

Environment and Natural Resources Trust Fund

2025 Request for Proposal

General Information

Proposal ID: 2025-087

Proposal Title: Enhancing Degradation of Emerging Contaminants via Microbial Starvation

Project Manager Information

Name: Paige Novak Organization: U of MN - College of Science and Engineering Office Telephone: (612) 626-9846 Email: novak010@umn.edu

Project Basic Information

Project Summary: Our research will provide concrete data to inexpensively improve the design of wastewater systems to biodegrade mixtures of pharmaceuticals, pesticides, and other contaminants of emerging concern, protecting our water resources.

ENRTF Funds Requested: \$390,000

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?

During the Project and In the Future

Narrative

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

Contaminants of emerging concern (CECs), such as pesticides and pharmaceuticals, are present in the environment and cause ecological or human health impacts (antimicrobial resistance, endocrine disruption) at low concentrations. CECs are found in water throughout lakes and rivers Minnesota. We must improve our wastewater treatment to remove or degrade CECs to protect our water supplies and enable industrial reuse of water whenever possible. Though bacteria can biodegrade many CECs, it is unpredictable and highly dependent on treatment conditions. The conditions of current biological treatment methods are often not optimal for CEC removal, and thus insufficient to prevent release of CECs or safeguard health during water reuse. Improvements are needed to protect ecological and human health from the impacts of CECs.

Interestingly, research has shown that when bacteria are "starved", they will adapt by degrading a larger variety of compounds, including CECs, and degrading them to much lower residual concentrations—essentially, "eating everything on their plate" while simultaneously "cleaning their plate." Unfortunately, although observed, the phenomenon is not understood sufficiently to utilize it for the development, design, and implementation of treatment systems for a wide variety of CECs in wastewater.

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.

CEC biodegradation is an optimal choice for treatment because it is inexpensive, it transforms CECs to non-harmful products, takes advantage of existing infrastructure, and is less energy intensive than other physical and chemical treatment options. Our proposed solution to the challenge of CECs in waste- and surface water is to stimulate better and more complete CEC biodegradation by determining appropriation treatment conditions that enable bacteria to effectively degrade more of these compounds to lower residual concentrations.

We need to better understand "starvation behavior" in bacteria so that treatment systems can be designed to stimulate this phenomenon predictably. We must understand (1) which types of CECs commonly found in Minnesota can be biodegraded simultaneously by bacteria under carbon-limited (starvation) conditions, (2) to what residual concentration are these CECs degraded, and (3) the carbon (i.e., bacterial food) concentration that induces this kind of behavior, essentially, how "hungry" do the bacteria need to be to effectively and efficiently degrade CECs.

This research will answer these fundamental questions, providing guidance that can be used to make simple and concrete engineering decisions to improve treatment systems to optimize them for CEC removal.

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?

We will understand classes/categories of CECs commonly found in Minnesota waters that degrade readily under biological starvation conditions, enabling appropriate treatment decisions to be made. We will also determine the required conditions to elicit this response from bacteria. These outcomes will provide specific data (e.g., target residence times or carbon concentrations) that can be used to improve design by changing the residence time in biological treatment systems, manage polishing ponds and natural treatment systems, or use new technologies that create bacterial starvation conditions, such as membrane bioreactor systems, to effectively remove 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: \$260,000

Activity Description:

We will establish microbial communities in triplicate aerobic continuous-flow reactors cultured under either (1) starvation conditions or (2) typical growth 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 to see how it changes over time and when it stabilizes.

After the system has stabilized, the reactor influent will be amended with a mixture of 9 CECs, all commonly found in Minnesota waters (atenolol, atrazine, carbamazepine, DEET, estrone, imidacloprid, metformin, metolachlor, sulfamethoxazole). These CECs represent different likely degradation pathways (oxidation, substitution, or reduction), allowing us to extend what we learn to other CECs. The residual concentration of each CEC in the mixture will be determined from the effluent of the reactors. The microbial community and the activity of certain known CEC biodegradation enzymes will be monitored to determine the extent that community shifts versus behavior changes are responsible for the CEC degradation patterns observed. Effluent will be collected and placed in batch reactors to assess if additional time leads to further CEC degradation.

