Final Abstract

Final Report Approved on August 26, 2026

M.L. 2022 Project Abstract

For the Period Ending June 30, 2026

Project Title: High Temperature Anaerobic Digestion of Sewage Sludge

Project Manager: Timothy LaPara

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Funding Source:

Fiscal Year:

Legal Citation: M.L. 2022, Chp. 94, Sec. 2, Subd. 04b

Appropriation Amount: $208,000

Amount Spent: $187,641

Amount Remaining: $20,359

Sound bite of Project Outcomes and Results

The sewage sludge produced during municipal wastewater treatment requires additional treatment to reduce the organic and pathogen content before it can be applied to agricultural soils. This research project demonstrated that high temperature anaerobic digestion only offers marginal improvement compared to conventional anaerobic digestion.

Summary of Project and Outcomes

The municipal wastewater treatment process generates “sewage sludge” that requires treatment prior to disposal (usually on agricultural soil). Sewage sludge is usually comprised of a mixture of primary solids (physically skimmed from untreated sewage) and secondary solids. Anaerobic digestion is the most popular technology used to treat sewage sludge, although how temperature affects process performance is poorly understood due to a myriad of historical factors. In this research project, triplicate bench-scale anaerobic digesters were operated at 95 degrees (Fahrenheit) and 125 degrees and their performance was compared with respect to biogas production, volatile solids destruction, pathogen inactivation, and the reduction in genes encoding antibiotic resistance.

The high temperature anaerobic digesters exhibited very similar performance compared to the low temperature

anaerobic digesters with respect to "traditional" metrics of anaerobic digester performance (volatile solids destruction, biogas production). The high temperature digesters, however, exhibited a small but statistically significant improvement with respect to the levels of antibiotic resistance genes and microbial pathogens. Microbiome profiling was consistent with this result as anaerobic digester temperature caused a substantial shift in microbial community composition.

This research project refutes two long-held beliefs regarding the performance of high temperature anaerobic digestion compared to conventional anaerobic digestion. First, high temperature anaerobic digestion performs very similarly with respect to standard performance metrics (biogas production, volatile solids destruction). Second, high temperature anaerobic digestion offers only a marginal improvement in the elimination of microbial pathogens and antibiotic resistance genes.

In conclusion, this research demonstrates that thermophilic anaerobic digestion unexpectedly offers similar performance as conventional anaerobic digestion. The engineering decision to utilize this technology for treating sewage sludge should be primarily based on other factors, particularly the cost of operation.

Project Results Use and Dissemination

The main outcome of this project is the understanding that high temperature anaerobic digestion offers similar performance to conventional anaerobic digestion. These research contributions are currently in the process of being published in the peer-reviewed literature. Additional dissemination of this research will occur, if feasible, via presentations at local and regional conferences focused on municipal wastewater treatment.

Environment and Natural Resources Trust Fund

M.L. 2022 Approved Final Report

General Information

Date: September 15, 2026

ID Number: 2022-087

Staff Lead: Lisa Bigaouette

Project Title: High Temperature Anaerobic Digestion of Sewage Sludge

Project Budget: $208,000

Project Manager Information

Name: Timothy LaPara

Organization: U of MN - College of Science and Engineering

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Project Reporting

Final Report Approved: August 26, 2026

Reporting Status: Project Completed

Date of Last Action: August 26, 2026

Project Completion: April 30, 2026

Legal Information

Legal Citation: M.L. 2022, Chp. 94, Sec. 2, Subd. 04b

Appropriation Language: $208,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to demonstrate that high temperature anaerobic digestion is effective at treating sewage sludge and preventing disease-causing microorganisms and antibiotic resistance genes from being released into the environment.

Appropriation End Date: June 30, 2025

Narrative

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.

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? 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 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 genes. 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) and rates of pathogen inactivation (e.g., E. coli, SARS-CoV-2).

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.

