Environment and Natural Resources Trust Fund

M.L. 2024 Approved Work Plan

General Information

ID Number: 2024-073

Staff Lead: Lisa Bigaouette

Date this document submitted to LCCMR: June 10, 2024

Project Title: Enhancing Wastewater Treatment through Genetic Sequencing

Project Budget: $553,000

Project Manager Information

Name: Timothy LaPara

Organization: U of MN - College of Science and Engineering

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Project Reporting

Date Work Plan Approved by LCCMR: June 20, 2024

Reporting Schedule: June 1 / December 1 of each year.

Project Completion: June 30, 2027

Final Report Due Date: August 14, 2027

Legal Information

Legal Citation: M.L. 2024, Chp. 83, Sec. 2, Subd. 04d

Appropriation Language: $553,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to generate genome sequences for and assess the ability of bacteria growing in wastewater treatment bioreactors to improve phosphorus and nitrogen removal from wastewater in Minnesota and to produce novel pharmaceutical compounds. This appropriation is subject to Minnesota Statutes, section 116P.10.

Appropriation End Date: June 30, 2027

Narrative

Project Summary: We will generate genome sequences of bacteria growing in wastewater treatment bioreactors, allowing us to improve phosphorus and nitrogen removal from wastewater in Minnesota and to discover novel pharmaceutical compounds.

Describe the opportunity or problem your proposal seeks to address. Include any relevant background information.

Municipal wastewater treatment is essential for protecting the quality of Minnesota's lakes and rivers. Historically, municipal wastewater treatment focused on the removal of biodegradable organic carbon (measured as "biochemical oxygen demand"), but modern wastewater treatment also focuses on removing phosphorus and nitrogen because of their ability to stimulate the growth of nuisance photosynthetic organisms (plants, algae, and cyanobacteria) in inland and coastal waters, respectively. Many municipal wastewater treatment facilities are already intentionally removing phosphorus and the Minnesota Pollution Control Agency is considering opportunities to reduce Minnesota's contribution of nitrogenous pollution to the hypoxic zone in the Gulf of Mexico from both point and non-point sources.

While there are numerous bioreactors designs that can successfully remove phosphorus and nitrogen from municipal wastewater, we have a relatively poor understanding of the microorganisms responsible for removing these contaminants. For example, our knowledge of ammonia-oxidizing bacteria (necessary for nitrogen removal) is based on a few model organisms that were isolated many years ago; even worse, the organism(s) necessary for phosphorus removal have never been isolated. Because of this limited knowledge, phosphorus and nitrogen removal from municipal wastewater can be unreliable, particularly in the cold climate that we enjoy in Minnesota.

What is your proposed solution to the problem or opportunity discussed above? Introduce us to the work you are seeking funding to do. You will be asked to expand on this proposed solution in Activities & Milestones.

The ideas for this proposed research come from two past/ongoing projects funded by LCCMR/ENRTF (M.L. 2016, Chp. 186, Sec. 2, Subd. 04k and M.L. 2021, Chp. 6, Art. 6, Sec. 2, Subd. 04j).

We propose to use proximity-ligation DNA sequencing to produce genome sequences of numerous bacteria from 30 municipal wastewater treatment bioreactor samples. These samples will be collected from 10 wastewater treatment facilities that perform enhanced biological phosphorus removal or that support novel/important nitrogen-removing microorganisms. We expect to obtain dozens of bacterial genome sequences from each of these samples; these genome sequences will then inform us on the optimal wastewater treatment conditions that are required to support these critically important organisms.

In addition, our prior work suggests that many of the organisms growing in wastewater treatment bioreactors can produce novel secondary metabolites that could have significant value to the pharmaceutical industry. We therefore also propose to "bioprospect" these genomic sequences for the potential to produce novel pharmaceuticals and then to use these same genomic sequences to guide the culturing of these organisms in the laboratory. These cultured organisms could then be used to produce these novel molecules on an industrially relevant scale.

What are the specific project outcomes as they relate to the public purpose of protection, conservation, preservation, and enhancement of the state's natural resources?

