



Environment and Natural Resources Trust Fund

2022 Request for Proposal

General Information

Proposal ID: 2022-141

Proposal Title: Enhancing biodegradation of emerging contaminants via microbial starvation

Project Manager Information

Name: Paige Novak

Organization: U of MN - College of Science and Engineering

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Project Basic Information

Project Summary: Our research will provide concrete data to improve the design of waste-, storm-, or drinking water systems to biodegrade mixtures of pharmaceuticals, hormones, and other contaminants of emerging concern

Funds Requested: \$263,000

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.

Pharmaceuticals, hormones, and other contaminants of emerging concern (CECs) are found in water throughout Minnesota. CECs can disrupt the endocrine system of fish, wildlife, and humans and encourage antimicrobial resistance. Bacteria can biodegrade many CECs. Nevertheless, CEC biodegradation varies and may not be complete enough to protect ecological and human health.

Interestingly, research has shown that when bacteria are “starved” and carbon-limited, they will adapt by degrading a much larger variety of compounds and degrading them to much lower residual concentrations--essentially, “eating everything on their plate” while simultaneously “cleaning their plate.” This phenomenon has been studied in most detail with pure cultures of bacteria degrading a range of harmless compounds; nevertheless, it has also been studied in environmentally relevant systems by ourselves and other researchers. For example, in wastewater treatment plants, the bacterial oxidation of CECs was stimulated under starvation conditions. Changes in both the bacterial community and the function of individual bacteria were observed, suggesting that “starvation behavior” is active in environmentally relevant systems where it is also enhanced by shifts in the bacterial community. Unfortunately, although observed, the phenomenon is not understood sufficiently to utilize it for the development and design of treatment systems.

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.

CEC biodegradation is an optimal choice for treatment because it is inexpensive, it can transform CECs to non-harmful products, and it is less energy intensive than other physical and chemical treatment options.

Our proposed solution to the challenge of CECs in waste-, storm-, surface water is to stimulate better and more complete CEC biodegradation.

To do this we need to better understand “starvation behavior” in bacteria so that treatment systems can be designed to stimulate this phenomenon predictably. To do this, we must understand (1) which CECs can be biodegraded simultaneously by bacteria under carbon-limited conditions, (2) to what residual concentration are these CECs degraded, and (3) what carbon concentration induces this kind of behavior, essentially, how “hungry” do the bacteria need to be to exhibit this behavior.

This research will answer these fundamental questions, providing guidance that can be used to make simple and concrete engineering decisions, such as changes to the residence time in biological treatment systems, better managing polishing ponds and natural treatment systems to minimize carbon input, or making use of new technologies that naturally operate under starvation, such as membrane bioreactor systems, similar to the system currently used in Hutchinson, MN.

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?

Three primary project outcomes are expected. We will understand which CECs, including which classes/categories of CECs, degrade most readily under conditions of nutrient limitation. We will also know to what concentrations these CECs are degraded so that treatment decisions can be made based on desired or required effluent CEC concentrations. Finally, we will know what conditions are required to elicit this response from bacteria so that systems can be designed accordingly. These outcomes will provide specific data (e.g., target residence times or carbon concentrations) that can be used to design biological treatment systems to degrade specific CECs.

Activities and Milestones

Activity 1: Determine how bacteria grown with abundant versus limited food sources differ in the rate and extent of CEC biodegradation

Activity Budget: \$173,500

Activity Description:

We will establish microbial communities in triplicate aerobic continuous-flow reactors cultured under starvation versus more typical growth conditions. These conditions will be established by varying the residence time in the reactors such that some reactors have a high turn-over and higher concentrations of a mixed carbon feed whereas others will have a much lower turn-over, resulting in starvation conditions. Reactors will be seeded with biomass from a membrane bioreactor in Hutchinson, MN, which should be adapted to starvation conditions. The carbon concentration flowing into and out of the reactors and the bacteria flowing out will be measured. The microbial community will be analyzed.

After the system has stabilized, effluent will be collected, amended with a mixture of 10 CECs (atenolol, bisphenol A, caffeine, DEET, diuron, estrone, fluoxetine, gemfibrozil, sulfamethoxazole, TCEP) and the CEC degradation rate and residual concentration will be determined. These CECs were selected because of their prevalence in Minnesota surface and wastewaters and their representation of likely degradation pathways (e.g., oxidation or reduction).

The microbial community and the activity of certain known CEC biodegradation enzymes will also be monitored to determine the extent that community shifts versus behavior changes are responsible for the CEC degradation patterns observed.