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

Activities and Milestones

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

Activity Budget: $132,500

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	Approximate 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 as well as antibiotic resistant bacteria. Conventional anaerobic digestion, however, is typically operated at approximately the temperature of the human body, which is not particularly effective at inactivating microorganisms that can grow inside a human body. In contrast, high temperature anaerobic digestion is operated at higher temperatures, which should make it superior to conventional anaerobic digestion with respect to inactivating microorganisms that cause disease in humans. This activity, therefore, will involve collecting biomass samples from bench-scale anaerobic digesters (see Activity 1) and then extracting and purifying DNA and RNA from these samples. Finally, quantitative polymerase chain reaction (qPCR) will be used to quantify pathogens and antibiotic resistance genes in these samples, allowing us to directly (and statistically) compare the effectiveness of high temperature anaerobic digestion versus conventional anaerobic digestion at inactivating pathogenic microorganisms and antibiotic resistance genes.

Activity Milestones:

Description	Approximate 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 pathogens and antibiotic resistance genes in anaerobic digesters operated at different temperatures	December 31, 2024

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.
This project will primarily involve bench-scale research. I will help disseminate the research results by making presentations to the local wastewater treatment community and by publishing the results in the peer-reviewed literature. Research publications will be open access, which will help LCCMR staff (and other) promote this work. That is, I will retain the copyright of all published material, which will allow anyone to post the articles anywhere that they would like. I will also acknowledge the Environment and Natural Resources Trust Fund during all dissemination activities. I will use of the trust fund logo or attribution language on project print and electronic media, publications, signage, and other communications per 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 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

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount	$ Amount Spent	$ Amount Remaining
Personnel										
Timothy LaPara		Project Manager			26.7%	0.51		$116,022	-	-
Undergraduate Research Assistants		Perform experiments and analyze data			0%	0.24		$7,478	-	-
Laboratory Technician		Perform laboratory experiments			7.1%	0.46		$31,003	-	-
							Sub Total	**$154,503**	**$154,503**	**-**
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.				0		$15,000	$8,540	$6,460
							Sub Total	**$15,000**	**$8,540**	**$6,460**
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	$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.					$9,097	$6,814	$2,283
	Tools and Supplies	DNA and RNA extraction kits	Extract and purify DNA and RNA from anaerobic digester samples. DNA					$2,400	$2,400	-

			extraction kits are about $600 for 100 preps (i.e., $6 per sample) and RNA extraction kits are a similar price (i.e, $6 per sample). This will allow us to process 200 samples for DNA/RNA analysis.							
	Tools and Supplies	Shaker-Incubators	We will need at least 2 incubators ($3500 each) capable of shaking and controlling temperature to perform lab-scale anaerobic digester experiments					$7,000	$6,392	$608
							Sub Total	**$25,997**	**$23,106**	**$2,891**
Capital Equipment										
		Steam generator	Repair pre-existing autoclave (i.e., sterilizer) that is needed for this project	X				$1,800	-	$1,800
							Sub Total	**$1,800**	**-**	**$1,800**
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					$3,000	$761	$2,239
	Conference Registration Miles/ Meals/ Lodging	I will identify a conference in Minnesota to attend to present our research results	Dissemination					$1,000	-	$1,000

							Sub Total	**$4,000**	**$761**	**$3,239**
Travel Outside Minnesota										
							Sub Total	**-**	**-**	**-**
Printing and Publication										
	Printing	Poster printing	We will present our research results in poster format, which will require printing					$500	-	$500
	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					$5,000	-	$5,000
							Sub Total	**$5,500**	**-**	**$5,500**
Other Expenses										
		Equipment repair	We will heavily use equipment that is already owned by the University of Minnesota; this money will be used to cover repair costs if this equipment breaks					$1,200	$731	$469
							Sub Total	**$1,200**	**$731**	**$469**
							Grand Total	**$208,000**	**$187,641**	**$20,359**

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
Capital Equipment		Steam generator	My department has a large autoclave that is needed for this project. The autoclave needs about $20,000 in repairs. The $1,800 that is requested is an estimate of the amount this project will use the autoclave during the next two calendar years. We need to request this as "capital equipment" because we will be purchasing a steam generator that the University classifies as capital equipment (i.e., the steam generator will cost >$15,000 but the ENRTF portion of this capital equipment will only be $1,800). **Additional Explanation :** My department has a large autoclave that is needed for this project. The autoclave needs about $20,000 in repairs. The $1,800 that is requested is an estimate of the amount this project will use the autoclave during the next two calendar years. We need to request this as "capital equipment" because we will be purchasing a steam generator that the University classifies as capital equipment (i.e., the steam generator will cost >$15,000 but the ENRTF portion of this capital equipment will only be $1,800).