This project will generate genome sequences from bacteria growing in municipal wastewater treatment bioreactors. This data will help us identify the optimal bioreactor conditions for removing phosphorus and nitrogen from wastewater, which are current (phosphorus) and future (nitrogen) priorities for the State of Minnesota. In addition, we will also "bioprospect" these genome sequences for the ability to produce biomolecules of potential value to the pharmaceutical industry. Simultaneously, these genome sequences will suggest methods to cultivate these organisms in the laboratory.

Project Location

What is the best scale for describing where your work will take place?

Statewide

What is the best scale to describe the area impacted by your work?

Statewide

When will the work impact occur?

In the Future

Activities and Milestones

Activity 1: Collect samples from municipal wastewater treatment bioreactors for proximity-ligation DNA sequence analysis

Activity Budget: $80,000

Activity Description:
Bioreactor samples will be collected from 10 different wastewater treatment facilities within the State of Minnesota on two separate occasions (i.e., a “summer” sample and a “winter” sample). These wastewater treatment facilities will be selected based on prior research (M.L. 2016, Chp. 186, Sec. 2, Subd. 04k) in which microbiome profiles identified the prominent bacteria involved in nitrogen removal and phosphorus removal. In addition, monthly samples will be collected from a single wastewater treatment bioreactor so that community dynamics can be tracked for an entire calendar year. These samples will be sent to Phase Genomics, who will perform their proprietary proximity-ligation DNA sequencing and subsequent analysis. That is, Phase Genomics will sequence DNA from our samples, and in return, they will send us 20-50 genome sequences from each sample.

Activity Milestones:

Description	Approximate Completion Date
Collect "summer" wastewater samples from 10 wastewater treatment facilities.	September 30, 2024
Collect "winter" samples from 10 different wastewater treatment facilities	March 31, 2025
Collect "monthly" samples from a single wastewater treatment facility	September 30, 2025
Send samples to Phase Genomics for metagenomic sequence analysis	September 30, 2025
Obtain metagenomic DNA sequence data from Phase Genomics	February 28, 2026

Activity 2: Analyze genomes sequences for the genetic ability for phosphorus and nitrogen removal

Activity Budget: $236,919

Activity Description:
The first step of this activity will be to compare the genome sizes and sequences of specific populations of bacteria that are responsible for nitrogen (Nitrosomonas spp., Nitrosospira spp., Nitrotoga spp., and Nitrospira spp.) and for phosphorus removal (Tetrasphaera-like and Rhodocyclus-like); these genome sequences will also be compared to previously sequenced genomes that are already published in public databases. In the second step of this activity, we analyze the metabolic pathways that are found in these genome sequences, which should suggest the ideal bioreactor conditions (e.g., dissolved oxygen concentrations, pH, temperature, etc.) for each of these organisms to proliferate during municipal wastewater treatment. Finally, we will analyze the time-series data from a single wastewater treatment facility to determine how these organisms wax and wane in population density throughout the calendar year.

Activity Milestones:

Description	Approximate Completion Date
Perform comparative genomics between wastewater genomes and previously described bacterial genomes	August 31, 2026
Analyze metabolic pathways within bacterial genomes to determine optimal conditions for wastewater treatment	June 30, 2027

Activity 3: Bioprospecting for genes associated with novel pharmaceuticals

Activity Budget: $93,041

Activity Description:
Microorganisms live in complex and competitive communities and they naturally produce molecules to inhibit the growth of their neighbors. These are some of the antibiotics and antifungals we rely on in both medicine and agriculture, however, these therapeutic molecules are losing their effectiveness as microorganisms are becoming resistant to their effects. Antibiotic and antifungal discovery has long depended on bioprospecting, with much success derived from soil microorganisms. Wastewater treatment bioreactors undoubtedly harbor numerous bioactive compounds that await discovery. Our previous analysis of two representative wastewater samples has yielded multiple potential molecules. In this activity, therefore, we will use the genome sequence data to identify novel pharmaceuticals. As a result of the work done in Activity 1, we will have DNA sequence data equivalent thousands of bacterial genomes, many of which will be novel. We will use the antiSMASH software package, which has the capability of identifying 71 different types of DNA sequence signatures, each associated with a different type of potential pharmaceutical molecule. We will catalog the genes according to the novelty of the predicted molecules they produce to prioritize those that are most likely to yield potentially novel pharmaceuticals.

