 different 'types' of bacteria in each sample. These quantitative values will allow us to directly compare the 'treatment capacity' of each biological wastewater treatment process (i.e., the ability to treat wastewater is usually proportional to the quantity of organisms of each type). This research will also allow us to compare how different treatment processes are affected by seasonal variation in temperature, process design, etc.

A very similar process will be used for Activity 2 as for Activity 1. We will use the same samples as used in Activity 1. We will perform these assays using the 384-well real time PCR system at the University of Minnesota Genomics Center (approximately 40,000 quantifications). Data will then be correlated to the process performance data provided to us from the treatment facilities.

Summary Budget Information for Activity 2:

ENRTF Budget: $ 194,000
Amount Spent: $ 10,774
Balance: $ 183,226

Outcome	Completion Date
1. *Sample collection and Genomic DNA extractions*	December 31, 2017
2. *qPCR targeting specific nutrient-removing microorganisms*	June 30, 2018
3. *Data Analysis at the Minnesota Supercomputing Institute*	April 30, 2019

Activity Status as of January 1, 2017:
As with Activity 1, the primary accomplishments Activity 2 have included sample collection and DNA extraction/purification from numerous wastewater treatment facilities. These DNA samples have been stored

and will be used for Illumina sequencing in subsequent project periods. Numerous samples have been used to quantify total bacteria (16S rRNA genes: 10^9/mL), ammonia-oxidizing bacteria (*amoA* genes: 10^6/mL), ammonia-oxidizing archaea (*amoA* genes: < 10^6/mL), denitrifying bacteria (*nosZ*: 10^7/mL; *nirS*: 10^7/mL;*nirK* genes: 10^5/mL), and polyphosphate accumulating organisms (16S rRNA genes specific to these organisms: 10^6/mL). In general, the quantities of these populations were stable over time.

Activity Status as of July 1, 2017:

Activity Status as of January 1, 2018:

Activity Status as of July 1, 2018:

Activity Status as of January 1, 2019:

Final Report Summary:

ACTIVITY 3: Disseminate our results to Minnesota's wastewater treatment facilities.
Description:

The first two activities will demonstrate the value of tracking bacterial populations in Minnesota's wastewater treatment facilities. The final activity will be to disseminate these results at local conferences and then to work with Minnesota's wastewater treatment facilities to perform these assays in-house. The techniques used in this project are relatively new, but they are ready to be used at wastewater treatment laboratories throughout the State for process monitoring and control. The cost of these assays is similar to those used for other laboratory assays used by wastewater treatment operators and managers. The skills required are similar to those needed by laboratory technicians.

Ideally, we will convince some facilities to purchase the equipment to perform these assays; in this case, we would provide training at no cost to the participating utility.

Summary Budget Information for Activity 3:

ENRTF Budget: $ 10,000
Amount Spent: $ 0
Balance: $ 10,000

Outcome	Completion Date
1. *Presentations at local wastewater treatment conferences*	December 31, 2018
2. *Train laboratory technicians at wastewater treatment facilities to use qPCR*	June 30, 2019

Activity Status as of January 1, 2017:

There has been no progress with this activity at this time.

Activity Status as of July 1, 2017:

Activity Status as of January 1, 2018:

Activity Status as of July 1, 2018:

Activity Status as of January 1, 2019:

Final Report Summary:

V. DISSEMINATION:

Description:

Findings will be disseminated and archived via reports to LCCMR, peer-reviewed publications, and presentations at conferences. We will also, when appropriate, disseminate results via press releases to the media. The audience is not only the scientific community, but also the public, policymakers, and practitioners. The work will also be of interest to the wastewater treatment community and we will seek avenues to share the results with this community (such as the Minnesota Wastewater Operators Conference). We would also like to work one-on-one with individual wastewater utilities to start performing these assays; our hope is that we can get actual wastewater treatment plants performing the analyses that we explore herein.

Status as of January 1, 2017:

Status as of July 1, 2017:

Status as of January 1, 2018:

Status as of July 1, 2018:

Status as of January 1, 2019:

Status as of July 1, 2019:

Final Report Summary:

VI. PROJECT BUDGET SUMMARY:
A. ENRTF Budget Overview:

Budget Category	$ Amount	Overview Explanation
Personnel:	$ 311,524	For Drs. LaPara ($77,844) and Behrens ($44,292) for directing the project, for a year of graduate student support at the University of Minnesota ($47,451), and for two years of post-doctoral research associate support ($114,704) at the University of Minnesota. Dr. LaPara will also effectively serve as a "post-doctoral" researcher during the first year of the project while he is on sabbatical from the University of Minnesota.
Equipment/Tools/Supplies:	$81,976	General lab supplies ($5,000), reagents for qPCR ($17,500), use of UMGC's facilities for qPCR and Illumina sequencing ($36,976), DNA extraction kits ($12,500), PCR purification kits ($10,000)
Travel Expenses in MN:	$4,500	Travel to wastewater treatment facilities in Minnesota to collect bioreactor samples; travel to MN wastewater conferences to present research; travel to wastewater treatment facilities to train lab personnel
TOTAL ENRTF BUDGET:	**$398,000**	

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: 1.75

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

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
National Science Foundation fellowship to Julie Johnston (informational only; not intended to represent committed cost share)	$121,225	$	NSF fellowship for graduate student working on this project
State			
	$197,064	$	In-kind contribution; indirect costs not charged to this project
TOTAL OTHER FUNDS:	**$318,289**	**$**	

VII. PROJECT STRATEGY:
A. Project Partners: N/A

B. Project Impact and Long-term Strategy:

The goal of the project is to get Minnesota's wastewater treatment plants to monitor their biomass for specific bacterial populations by qPCR. This will be achieved by demonstrating and verifying the technology and then presenting the results (including the costs for the analyses) to Minnesota's wastewater treatment facilities. If needed (or requested), we will even visit treatment facilities to train their personnel to perform the assays.

C. Funding History: N/A

VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS: N/A

IX. VISUAL COMPONENT or MAP(S): See attached visual

X. RESEARCH ADDENDUM: See attached

XI. REPORTING REQUIREMENTS:
Periodic work plan status update reports will be submitted no later than January 1, 2017, July 1, 2017, January 1, 2018, July 1, 2018, and January 1, 2019. A final report and associated products will be submitted between June 30 and August 15, 2019.