Non ENRTF Funds

Category	Specific Source	Use	Status	$ Amount	$ Amount Spent	$ Amount Remaining
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	$114,400	$103,203	$11,197
			Non State Sub Total	**$114,400**	**$103,203**	**$11,197**
			Funds Total	**$114,400**	**$103,203**	**$11,197**

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....

Supplemental Attachments

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

Title	File
Research Addendum	950e83a8-c07.docx
Background Check Certification Form	ca29dda8-b25.pdf

Difference between Proposal and Work Plan

Describe changes from Proposal to Work Plan Stage

Because of the reduced budget, I had to make numerous changes to this work plan. I delete Activity 3 from this work plan to reduce costs. I also changed the personnel substantially from involve me plus a graduate student to me plus undergraduate assistants. In doing this, I am hoping to take advantage of a year of sabbatical "leave" during the 2023-2024 academic year, which will allow me to focus on this project (i.e., I will not be teaching so I will be the technician doing most of the work -- the undergraduates will assist me). All of these changes are consistent with the Research Addendum that is currently under review.

I also substantially reduced the focus on antibiotic resistance genes (the primary focus of Activity 3). I will still analyze antibiotic resistance genes as part of Activity 2, but the extent of this work will be relatively minor.

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?

Yes

Do you understand that travel expenses are only approved if they 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 understand the UMN Policy on travel applies.

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

Do you understand that a named service contract does not constitute a funder-designated subrecipient or approval of a sole-source contract? In other words, a service contract entity is only approved if it has been selected according to the contracting rules identified in state law and policy for organizations that receive ENRTF funds through direct appropriations, or in the DNR's reimbursement manual for non-state organizations. These rules may include competitive bidding and prevailing wage requirements

N/A

Work Plan Amendments

Amendment ID	Request Type	Changes made on the following pages	Justification For Amendment Request (word limit 75)	Date Submitted	Approved	Date of LCCMR Action
1	Amendment Request	• Budget - Personnel	I would like to hire a professional lab technician for Summer 2023 and Summer 2024 rather than an undergraduate researcher. I have had trouble hiring undergraduate assistants because the job market is sufficiently attractive that undergraduate students do not especially want to work for their professors in the summer. This is a budget-neutral change; the overall cost of the project does not change.\	May 2, 2023	Yes	May 5, 2023
2	Amendment Request	• Budget - Capital, Equipment, Tools, and Supplies • Budget - Other	We need to fix our autoclave/sterilizer, which is needed for this project. This repair will involve buying a steam generator that will cost > $15,000, which the University classifies as capital equipment. I am requesting a shift of funds from the line item for "equipment repair" to "equipment purchase" because the repair requires the purchase of capital equipment. This is still for "equipment repair" and it will not change the total project cost.	June 6, 2023	Yes	June 9, 2023
3	Completion Date	Previous Completion Date: 06/30/2025 New Completion Date: 04/30/2026	I have not yet been able to produce a draft manuscript per the dissemination requirements of this project. I should be able to have a draft manuscript submitted by the new completion date. No new funds are requested and additional funds will not be needed.	October 26, 2025	Yes	October 28, 2025

