Environment and Natural Resources Trust Fund

2025 Request for Proposal

General Information

Proposal ID: 2025-243

Proposal Title: Optimizing Anaerobic Digestion to Eliminate Antibiotic Resistance Genes

Project Manager Information

Name: Timothy LaPara

Organization: U of MN - College of Science and Engineering

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Project Basic Information

Project Summary: This project will investigate anaerobic digestion of sewage sludge to also eliminating antibiotic resistance genes. This should be achievable by operating anaerobic digesters at slightly warmer temperatures than typical operation.

ENRTF Funds Requested: $290,000

Proposed Project Completion: June 30, 2028

LCCMR Funding Category: Water Resources (B)

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

Narrative

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

The State of Minnesota has more than 500 municipal wastewater treatment plants that generate more than 150,000 tons (300 million pounds) of sewage sludge each year. In Minnesota, only 1/3 of this sewage sludge is treated and beneficially re-used as a soil additive for agricultural purposes.

Anaerobic digestion is a critically important technology for treating sewage sludge. Anaerobic digestion is typically operated at approximately human body temperature, which limits its effectiveness at eliminating microbes that are known to make humans sick (pathogens) and those that are resistant to antibiotics. Prior research has demonstrated that anaerobic digestion at very high temperature (130 degrees) is effective at eliminating both pathogens and antibiotic resistance. However, high temperature anaerobic digestion requires substantially more energy/cost to operate at these high temperatures.

This research will test *slightly* higher temperatures during the anaerobic digestion of municipal sewage sludge. Although this seems illogical, the "old" environmental engineering literature (prior to 1950) suggests (without evidence) that anaerobic digestion is only effective at human body temperature and at temperatures greater than 125 degrees. We believe that slightly higher temperatures (108 and 120 degrees) should be as effective as very high temperatures without the additional cost/energy requirements.

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.

High temperature anaerobic digestion (i.e., 125-130 degrees) has had a poor reputation since the 1930s, when initial attempts at using the technology were unsuccessful. Recent research and real-world successes (for example, at the wastewater treatment facility in Duluth, MN), however, have refuted this poor reputation. Indeed, high temperature anaerobic digestion has been shown to be highly effective at treating sewage sludge while also eliminating microorganisms that make humans sick and/or are antibiotic resistant.

Virtually all modern anaerobic digesters, therefore, are either operated at conventional temperatures (95-98 degrees) or very high temperatures (at least 125 degrees) because of a curious assumption that microorganisms cannot thrive at temperatures ranging from 100-120 degrees. There is no experimental evidence to support this assumption.

This proposed research will optimize temperature for anaerobic digestion by comparing performance at 95, 105, 115, and 130 degrees. Our hypothesis is that slightly higher temperatures (105 degrees) will be very effective at treating sewage sludge while eliminating both pathogenic microorganisms and antibiotic resistance.

This project is a continuation of a previous project supported by the Minnesota Environment and Natural Resources Trust Fund (High Temperature Anaerobic Digestion of Sewage Sludge; M.L. 2022, , Chp. 94, Art. , Sec. 2, S

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?

On a regular basis (every 10-20 years), each of the 500+ wastewater treatment facilities in Minnesota is analyzed to ensure that ever-evolving regulations are satisfied and to upgrade facilities with newly developed technologies. This project should lead to more frequent use of slighlty higher temperature anaerobic digestion at these facilities because it results in better sewage sludge treatment, greater use of treated sewage sludge for agricultural purposes, and improved public health.

Activities and Milestones

Activity 1: Compare anaerobic digester performance at temperatures of 95, 105, 115, and 130 degrees Fahrenheit

Activity Budget: $116,000

Activity Description:
This activity will involve the establishment of 12 bench-scale anaerobic digesters operated in parallel at temperatures of 95, 105, 115, and 130 degrees (Fahrenheit) (each digester will be triplicated for statistical purposes). We will then measure and compare the performance of these digesters using conventional parameters including the destruction of total solids and volatile solids as well as the production of biogas (which includes both volume of biogas and the methane content of the biogas). In addition, we will explore novel performance parameters including the destruction of carbohydrates, proteins, ATP, and RNA. Bench-scale bioreactors will be fed untreated sewage sludge collected from a local wastewater treatment facility. This experimental design will be novel as prior studies have not directly compared anaerobic digester performance as a function of temperature and most studies have only performed anaerobic digestion at 95 degrees (approximately the temperature of the human body) and at ~55 degrees (i.e., so-called "thermophilic" digestion); indeed, there is an old (but NOT justified by experimental data) adage in the environmental engineering literature that suggests that anaerobic digestion between these two temperatures is not feasible.

Activity Milestones:

Description	Approximate Completion Date
Purchase equipment and supplies necessary to perform experiments	December 31, 2025
Initiate bench-scale anaerobic digester experiments	May 31, 2026
Complete bench-scale anaerobic digester experiments	September 30, 2026
Complete biochemical analyses (protein, RNA, ATP, etc)	July 31, 2027

Activity 2: Compare the ability of anaerobic digesters operated at different temperatures to eliminate antibiotic resistance genes

Activity Budget: $174,000

Activity Description:
My hypothesis is that higher temperatures lead to greater destruction of antibiotic resistance during anaerobic digestion; this hypothesis has been strongly supported by my prior research. This project, however, will test a more subtle hypothesis in that only slightly higher anaerobic digestion temperatures should be sufficient to destroy antibiotic resistance (ARGs). By analogy, sick humans significantly benefit from slightly elevated body temperature (i.e., a fever), such that the energy required to heat anaerobic digesters to 130 degrees to eliminate ARGs is unnecessarily expensive.

