



Environment and Natural Resources Trust Fund

2022 Request for Proposal

General Information

Proposal ID: 2022-087

Proposal Title: High temperature anaerobic digestion of sewage sludge

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 research project will demonstrate that high temperature anaerobic digestion is highly effective at treating sewage sludge, particularly with respect to destroying disease-causing microorganisms and antibiotic resistance genes.

Funds Requested: \$302,000

Proposed Project Completion: June 30 2025

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?

During the Project and In the Future

Narrative

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

This proposed project will investigate that the use of high temperature anaerobic digestion as a better technology for treating the sewage sludge generated by Minnesota's municipal wastewater treatment facilities.

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.

This proposed research project would directly compare the performance of high temperature anaerobic digestion (operated at temperatures of 120 to 140 degrees) versus conventional anaerobic digestion (operated at 95-98 degrees). Although high temperature anaerobic digestion is already in use within the State of Minnesota (for example, at the Western Lake Superior Sanitary District in Duluth), its use is uncommon because of a long-standing reputation for process instability. This proposed research will provide scientific evidence that this reputation for process instability is unfounded and that the performance of high temperature anaerobic digestion far exceeds other, more commonly used sewage sludge treatment technologies.

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

High temperature anaerobic digestion has had a poor reputation since the 1930s, when initial attempts at using the technology were unsuccessful; this poor reputation has largely stuck with this technology ever since, even though a similarly poor reputation associated with conventional anaerobic digestion has dissipated. This poor reputation is the primary cause for this technology being rarely used. The reality, however, is that because it operates at higher temperatures, high temperature anaerobic digestion should far exceed the performance of conventional anaerobic digestion (operated at human body temperature), particularly with respect to the inactivation of disease-causing organisms and antibiotic resistance. Simply put, the wide-spread application of high temperature anaerobic digestion for sewage sludge treatment would benefit both Minnesota's environment and the health of Minnesota's residents while costing approximately the same as conventional anaerobic digestion.

This proposed research will directly compare high temperature anaerobic digestion to conventional anaerobic digestion in laboratory-scale systems that simulate full-scale installations. We will directly compare rates of treatment (e.g., biogas production and solids destruction rates), rates of pathogen inactivation (e.g., E. coli, SARS-CoV-2), and rates of destruction of antibiotic resistance genes.

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 high 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. Remarkably, because the fuel for heating the digesters comes from biogas produced during treatment, high temperature anaerobic digestion can be installed at a similar cost as conventional anaerobic digestion.

Activities and Milestones

Activity 1: Compare effectiveness of anaerobic digestion as a function of temperature

Activity Budget: \$151,000

Activity Description:

This activity will involve the establishment of 6 bench-scale anaerobic digesters operated in parallel at different temperatures (ranging from 75 to 140 degrees). We will then measure and compare the performance of these digesters using conventional parameters like 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, RNA, and DNA. Bench-scale bioreactors will be fed untreated sewage sludge collected from a local wastewater treatment facility. This experimental design will be very novel as prior studies have not directly compared anaerobic digester performance as a function of temperature.

Activity Milestones:

Description	Completion Date
Initiate laboratory-scale anaerobic digesters	August 31 2022
Acclimate anaerobic digesters and establish baseline performance standards	December 31 2022
Determine performance characteristics as a function of temperature	August 31 2023
Characterize microbial community composition as functions of temperature	March 31 2024

Activity 2: Characterize the microbial community composition of the biomass from anaerobic digesters operated at different temperatures, especially the disease-causing organisms

Activity Budget: \$75,500

Activity Description:

Because it is derived from human fecal material, one of the primary environmental problems posed by sewage sludge is that it contains substantial quantities of microorganisms that cause disease. For this reason, thermophilic anaerobic

Activity Milestones:

Description	Completion Date
Simulate anaerobic digestion at different temperatures (same as Activity 1)	August 31 2023
Extract and purify genetic material for analysis via PCR	December 31 2023
Characterize the microbiome of anaerobic digesters operated at different temperatures	June 30 2024
Quantify specific disease causing organisms in anaerobic digesters operated at different temperatures	December 31 2024

Activity 3: Characterize the ability of high temperature anaerobic digesters to destroy antibiotic resistant bacteria and their genes that encode antibiotic resistance.

Activity Budget: \$75,500

Activity Description:

Prior research has demonstrated that antibiotic resistance is more efficiently inactivated by anaerobic digesters operated at high temperatures (> 125 degrees) than those operated at more conventional temperatures (< 98 degrees). This prior research, however, also previously demonstrated that unexpectedly large quantities of residual antibiotic resistance genes remained even after high temperature anaerobic digestion. In this activity, therefore, we will investigate the status of these residual antibiotic resistance genes. Our hypothesis is that these residual antibiotic

resistance genes are not intact and harbored by living organisms, but rather they have been sheared and are existing outside living cells. That is, the majority of antibiotic resistance genes that we have detected in our prior research were not genuine antibiotic resistance genes, but rather sheared pieces of DNA that were still detectable (i.e., a "false positive") by the methods that we were using (quantitative PCR).