Status Update Reporting

Final Status Update June 14, 2026

Date Submitted: July 2, 2026

Date Approved: July 9, 2026

Overall Update
Triplicate bench-scale anaerobic digesters were operated at 95 degrees and 125 degrees from June/July 2023 until January 2024, during which time performance stabilized with respect to biogas production, volatile solids destruction, total solids destruction, and methane content of the biogas. In addition, a "batch" experiment was performed in which each digester was fed untreated sewage sludge, allowing us to track key parameters (volatile solids destruction, ATP, protein, and specific genetic targets) over time. The high temperature anaerobic digesters exhibited very similar performance compared to the low temperature anaerobic digesters with respect to "traditional" metrics of anaerobic digester performance (solids destruction, methane production, and protein levels). The high temperature digesters, however, exhibited superior performance with respect to the levels of antibiotic resistance genes and microbial pathogens. Microbiome profiling was consistent with this result as anaerobic digester temperature caused a substantial shift in microbial community composition. These results refute long-held beliefs that the performance of high temperature anaerobic digestion is poor compared to low temperature digestion. In fact, high temperature anaerobic digestion exceeds the performance of low temperature anaerobic digestion with respect to the removal of unwanted microorganisms (pathogens, antibiotic resistance genes).

Activity 1
Activity 1 is complete. That is, the bench-scale anaerobic digesters were successfully operated for ~6 months and more than 250 samples were collected/preserved. We completed the analyses for protein, total solids, ATP, and volatile solids. Samples for carbohydrate and RNA were not obtained during the experimental phase because of time constraints (i.e., sample collection took longer than expected and these sample types were determined to be least important).
(This activity marked as complete as of this status update)

Activity 2
Activity 2 is complete. The samples for this activity were collected and preserved. Extraction and purification of the genetic material occurred in May 2024, which was followed by genetic analysis via polymerase chain reaction (PCR). We then queried these samples for approximately 10 bacterial and protozoan pathogens and 6 different genes associated with antibiotic resistance. Microbial community composition was tracked by sequencing PCR-amplified 16S rRNA gene sequences. These results demonstrated that high temperature anaerobic digesters have completely different microbial communities compared to "normal" temperature anaerobic digestion. This shift in community composition is undoubtedly the driving mechanism connected to the concomitant decrease in pathogen and antibiotic resistance levels.
(This activity marked as complete as of this status update)

Dissemination
I submitted the first research manuscript connected to this work in June 2026. This manuscript focuses on the performance of the anaerobic digesters at two different temperatures as well as the microbial community composition (i.e., sequencing of 16S rRNA gene sequences). I also anticipate submitted a second manuscript in 2026, this one focusing on the ability of anaerobic digesters at these temperatures to reduce the quantities of antibiotic resistance genes and of microbial pathogens.

Status Update Reporting

Status Update March 1, 2026

Date Submitted: July 2, 2026

Date Approved: July 9, 2026

Overall Update
This project has been completed but it was extended to April 30, 2026 to give me time to submit at least one research manuscript to satisfy the requirements for dissemination. As of this time, the manuscript is about half-way complete. I envision no difficulty submitting this manuscript for publication by April 30, 2026.

Activity 1
Activity 1 is now complete. That is, the bench-scale anaerobic digesters were successfully operated for ~6 months and more than 250 samples were collected/preserved. We completed the analyses for protein, total solids, ATP, and volatile solids. Samples for carbohydrate and RNA were not obtained during the experimental phase because of time constraints (i.e., sample collection took longer than expected and these sample types were determined to be least important).
(This activity marked as complete as of this status update)

Activity 2
This activity was previously marked complete.
(This activity marked as complete as of this status update)

Dissemination
I anticipate submitted a research manuscript on this work by April 30, 2026.