Activity Milestones:

| Description | Approximate Completion Date |
|---------------------------------------------------------------------------------------------------------|--------------------------------|
| Verification of CEC extraction and analysis method | December 31, 2025 |
| Development and analysis of microbial communities under conditions of abundant vs. limiting food | May 31, 2026 |
| sources | May 21, 2020 |
| Isolation of wastewater bacteria | May 31, 2026 |
| Determination of biodegradation extent of the CEC mixture when microbial communities are starved or not | December 31, 2026 |
| Determination of biodegradation extent of the CEC mixture when microbial isolates are starved or not | March 31, 2027 |
| Determine how changes in the microbial community structure versus activity correspond to enhanced | May 31, 2027 |
| CEC biodegradation | |

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

Activity Budget: \$130,000

Activity Description:

Using the same continuous-flow reactor approach described in Activity 1, we will alter the residence time systematically between 3 hours (ample food available) and 100 hours (starvation). Once the carbon concentration and the bacteria flowing out of the reactors are stabilized for each condition, they will be amended with the CEC mixture, and the residual concentration of each CEC in the mixture will be determined from the effluent. The carbon concentration and microbial concentration will be monitored throughout. Six different residence times will initially be tested (3, 6, 10, 20, 40, and 100 hours), which will be altered as needed. Residual CEC and organic carbon concentrations will be measured over time, allowing for replication over time.

We expect that as we increase the residence time to be longer than 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 both mixed and pure cultures. Through our careful selection of CECs, focusing on their degradation mechanisms and compound classes, the results should be generalizable to additional compounds beyond those that we are testing.

Activity Milestones:

| Description | Approximate Completion Date |
|--------------------------------------------------------------------------------------------------------------------|--------------------------------|
| Development of microbial communities under a range of conditions, from starved to grown under abundance | November 30, 2027 |
| Development of microbial isolates under a range of conditions, from starved to grown under abundance | January 31, 2028 |
| Determination of CEC biodegradation extent ranging from starvation to abundance for isolates and mixed communities | May 31, 2028 |

Project Partners and Collaborators

| Name | Organization | Role | Receiving Funds |
|----------------|---------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|
| John | Short Elliott | SEH [®] is a regional engineering firm with strong Minnesota contacts. We will | |
| Glatzmaier | Hendrickson Inc. (SEH®) | share results with them in a timely manner and they will help to disseminate the results to their clients and regulatory contacts. | |
| Scott Kyser | Minnesota Pollution Control Agency | We will share results with Scott and the MPCA in a timely manner and they will help to disseminate the results to wastewater treatment facilities across Minnesota. | No |
| William Arnold | University of Minnesota | Co-PI. | Yes |

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?

We will disseminate results through publications, conferences and contacts to Minnesota Pollution Control Agency personnel, wastewater treatment plants, and consultants. This complements our current research and is also an extension of our previous research on the biodegradation of the human hormone estrone in wastewater. The UMN and Novak are partners on a large grant focused on water technology and funded by the National Science Foundation at \$15M/2 years, with the potential for additional investments of up to \$145M/8 years. This effort will be leveraged across work at UMN and in Novak's laboratory on water technology.

| Name | Appropriation | Amount Awarded |
|--------------------------------------------------------------------------------|------------------------------------------------------------------------|-------------------|
| Determining Influence of Insecticides on Algal Blooms | M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04a | \$350,000 |
| Benign Design: Environmental Studies Leading to Sustainable Pharmaceuticals | M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04b | \$415,000 |
| 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 |
| Technology For Energy-Generating Onsite Industrial Wastewater Treatment | M.L. 2021, First Special Session, Chp. 6, Art. 5, Sec. 2, Subd. 04b | \$450,000 |
| Finding, Capturing, and Destroying PFAS in Minnesota Waters | M.L. 2023, , Chp. 60, Art. 2, Sec. 2, Subd. 04d | \$478,000 |
| Removing CECs from Stormwater with Biofiltration | M.L. 2023, , Chp. 60, Art. 2, Sec. 2, Subd. 04j | \$641,000 |

Other ENRTF Appropriations Awarded in the Last Six Years

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 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 25 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 ENRTF-funded 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 ENRTF and the U.S. federal government.