Activity Milestones:

Description	Completion Date
Development and analysis of microbial communities under conditions of abundant vs. limiting food sources	May 31 2023
Determination of CEC biodegradation rate and extent when microbial communities are starved or not	December 31 2023
Determine how changes in the microbial community structure versus activity correspond to enhanced CEC biodegradation	May 31 2024

Activity 2: Determine the “starvation threshold” at which bacteria develop an ability to biodegrade more CECs simultaneously and to a lower concentration

Activity Budget: \$89,500

Activity Description:

Using the same continuous-flow reactor approach described in Activity 1, we will set up multiple treatments of triplicate aerobic reactors in which the residence time is varied systematically between 1 hr (ample food available) and 40 hours (starvation). Once the carbon concentration and the bacteria flowing out of the reactors are stabilized for each condition, effluent will be collected, amended with the mixture of 10 CECs described above, and the CEC degradation rate and residual concentration will be determined. The carbon concentration, microbial concentration, and microbial community structure will be monitored throughout.

We expect that as we increase the residence time above about 10 hours, enhanced CEC degradation will be observed. This expectation is based on what we have observed in wastewater treatment systems with estrone and what others have observed in laboratory studies with pure cultures. By studying CEC biodegradation in this way, we are studying communities that have not been previously adapted to particular CECs, enabling the results to be generalizable.

Activity Milestones:

Description	Completion Date
Development of microbial communities under a range of conditions, from starved to grown under abundance	November 30 2024
Determination of CEC biodegradation rate and extent when conditions range from starvation to abundance	May 31 2025

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
John Glatzmaier	Short Elliott Hendrickson Inc. (SEH®)	SEH® is a regional engineering firm with strong Minnesota contacts. I will share results with them in a timely manner and they will help to disseminate the results to their clients and regulatory contacts.	No
Tim Gratke and John Paulson	Hutchinson, MN Wastewater Treatment Facility	I will share results with the Hutchinson MN WWTF personnel in a timely manner and they will help to disseminate the results to their relevant local partners and their regulatory contacts. They will also provide biomass for experiments from their Membrane Bioreactor.	No

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?

I will disseminate results through publications and conferences and will work with existing contacts to disseminate findings to agency personnel. I will present results at local/regional conferences to reach a broad audience. I plan to submit a proposal to the National Science Foundation to further study this phenomenon. This research complements other work in my laboratory on the effect of low carbon concentrations on bacteria that biologically dechlorinate toxic chemicals at contaminated sites. It is also an extension of our previous research on the effect of carbon on the biodegradation of the human hormone estrone during wastewater treatment.

Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Evaluation of Wastewater Nitrogen and Estrogen Treatment Options	M.L. 2014, Chp. 226, Sec. 2, Subd. 03d	\$500,000
Wastewater Nitrogen Removal Technology to Protect Water Quality	M.L. 2017, Chp. 96, Sec. 2, Subd. 04b	\$450,000
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
Degrading Chlorinated Industrial Contaminants with Bacteria	M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04s	\$150,000

Project Manager and Organization Qualifications

Project Manager Name: Paige Novak

Job Title: Professor

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

Dr. Paige Novak is a Professor and the Joseph T. and Rose S. Ling Chair of Environmental Engineering in the Department of Civil, Environmental, and Geo- Engineering at the University of Minnesota. She received her B.S. in Chemical Engineering from The University of Virginia in 1992 and her M.S. and Ph.D. in Environmental Engineering from The University of Iowa in 1994 and 1997, respectively. Dr. Novak has been studying the biological treatment of water and wastewater for over 20 years. Recent work has focused on the generation of energy from high-strength wastewater and the degradation of pollutants in wastewater and surface water. She has completed several LCCMR projects on understanding and enhancing the biodegradation of contaminants of emerging concern, including phytoestrogens

(plant-based estrogens found in high concentrations in soy) and the potent human estrogen, estrone. She has also completed projects on understanding and enhancing the biodegradation of other toxic contaminants, supported by both the LCCMR and the U.S. federal government.

If funded, Dr. Novak will oversee the student researcher on the project. She will work closely with the researcher to design experiments and analyze and interpret the data produced. She will be responsible for generating reports and leading dissemination efforts. She will work with project partners to communicate findings broadly.