Additional Status Update Reporting

Additional Status Update August 14, 2025

Date Submitted: November 19, 2025

Date Approved: November 25, 2025

Overall Update
Triplicate bench-scale anaerobic digesters were operated at 95 degrees and 125 degrees from June/July 2023 until January 2024, during which time performance stabilized with respect to biogas production, volatile solids destruction, total solids destruction, and methane content of the biogas. In addition, a "batch" experiment was performed in which each digester was fed untreated sewage sludge, allowing us to track key parameters (volatile solids destruction, ATP, protein, and specific genetic targets) over time. The high temperature anaerobic digesters exhibited very similar performance compared to the low temperature anaerobic digesters with respect to "traditional" metrics of anaerobic digester performance (solids destruction, methane production, and protein levels). The high temperature digesters, however, exhibited superior performance with respect to the levels of antibiotic resistance genes and microbial pathogens. Microbiome profiling was consistent with this result as anaerobic digester temperature caused a substantial shift in microbial community composition. These results refute long-held beliefs that the performance of high temperature anaerobic digestion is poor compared to low temperature digestion. In fact, high temperature anaerobic digestion exceeds the performance of low temperature anaerobic digestion with respect to the removal of unwanted microorganisms (pathogens, antibiotic resistance genes).

Activity 1
Activity 1 is now complete. That is, the bench-scale anaerobic digesters were successfully operated for ~6 months and more than 250 samples were collected/preserved. We completed the analyses for protein, total solids, ATP, and volatile solids. Samples for carbohydrate and RNA were not obtained during the experimental phase because of time constraints (i.e., sample collection took longer than expected and these sample types were determined to be least important).
(This activity marked as complete as of this status update)

Activity 2
Activity 2 is now complete. The samples for this activity were collected and preserved. Extraction and purification of the genetic material occurred in May 2024, which was followed by genetic analysis via polymerase chain reaction (PCR). We then queried these samples for approximately 10 bacterial and protozoan pathogens and 6 different genes associated with antibiotic resistance. Microbial community composition was tracked by sequencing PCR-amplified 16S rRNA gene sequences. These results demonstrated that high temperature anaerobic digesters have completely different microbial communities compared to "normal" temperature anaerobic digestion. This shift in community composition is undoubtedly the driving mechanism connected to the concomitant decrease in pathogen and antibiotic resistance levels.
(This activity marked as complete as of this status update)

Dissemination
The dissemination of this work has not yet begun. We did not have the full dataset until May 2025. Now that we have the full dataset, we will begin drafting manuscripts for publication and begin presenting our results at local (and national, if feasible) conferences. These dissemination activities will be at no (additional) cost to the MN ENTRF.

Status Update Reporting

Status Update March 1, 2025

Date Submitted: March 26, 2025

Date Approved: May 9, 2025

Overall Update

As stated in the previous updates, triplicate bench-scale anaerobic digesters were operated at 95 degrees and 125 degrees from June/July 2023 until January 2024, during which time performance stabilized with respect to biogas production, volatile solids destruction, total solids destruction, and methane content of the biogas. In mid-January, samples were collected and preserved for analysis of ATP and protein; additional samples were collected and preserved from which DNA has been extracted and purified so that specific pathogens and antibiotic resistance genes can be quantified by polymerase chain reaction (PCR). In addition, a "batch" experiment was performed in which each digester was fed untreated sewage sludge, allowing us to track key parameters (volatile solids destruction, ATP, protein, and specific genetic targets) over time. The majority of samples have been analyzed. Due to supply issues, however, we still need to analyze the ATP samples. We also have a handful for PCR assays to complete. We also intend to do more microbiome profiling (sequencing of 16S rRNA genes). We have already begun drafting manuscripts for publication. This project is more or less "on schedule" and we envision no difficulties completing the work by the end of June.

Activity 1

The majority of effort for this Activity is now complete. That is, the bench-scale anaerobic digesters were successfully operated for ~6 months and more than 250 samples were collected/preserved. The remaining tasks is to perform the analyses on the samples that were collected and preserved. To date, we have completed the analyses for protein, total solids, and volatile solids. Analysis for ATP has been delayed due to issues with a chemical supplier (back-ordered until February 2025). Samples for carbohydrate and RNA were not obtained during the experimental phase because of time constraints (i.e., sample collection took longer than expected and these sample types were determined to be least important). Remaining work is in progress and should be completed by May 15, 2025.