Dr. William Arnold is a co-PI on the project. He received his B.S. in Chemical Engineering from MIT in 1994, his M.S. in Chemical Engineering from Yale in 1995, and a Ph.D. in Environmental Engineering from The Johns Hopkins University in 1999. He has been studying the fate of micropollutants, including pharmaceuticals, pesticides, and PFAS compounds in aquatic environments for twenty years. As part of these studies, he has evaluated the presence and removal of various CECs in Minnesota's waters, including past work supported by the ENRTF. Past ENTRF funded work has been impactful to Minnesota, particularly work on triclosan and quaternary ammonium compounds.

If funded, Drs. Novak and Arnold will work closely together to oversee the student researcher on the project. They will be responsible for generating reports, leading dissemination efforts, and working 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. Ineli gible | % Bene fits | # FTE | Class ified Staff? | \$ Amount |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|-------------------|----------|--------------------------|-----------|
| Personnel | | | | | | | | |
| Paige Novak, Pl | vak, Project supervisor. Novak, PI. Overall project supervision, experimental design, data analysis and interpretation. | | | | 37.1% | 0.21 | | \$75,519 |
| William Arnold, Co-PI | | Project co-supervisor. Arnold, Co-PI. Analytical method development, data analysis and interpretation. | | | 37.1% | 0.15 | | \$50,771 |
| Graduate Graduate Research Assistant. Will set up the flow- student student 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. | | | | 45% | 1.5 | | \$170,404 | |
| Undergraduate student researcher | | Undergraduate Research Assistant. Will assist graduate student with reactor operation, sampling, sample analysis. | | | 0% | 0.36 | | \$11,452 |
| | | | | | | | Sub Total | \$308,146 |
| Contracts and Services | | | | | | | | |
| | | | | | | | Sub Total | - |
| Equipment, Tools, and Supplies | | | | | | | | |
| | Equipment | Six low-flow pumps | Pumps will be purchased for the flow- through reactor. The flow-rate needed is very low, so new specialized pumps will be needed. | | | | | \$12,000 |
| | 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 | 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 | | | | | \$69,198 |

| | | | | Sub Total | - |
|-----------------------------|--------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|----------|
| Printing and Publication | | | | | |
| Detection | | | | Sub Total | - |
| Travel Outside Minnesota | | | | | |
| | | | | Sub Total | \$656 |
| | Miles/ Meals/ Lodging | Travel to Hutchinson, MN from the UMN campus is 70 miles one-way. Reimbursement rates are 67 cents/mile. We anticipate 3 trips a year for the first two years and one trip in the final year. | Project personnel will travel to pick up wastewater biomass samples from Hutchinson Minnesota. They operate a membrane bioreactor, which operates under starvation conditions. We have discussed using their bacteria in this research. | | \$656 |
| Travel In Minnesota | | | | Total | 4050 |
| and Stewardship | | | | Sub | - |
| Acquisitions | | | | Sub Total | - |
| Capital Expenditures | | | | Total | |
| | | construction, microbial analysis costs, analytical fees). These are all required and standard costs. | to study, including isotope-labeled standards to ensure reproducible and high quality analysis, particularly for the low concentrations expected. Services include sequencing so that changes in the microbial community versus microbial activity can be assessed. Analytical costs and repair costs are included for upkeep of the equipment required to analyze CECs, which is highly specialized. | Sub | \$81,198 |

| Other | | | | | |
|----------|--|--|--|-------|-----------|
| Expenses | | | | | |
| | | | | Sub | - |
| | | | | Total | |
| | | | | Grand | \$390,000 |
| | | | | Total | |

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 | | | | |
| | | | Non State | - |
| | | | Sub Total | |
| | | | Funds | - |
| | | | Total | |

Total Project Cost: \$390,000

This amount accurately reflects total project cost?

Yes

Attachments

Required Attachments

Visual Component File: <u>8e0c79c2-062.pdf</u>

Alternate Text for Visual Component

The graphic shows how bacteria that are starved are able to degrade contaminants of emerging concern more effectively than well-fed bacteria....

Supplemental Attachments

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

| Title | File |
|--------------------------------------------|-------------------------|
| University of Minnesota endorsement letter | <u>9b32405c-32f.pdf</u> |

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?

No

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:

William Arnold, Professor, and Katie Sauer, Accountant, Department of Civil, Environmental, and Geo-Engineering at the University of Minnesota; personnel in the Sponsored Projects Administration at the University of Minnesota.