This Activity will simultaneously test a second novel hypothesis of mine. Anaerobic digestion fundamentally involves "treating" dense suspensions of bacteria (i.e. the organisms that resistant to antibiotics and harbor ARGs) using other microorganisms that grow on these dense suspensions of bacteria. The existence of these two groups of bacteria complicates the analysis of antibiotic resistance during anaerobic digestion. My hypothesis is that a substantial fraction of the antibiotic resistance that we detect in anaerobic digesters (especially high-temperature anaerobic digesters) are from bacteria that are already dead (and thus harmless). To test this hypothesis, we will measure antibiotic resistance in samples collected from the digester as well as "washed" samples that will removed any non-cellular material.

Activity Milestones:

Description	Approximate Completion Date
Collect anaerobic digester samples	September 30, 2026
Extract and purify DNA from anaerobic digester samples	June 30, 2027
Quantify numerous antibiotic resistance genes by real time and/or digital polymerase chain reaction (PCR)	September 30, 2027

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 reducing antibiotic resistance levels during municipal wastewater treatment) and the National Science Foundation.

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	-	-
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

Project Manager and Organization Qualifications

Project Manager Name: Timothy LaPara

Job Title: Professor

Provide description of the project manager's qualifications to manage the proposed project.

Professor LaPara has worked at the University of Minnesota in the Department of Civil, Environmental, and Geo-Engineering since August 2000. He has worked on numerous research projects funded the Minnesota Environment and Natural Resources Trust Fund, the National Science Foundation, the National Institutes of Health, the Environmental Protection Agency, and other city, state, and federal agencies. His research has resulted in countless presentations at scientific conferences and more than 90 publications in the peer-reviewed literature. According to Web of Science, his research has been cited more than 3000 times.

Organization: U of MN - College of Science and Engineering

Organization Description:

The University of Minnesota is one of the largest, most comprehensive, and most prestigious public universities in the United States (http://www1.umn.edu/twincities/01_about.php). The laboratories directed by the project manager contains the majority of the equipment needed to perform the proposed project, including centrifuges, pumps, water meters, analytical balances, and a real-time PCR machine. The University of Minnesota also has "core facilities" that offer additional equipment, which can be used by University researchers "at cost." For this project, the core facility that is most germane is the University of Minnesota Genomics Center (UMGC; http://genomics.umn.edu). UMGC offers

state-of-the-art DNA sequencing capabilities, numerous real-time PCR machines, digital PCR machines, and experts available for consultation on an as-needed basis.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount
Personnel								
Timothy LaPara		Project Manager			27%	0.36		$98,577
Laboratory Technician		Perform laboratory experiments and analyses			25%	0.75		$96,557
							Sub Total	**$195,134**
Contracts and Services								
							Sub Total	**-**
Equipment, Tools, and Supplies								
	Equipment	Shaker-incubators	These are needed to perform anaerobic digester at precise temperatures					$50,000
	Tools and Supplies	Expendable supplies needed to perform the proposed research, including chemicals, glassware, DNA extraction kits, reagents for quantitative PCR, tedlar bags, syringes, etc.	Expendable supplies to perform the proposed research					$35,900
							Sub Total	**$85,900**
Capital Expenditures								
							Sub Total	**-**
Acquisitions and Stewardship								
							Sub Total	**-**
Travel In Minnesota								
	Miles/ Meals/ Lodging	5-10 trips to unnamed wastewater treatment facility to collect material	Travel to a wastewater treatment facility to collect untreated sewage					$966

			sludge and anaerobic digester material for inoculum					
	Conference Registration Miles/ Meals/ Lodging	1 person, 1 conference	Attend a conference within Minnesota to present research results to the public					$1,000
							Sub Total	**$1,966**
Travel Outside Minnesota								
							Sub Total	**-**
Printing and Publication								
	Printing	Poster (or similar) for conference presentations	One common method for presenting research at conferences is to print a poster for presentation					$500
	Publication	open access publishing charges	For a fee, most peer reviewed journals allow "open access" publishing, which means the authors keep the copyright. This will allow LCCMR to more broadly disseminate our research results					$3,500
							Sub Total	**$4,000**
Other Expenses								
		Equipment repair	This project will utilize equipment that is currently owned by the University of Minnesota. If this equipment were to break, funds will be required for repair so that the project can be successful.					$3,000
							Sub Total	**$3,000**
							Grand Total	**$290,000**

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request

Non ENRTF Funds

Category	Specific Source	Use	Status	Amount
State				
			State Sub Total	**-**
Non-State				
In-Kind	Indirect costs not charged as part of this project	These are costs for maintaining buildings and other basic infrastructure needed to host a research laboratory	Secured	$132,019
			Non State Sub Total	**$132,019**
			Funds Total	**$132,019**

Total Project Cost: $422,019

This amount accurately reflects total project cost?

Yes

Attachments

Required Attachments

Visual Component

File: 241f3af3-805.pdf

Alternate Text for Visual Component

The visual shows a photograph of a full-scale anaerobic digester. The visual also shows data from past research that demonstrates that anaerobic digestion at 130 degrees (F) reduces antibiotic resistance by more than 90% compared to anaerobic digestion at "normal" temperatures (approximately 95 degrees F)...

Supplemental Attachments

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

Title	File
LaPara letter	6041ecc8-1e5.pdf

Administrative Use

Does your project include restoration or acquisition of land rights?

No

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

No

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

N/A

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

N/A

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

Provide the name(s) and organization(s) of additional individuals assisting in the completion of this proposal:

Katie Sauer, University of Minnesota (accountant)