Activity 2

The samples for this activity have been collected and preserved. Extraction and purification of the genetic material occurred in May 2024, which has been followed by genetic analysis via polymerase chain reaction (PCR). To date, we have queried our samples for approximately 10 bacterial and protozoan pathogens. Additional analysis is on-going using PCR to quantify genes encoding antibiotic resistance. We sent samples to the University of Minnesota Genomics Center (UMGC) to characterize the microbial community composition and have received results. We anticipate sending additional samples for sequence analysis by April 15, 2025. We have completed the genetic analysis for microbial pathogens (as of September 2024) but similar analyses for quantifying antibiotic resistance genes are on-going. We envision have all results by May 15, 2025.

Dissemination

We have not performed any dissemination activities to date. Now that we have the majority of data from this projects, manuscripts have been started and we are looking for opportunities to present our work at conferences within the State of Minnesota.

Status Update Reporting

Status Update September 1, 2024

Date Submitted: September 13, 2024

Date Approved: September 19, 2024

Overall Update

As stated in the previous update, triplicate bench-scale anaerobic digesters were operated at ~95 degrees and ~125 degrees from June/July 2023 until January 2024, during which time performance stabilized with respect to biogas production, volatile solids destruction, total solids destruction, and methane content of the biogas. In mid-January, samples were collected and preserved for analysis of ATP and protein; additional samples were collected and preserved from which DNA has been extracted and purified so that specific pathogens and antibiotic resistance genes can be quantified by polymerase chain reaction (PCR). In addition, a "batch" experiment was performed in which each digester was fed untreated sewage sludge, allowing us to track key parameters (volatile solids destruction, ATP, protein, and specific genetic targets) over time. We anticipate completing all laboratory analyses by the end of 2024. Once these data are generated, we will initiate the authoring of manuscripts. This project is more or less "on schedule" and we envision no difficulties completing the work by the end of June 2025.

Activity 1

The majority of effort for this Activity is now complete. That is, the bench-scale anaerobic digesters were successfully operated for ~6 months and more than 50 samples were collected/preserved. The remaining tasks is to perform the analyses on the samples that were collected and preserved. To date, we have completed the analyses for protein, total solids, and volatile solids. Analysis for ATP has been delayed due to issues with a chemical supplier (back-ordered until at least mid-September 2024). Samples for carbohydrate and RNA were not obtained during the experimental phase because of time constraints (i.e., sample collection took longer than expected and these sample types were determined to be least important). Remaining work is in progress and should be completed by the end of 2024.

Activity 2

The samples for this activity have been collected and preserved. Extraction and purification of the genetic material will occurred in May 2024, which has been followed by genetic analysis via polymerase chain reaction (PCR). To date, we have queried our samples for approximately 10 bacterial and protozoan pathogens. We have sent samples to the University of Minnesota Genomics Center (UMGC) to characterize the microbial community composition (we anticipate receiving these results by the end of September 2024). We also anticipate completing the genetic analysis for microbial pathogens by the end of September 2024. We envision analyzing samples for antibiotic resistance genes by the end of November 2024.

Dissemination

We have not performed any dissemination activities to date. Because we anticipate having the majority (if not the entirety) of our data by the end of the summer, we will now begin looking for dissemination opportunities.

Status Update Reporting

Status Update March 1, 2024

Date Submitted: March 5, 2024

Date Approved: April 11, 2024

Overall Update

As stated in the previous update, triplicate bench-scale anaerobic digesters were operated at ~95 degrees and ~125 degrees from June/July 2023 until January 2024, during which time performance stabilized with respect to biogas production, volatile solids destruction, total solids destruction, and methane content of the biogas. In mid-January, samples were collected and preserved for analysis of ATP, protein, and RNA; additional samples were collected and preserved from which DNA will be extracted and purified so that specific pathogens can be quantified by polymerase chain reaction (PCR). Then, a "batch" experiment was performed in which each digester was fed untreated sewage sludge, allowing us to track key parameters (volatile solids destruction, ATP, protein, and specific genetic targets; note: we were unable to collect samples for RNA analysis) over time. We anticipate completing all laboratory analyses during the summer of 2024. Once these data are generated, we will then spend Fall 2024 analyzing the data with the plan to initiate the authoring of manuscripts by that time. This project is more or less "on schedule" and we envision no difficulties completing the work by the end of June 2025.