This activity, therefore, will perform metagenomic DNA sequence analysis of the DNA from high temperature anaerobic digesters after separating the DNA based on size. That is, we anticipate that antibiotic resistance genes will be almost exclusively in the small DNA size fractions (representing "dead" DNA) and not in the large DNA size fractions (representing "live" organisms).

Activity Milestones:

Description	Completion Date
Simulate anaerobic digestion operated at different temperatures (Activity 1)	August 31 2023
Extract, purify, and size-fractionate genetic material	December 31 2023
Metagenomic DNA sequencing of size-fractionated DNA	June 30 2024
Bioinformatic analysis of metagenomic DNA sequence data	March 31 2025

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 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 National Science Foundation and the Department of Energy (the DOE has recently become interested in more energy-efficient infrastructure). This research should also lead to increased usage of this technology at full-scale municipal wastewater treatment facilities, which will create opportunities for research at full-scale installations.

Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Triclosan Impacts on Wastewater Treatment	M.L. 2014, Chp. 226, Sec. 2, Subd. 03c	\$380,000
Wastewater Treatment Process Improvements	M.L. 2016, Chp. 186, Sec. 2, Subd. 04k	\$398,000
Emerging Issues Account	M.L. 2018, Chp. 214, Art. 4, Sec. 2, Subd. 10	\$439,000
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

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.

Timothy LaPara has a PhD from the School of Civil Engineering at Purdue University (1999) and has been a member of the faculty of the University of Minnesota in the Department of Civil, Environmental, and Geo- Engineering since August of 2000. His expertise is in environmental microbiology, with a special emphasis in municipal wastewater treatment and in the treatment of public water supplies. He has authored or co-authored more than 75 manuscripts in the peer-reviewed literature; these manuscripts have been cited more than 3000 times according to Web of Science.

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 managers contain 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, droplet 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			26.7%	0.36		\$69,459
Graduate Research Assistant		Perform experiments and analyze data			43%	1.5		\$155,240
							Sub Total	\$224,699
Contracts and Services								
University of Minnesota Genomics Center	Internal services or fees (uncommon)	UMGC provides at-cost access to state-of-the-art molecular/genetic equipment (e.g., next-gen DNA sequencing, droplet digital PCR), supplies, and technical expertise.				-		\$22,500
							Sub Total	\$22,500
Equipment, Tools, and Supplies								
	Tools and Supplies	Expendable reagents for quantitative polymerase chain reaction	These reagents are needed to characterize specific genes and organisms of importance to anaerobic digestion					\$7,500
	Tools and Supplies	Miscellaneous chemicals, laboratory supplies (e.g., glassware)	Numerous chemical reagents will need to be purchased to collect samples, process these samples, analyze the samples, and preserve the samples.					\$15,000
	Tools and Supplies	DNA extraction kits	Extract and purify DNA from anaerobic digester samples					\$7,500
	Tools and Supplies	Shaker-Incubators	We will need incubators capable of shaking and controlling temperature to perform lab-scale anaerobic digester experiments					\$15,000
							Sub Total	\$45,000

Capital Expenditures									
								Sub Total	-
Acquisitions and Stewardship									
								Sub Total	-
Travel In Minnesota									
	Miles/ Meals/ Lodging	We will need to travel to a nearby wastewater treatment plant to collect untreated sewage sludge	Our research investigates the treatment of sewage sludge; we can only obtain this material from full scale treatment facilities						\$1,500
	Conference Registration Miles/ Meals/ Lodging	The graduate student and I will identify a conference in Minnesota to attend to present our research results	Dissemination						\$1,000
								Sub Total	\$2,500
Travel Outside Minnesota									
								Sub Total	-
Printing and Publication									
	Printing	Poster printing	We will present our research results in poster format, which will require printing						\$301
	Publication	Open access publication charges	We will publish our results in "open access" format so that LCCMR staff can better help us promote our research						\$4,500
								Sub Total	\$4,801
Other Expenses									
		Equipment repair	We will heavily use equipment that is already owned by the University of						\$2,500

			Minnesota; this money will be used to cover repair costs if this equipment breaks					
							Sub Total	\$2,500
							Grand Total	\$302,000

Classified Staff or Generally Ineligible Expenses

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

Category	Specific Source	Use	Status	Amount
State				
			State Sub Total	-
Non-State				
In-Kind	The University of Minnesota typically charges a 55% overhead rate for all research expenditures except for those on capital equipment and graduate student tuition. By law, the University of Minnesota does not charge this overhead on projects funded by the State of Minnesota.	Overhead.	Secured	\$134,980
			Non State Sub Total	\$134,980
			Funds Total	\$134,980

Attachments

Required Attachments

Visual Component

File: [1d21f43a-58c.pdf](#)

Alternate Text for Visual Component

The visual shows a photo of a full-scale anaerobic digester and a map of the State of Minnesota, with a star located over the City of Duluth, where a full-scale, high-temperature anaerobic digester is already in operation....

Administrative Use

Does your project include restoration or acquisition of land rights?

No

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

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

High Temperature Anaerobic Digestion of Sewage Sludge



Anaerobic digestion is commonly used to treat sewage sludge, but high temperature anaerobic digestion is rare. An exception to this rarity can be found in Duluth (shown above), where high temperature anaerobic digestion has been successfully used for decades.