Activity Milestones:

Description	Approximate Completion Date
Identify potential biosynthetic gene clusters	July 31, 2026

Activity 4: Growth and selection for bacteria with antibiotic and antifungal activity

Activity Budget: $93,040

Activity Description:
While computer analysis of genetic information can make predictions about the potential for the synthesis of novel pharmaceuticals, a critical next step is to isolate the bioactive compounds from the actual organisms. In this activity, we will grow organisms from the wastewater bioreactor samples from which sequence data was obtained. Culturing conditions that select for the organisms of interest will be chosen, varying nutrient levels and environmental conditions such as pH, temperature, and oxygen availability. Once bacteria are isolated, genetic markers from organisms that grow will be compared to those observed through antiSMASH analysis of genomic sequencing data (Activity 3) to focus our study on those organisms predicted to produce novel compounds. In the laboratory, these bacterial candidates will be tested for the production of antibiotic or antifungal activity using competition assays. In the simplest assay, newly isolated bacteria will be grown alongside tester strains that mimic some of the most recalcitrant bacterial superbugs. Bacteria that are found to inhibit their competition will be further studied by chemically isolating and identifying the inhibitor molecule to determine if it is indeed a novel potential pharmaceutical.

Activity Milestones:

Description	Approximate Completion Date
Grow bacteria isolated from bioreactors using culture conditions informed by genomic data	December 31, 2026
Screen isolated bacteria for antibiotic and antifungal activity	June 30, 2027

Activity 5: Dissemination of research results via research publications, presentations at conferences, and communication with personnel at the Minnesota Pollution Control Agency

Activity Budget: $50,000

Activity Description:
We will author manuscripts for peer-reviewed publication and make formal presentations at conferences within the

State of Minnesota. The potential conferences will be the Conference on the Environment, the Minnesota Wastewater Treatment Operators Conference, and/or the Minnesota Water Resources Conference. We will also communicate with personnel at the Minnesota Pollution Control Agency throughout this project.

Activity Milestones:

Description	Approximate Completion Date
Presentation of research results at in-state conferences.	June 30, 2027
Publication of peer-reviewed manuscripts	June 30, 2027
On-going communication with personnel from the Minnesota Pollution Control Agency	June 30, 2027

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Justin Donato	University of St. Thomas	Co-Investigator/Project Manager	Yes
Joanna Klein	University of St. Thomas	Co-Investigator/Project Manager	Yes

Dissemination

Describe your plans for dissemination, presentation, documentation, or sharing of data, results, samples, physical collections, and other products and how they will follow ENRTF Acknowledgement Requirements and Guidelines.
We will directly share our results with all of the participating utilities and the Minnesota Pollution Control Agency. We will also present our results at in-state conferences, such as the Conference on the Environment, the Minnesota Wastewater Operators Conference, and/or the Minnesota Water Resources Conference. Finally, we will publish our results in the peer-reviewed literature; we will attempt to publish our research in an open-access format to allow LCCMR staff to help further promote our research.

We will properly acknowledge financial support from the Minnesota Environment and Natural Resources Trust Fund with all project print and electronic media, publications, signage, and other communications. This will be achieved through the use of the trust fund logo and/or the appropriate attribution language. These attributions will be made by all project personnel, whether they work at the University of Minnesota or the University of St. Thomas (sub award recipient). All attributions will meet or exceed the ENTRF Acknowledgment Guidelines.

Long-Term Implementation and Funding

Describe how the results will be implemented and how any ongoing effort will be funded. If not already addressed as part of the project, how will findings, results, and products developed be implemented after project completion? If additional work is needed, how will this work be funded?
This project should result in several high visibility research publications that will attract the attention of other scientists and engineers. This will, in turn, make receiving funding for additional research much easier from federal sources such as the Environmental Protection Agency, which is keenly interested in improving nutrient removal from municipal wastewater. The bioprospecting portion of this research would lead to array of new research to better understand the activity of and production of any discovered biomolecules.

Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Evaluating Coronavirus And Other Microbiological Contamination Of Drinking Water Sources From Wastewater	-	-
Evaluate Emerging Pathogens in Lakes, Rivers, and Tap Water to Keep Drinking Water Safe	M.L. 2018, Chp. 214, Art. 4, Sec. 2, Subd. 04f	$325,000
Mapping Antibiotic Resistance in Minnesota to Help Protect Environmental, Animal, and Human Health	M.L. 2018, Chp. 214, Art. 4, Sec. 2, Subd. 04h	$750,000
Improving Nitrogen Removal in Greater Minnesota Wastewater Treatment Ponds	M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04e	$325,000
Monitoring Emerging Viruses in Minnesota's Urban Water Cycles	M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 04c	$416,000

Evaluating Coronavirus And Other Microbiological Contamination Of Drinking Water Sources From Wastewater	M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 04g	$594,000
Antibiotic Resistance And Wastewater Treatment: Problems And Solutions	M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 04j	$432,000
High Temperature Anaerobic Digestion of Sewage Sludge	M.L. 2022, , Chp. 94, Art. , Sec. 2, Subd. 04b	$208,000

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount
Personnel								
Timothy LaPara		Project Manager			26.9%	0.24		$61,504
Graduate Student		Researcher			41%	1.5		$200,634
Laboratory Technician		Researcher			24%	0.25		$23,905
							Sub Total	**$286,043**
Contracts and Services								
University of St. Thomas	Sub award	Collaborate throughout the entire project and lead the bioprospecting work (Activity 3) and bacterial isolation (Activity 4). Personnel ($156,079; co-managers and undergrads; 7.4% fringe), equipment/freezer ($15,000), expendable supplies ($5,000; Petri dishes, growth media, etc), and DNA sequencing services from Phase Genomics ($65,000).		X		0.51		$251,081
TBD	Professional or Technical Service Contract	We envision needing to perform a limited amount of DNA sequencing to validate and improve our results. Some of the sequencing technology we will likely need (Sanger sequencing) is no longer performed by the University of Minnesota Genomics Center.				-		$5,000
							Sub Total	**$256,081**
Equipment, Tools, and Supplies								
	Tools and Supplies	General laboratory supplies	We will need to collect and process 30 wastewater samples. We will also need to perform miscellaneous laboratory analyses to support the primary data that will be generated.					$3,376
							Sub Total	**$3,376**
Capital Expenditures								

							Sub Total	**-**
Acquisitions and Stewardship								
							Sub Total	**-**
Travel In Minnesota								
	Miles/ Meals/ Lodging	30 trips to wastewater treatment facilities	Collect samples					$3,000
	Conference Registration Miles/ Meals/ Lodging	1 conference	Attend a conference within the State of Minnesota to disseminate research results	X				$1,500
							Sub Total	**$4,500**
Travel Outside Minnesota								
							Sub Total	**-**
Printing and Publication								
	Publication	Open access fees	This will allow us to retain the copyright of all publications; this will help us disseminate our results widely					$3,000
							Sub Total	**$3,000**
Other Expenses								
							Sub Total	**-**
							Grand Total	**$553,000**

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
Contracts and Services - University of St. Thomas	Sub award	Collaborate throughout the entire project and lead the bioprospecting work (Activity 3) and bacterial isolation (Activity 4). Personnel ($156,079; co-managers and undergrads; 7.4% fringe), equipment/freezer ($15,000), expendable supplies ($5,000; Petri dishes, growth media, etc), and DNA sequencing services from Phase Genomics ($65,000).	A laboratory-grade deep freezer (-80 degrees celsius) is required for Activity 4 of this project (cost = $15000). This will allow us to cryopreserve bacterial isolates indefinitely so that they can be isolated, preserved, and then studied in a reasonable time frame (i.e., otherwise, the bacteria would die). The University of St. Thomas does not currently have any freezer that fulfills this need. This freezer would be dedicated solely to this project. We acknowledge that we understand ENTRF requirements for repayment if the use of this capital equipment changes.
Travel In Minnesota	Conference Registration Miles/Meals/Lodging	1 conference	We will attend one or more conferences to help disseminate the results of this project.