Activity 1

The majority of effort done this Activity is now complete. That is, the bench-scale anaerobic digesters were successfully operated for ~6 months and samples were collected/preserved. The remaining tasks is to perform the analyses on the samples that were collected and preserved; this work should be performed during Summer 2024.

Activity 2

The samples for this activity have been collected and preserved. Extraction and purification of the genetic material will occur in May 2024, which will then be followed by a lot of genetic analysis via polymerase chain reaction (PCR).

Dissemination

We have not performed any dissemination activities to date. Because we anticipate having the majority (if not the entirety) of our data by the end of the summer, we will now begin looking for dissemination opportunities.

Status Update Reporting

Status Update September 1, 2023

Date Submitted: September 8, 2023

Date Approved: November 7, 2023

Overall Update

As stated would occur in the previous status update, the bench scale anaerobic digesters were established in our laboratory in June 2023. These bench-digesters were inoculated using anaerobic digester material from a full-scale wastewater treatment facility and these digesters have been fed weekly since then. Three of the bioreactors are operated at ~95 degrees Fahrenheit; three of these bioreactors are operated at ~130 degrees Fahrenheit. Each of these bioreactors is generating 1-3 liters of biogas each week. We are currently monitoring (weekly) total solids destruction. We have begun optimizing our analyses for volatile solids destruction, total coliforms, E. coli, protein, ATP, RNA, and methane content of the biogas.

We anticipate operating these bench-scale digesters until December/January, allowing them several months to stabilize their performance. At the same time, we will be optimizing our method for the assays described above. Then, once we are fully prepared, we will perform concurrent kinetic experiments (i.e., samples collected ~daily for 2-3 weeks) to allow us to quantitively compare the performance of high temperature (~130 F) versus conventional temperature (~95 F) anaerobic digesters.

Activity 1

The experimental work required to satisfy this activity has begun and is proceeding as expected. These digesters will continue to "acclimate" until the end of 2023, at which time experiments will be undertaken to compare the performance of anaerobic digesters operated at ~95 degrees Fahrenheit compared to anaerobic digesters operated at ~130 Fahrenheit. While these digesters are acclimating, we will perform preliminary analyses to ensure that our analytical methods (RNA, protein, total coliforms, E. coli, methane content of the biogas, etc) meet rigorous standards for quality assurance and quality control.

According to our original time line, these experiments were supposed to be completed in December 2022, which means that we are approximately 1 year behind schedule. This delay was forced by a shift in my sabbatical from Fall 2023/Spring 2023 to Spring 2024/Fall 2024. We still anticipate completing this project by Summer 2024, which would leave an additional year of project time to resolve an unexpected issues.

Activity 2

Work on this activity cannot commence until the bench-scale digesters have sufficiently acclimated, which I anticipate occurring in December 2023.

Dissemination

There has been no dissemination activity to date.

Status Update Reporting

Status Update March 1, 2023

Date Submitted: March 16, 2023

Date Approved: March 17, 2023

Overall Update
The primary activity on this project to date has been planning and preliminary experiments to establish a working experimental system. This means that I have investigated various suppliers to identify the laboratory materials (beakers, flasks, shaker-incubators, etc) that are needed to perform the proposed work.

This activity is about 3 months behind the originally-scheduled work plan. I had originally envisioned taking a sabbatical for the 2023-2024 academic year, but this sabbatical was shifted back by a semester. That is, my sabbatical will now occur for the Spring 2024 and Fall 2024 semesters rather than the Fall 2023 and Spring 2024 semesters. Ergo, I decided that it would be best to not initiate experiments until May 2023, once the Spring 2023 semester was complete.

Activity 1
Progress on this activity has primarily been in the realm of planning. These experiments are expected to begin in early May 2023.

Activity 2
Progress on this activity has primarily been in the realm of planning. These experiments are expected to begin in early May 2023.

Dissemination
There has been no dissemination at the present time.