Organization: U of MN - College of Science and Engineering

Organization Description:

The University of Minnesota (UMN) 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 College of Science and Engineering at the University of Minnesota is ranked among the top engineering and science academic programs in the country. The college includes 12 academic departments offering a wide range of degree programs at the baccalaureate, master's, and doctoral levels. Indeed, researchers within the College of Science and Engineering are on the leading edge of finding ways to solve some of the world's greatest problems by developing new forms of environment-friendly energy, designing new medical devices, improving digital and electronic technologies, and developing a strong national infrastructure. The College of Science and Engineering also offers students a rigorous, world-class education tailored to their interests and goals. The Department of Civil, Environmental, and Geo- Engineering (CEGE) at UMN is known for its pioneering work in analytical, computational, and experimental methods. We practice research excellence grounded in rigorous fundamentals for wide-ranging applications.

The PI has access to all of the facilities needed in CEGE to perform the described research.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	\$ Amount
Personnel								
Paige Novak, PI		Project supervisor. Novak, PI (6% time per year for three years, salary 73% of cost, fringe benefits 27% of cost). Overall project supervision, experimental design, data analysis and interpretation. Total estimated cost is \$54,231.			27%	0.18		\$54,231
Graduate student researcher		One Graduate Research Assistant (50% FTE per year for three years, salary 56% of cost, fringe benefits 11% of cost, tuition 33% of cost). Will set up the flow-through reactors and grow bacteria under a range of conditions, will perform the experiments described to determine how low concentrations of substrate enable better contaminant of emerging concern degradation. Will sequence and analyze the microbial community data. Will analyze samples and data as a whole to determine outcomes. Total estimated cost is \$152,097.			44%	1.5		\$152,097
							Sub Total	\$206,328
Contracts and Services								
							Sub Total	-
Equipment, Tools, and Supplies								
	Tools and Supplies	Laboratory supplies, services, and analytical costs (includes, but is not limited to, chemicals for all analyses, supplies to maintain analytical equipment and perform analysis, supplies for reactor construction, including pumps for lab-scale systems, microbial analysis costs, analytical fees). These are all required and standard costs.	Pumps will be purchased for the flow-through reactor. Reactor materials will be purchased to grow the organisms. Chemicals will be purchased to perform the needed CEC and microbial analyses and to culture the bacteria. CECs will be purchased to study. Services include sequencing so that changes in the microbial community versus microbial activity can be					\$56,672

			assessed. Analytical costs and repair costs are included for upkeep of the equipment required to analyze CECs.					
							Sub Total	\$56,672
Capital Expenditures								
							Sub Total	-
Acquisitions and Stewardship								
							Sub Total	-
Travel In Minnesota								
							Sub Total	-
Travel Outside Minnesota								
							Sub Total	-
Printing and Publication								
							Sub Total	-
Other Expenses								
							Sub Total	-
							Grand Total	\$263,000

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				
			Non State Sub Total	-
			Funds Total	-

Attachments

Required Attachments

Visual Component

File: [Oba47e0d-6bb.pdf](#)

Alternate Text for Visual Component

The visual shows a well-fed bacteria not degrading contaminants of emerging concern, such as pharmaceuticals and personal care products, well, whereas a bacteria that is starving is able to degrade them effectively....

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?

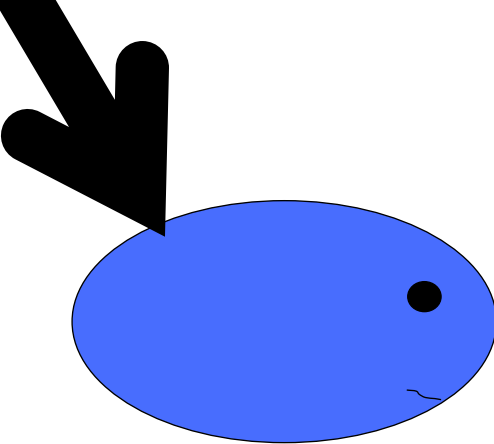
Yes

Does the organization have a fiscal agent for this project?

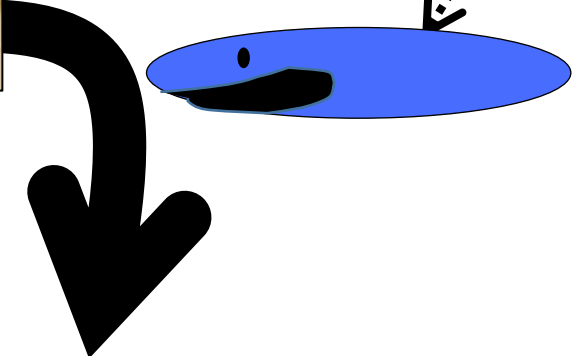
Yes, Sponsored Projects Administration

Bacterial starvation leads to survival instincts, triggering degradation of a greater number of contaminants of emerging concern to a lower residual concentration.

We will understand this response so that we can trigger it for water/wastewater treatment.



Limited degradation



Extensive degradation