Non ENRTF Funds

Category	Specific Source	Use	Status	$ Amount
State				
			State Sub Total	**-**
Non-State				
In-Kind	University of Minnesota	Indirect costs that are not allowed to be charged to LCCMR/ENTRF	Secured	$179,805
In-Kind	University of St. Thomas	Indirect costs that are not allowed to be charged to LCCMR/ENTRF	Secured	$97,737
			Non State Sub Total	**$277,542**
			Funds Total	**$277,542**

Attachments

Required Attachments

Visual Component

File: 9218902b-374.pdf

Alternate Text for Visual Component

A flow diagram that shows wastewater bioreactors leading to bacterial genomes that lead to better surface water quality. Another pathway shows wastewater bioreactors leading to bacterial genomes to the discovery of novel genes that lead to novel pharmaceutical compounds....

Supplemental Attachments

Capital Project Questionnaire, Budget Supplements, Support Letter, Photos, Media, Other

Title	File
Letter of Commitment	aeb2f574-f55.pdf
accepted Research Addendum	956a38a3-9b9.pdf

Difference between Proposal and Work Plan

Describe changes from Proposal to Work Plan Stage

I made changes per comments by LCCMR staff (probably Mike Campana).

Responses to comments: (2) We did not make changes to our research addendum. The comments by the peer reviewers were of a nature of "things that we should consider and anticipate" rather than "changes that we should make to our research plan." The comments were appreciated and helpful but they did not lead to any changes to our planned research, (3) thank you!, (4) we added milestones as suggested, (5) We added a milestone as suggested, (6) We made the changes as suggested, (7) We made the suggested change. Note: I was unable to DELETE the previous budget item under "other"; in response, I noted this and made the funded required zero, (8) We have made the suggested change, and (9) This is correct. St. Thomas has a pre-existing relationship with Phase Genomics, so we viewed this approach as "easier" (it is overall cost-neutral).

In addition: We had originally budgeted for the hiring of a post-doctoral researcher at the University of Minnesota. We did this because we knew that we would hire either a post-doctoral researcher or graduate student and the cost of a post-doctoral researcher is slightly higher. We recently identified a graduate student who would be a good fit for this position, so we have changed the budget accordingly. The cost of a graduate student is slightly less than a post-doctoral researcher (by $23,905); we re-allocated these funds to a laboratory technician category, which we will use to hire this same person during the Summer of 2024 (because he cannot begin graduate school until Fall of 2024) and/or another person who works in our department whose current appointment is only 9-months (i.e., she is a available during the summer to help with this project).

Addition on May 23, 2024: We were notified of a discrepancy as far as the number of samples that would be collected analyzed in Activity. This has been correct. We will collect monthly samples from a single wastewater treatment facility.

Additional Acknowledgements and Conditions:

The following are acknowledgements and conditions beyond those already included in the above workplan:

Do you understand and acknowledge the ENRTF repayment requirements if the use of capital equipment changes?

N/A

Do you agree travel expenses must follow the "Commissioner's Plan" promulgated by the Commissioner of Management of Budget or, for University of Minnesota projects, the University of Minnesota plan?

Yes, I agree to the UMN Policy.

Does your project have potential for royalties, copyrights, patents, sale of products and assets, or revenue generation?

Yes

Do you understand and acknowledge IP and revenue-return and sharing requirements in 116P.10?

Yes

Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF?

No

Does your project include original, hypothesis-driven research?

Yes

Does the organization have a fiscal agent for this project?

Yes, Sponsored Projects Administration

Does your project include the pre-design, design, construction, or renovation of a building, trail, campground, or other fixed capital asset costing $10,000 or more or large-scale stream or wetland restoration?

No

Do you propose using an appropriation from the Environment and Natural Resources Trust Fund to conduct a project that provides children's services (as defined in Minnesota Statutes section 299C.61 Subd.7 as "the provision of care, treatment, education, training, instruction, or recreation to children")?

No