Environment and Natural Resources Trust Fund

2025 Request for Proposal

General Information

Proposal ID: 2025-136

Proposal Title: Terminating PFAS-Type Pesticides via Enzyme Cocktails

Project Manager Information

Name: Hua Zhao

Organization: U of MN - College of Food, Agricultural and Natural Resource Sciences

Office Telephone: (612) 624-3028

Email: zhao1822@umn.edu

Project Basic Information

Project Summary: This project will examine selected enzymes and cocktails for biodegradation of pesticide-type PFAS, and will design a biofilter for effective elimination of pesticide PFAS from water samples collected near farmlands.

ENRTF Funds Requested: $301,000

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.

Per- and polyfluoroalkyl substances (PFAS) have been widely used in almost every industry and many consumer products, including 1,400 PFAS compounds found in >200 application categories and subcategories. PFAS are spreading through water and air to our bloodstream and lung causing serious health issues such as cancers, type 1 diabetes, lung diseases, and other disorders. PFAS are known as "forever chemicals" because carbon–fluorine (C–F) bond is one of the strongest covalent bonds, even stronger than corresponding carbon-hydrogen (C–H) bond. Simply capturing PFAS without terminating them is not enough because they will likely find ways to go back to the environment.

PFAS in pesticides could come from different sources: active ingredients being PFAS or fluorinated pesticides, formulation additives as anti-forming, dispersing, or wetting agents, and fluorinated plastic (such as HDPE) containers. In addition, there are many fluorinated pesticides that are not officially classified as PFAS, but show similar polluting impact on the environment as PFAS in terms of persistence, bioaccumulation potential, and ecotoxicological effects on soil and aquatic ecosystems. Between 2010 and 2020, fluorinated pesticides accounted for over half of all pesticides approved; during 2015-2020, ~70% of newly approved agrochemicals are fluorinated pesticides.

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.

Based on the narrow definition of PFAS by EPA, three currently used pesticides (i.e., broflanilide, pyrifluquinazon, and noviflumuron) are classified as PFAS, although based on the Organization for Economic Co-operation and Development (OECD) definition, over 200 fluorinated pesticide ingredients (e.g., bifenthrin) are considered as PFAS and most of them are banned by European Union. Microbial degradation of fluorinated pesticides typically leads to smaller fluorinated molecules due to the lack of defluorinating enzymes in the microbial consortia, although some fungi and activated sludge communities have been shown capable of defluorination.

Our goal is to develop enzyme cocktails that will break down pesticide-type and other PFAS in the environment (e.g., rivers and lakes), and to construct reactive biofilters from immobilized enzymes for a pilot-scale PFAS cleaning from water (see Visual Component). The enzyme cocktails consist of at least two types of enzymes to cleave both carbon-fluorine (C–F) and carbon-carbon (C–C) bonds so that PFAS molecules are converted into non-hazardous fluoride ions and smaller molecules. In addition, we aim to break down some aromatic C–F bonds in pesticides since among all organofluoride compounds, fluoroaromatics are most difficult to break due to the super strong C–F bond.

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?

The successful completion of this project will result in a green and effective method for permanent termination of pesticide-type and other PFAS from the environment especially from water. In particular, as a major agriculture state, Minnesota's rivers, lakes and underground water face serious contaminations from fluorinated pesticide runoff from farmlands. Our reactive filtration system will not only capture PFAS, but also convert them to environmentally benign components to avoid pesticide (and other PFAS) accumulation and contamination in Minnesota's water resources.

Activities and Milestones

Activity 1: Evaluate individual enzymes for breaking C–F and C–C bonds in pesticide-type PFAS

Activity Budget: $98,267

Activity Description:

We will investigate two types of enzymes: (1) two metalloenzymes (i.e., cytochromes P450 and cysteine dioxygenase) for breaking C–F bonds, and (2) peroxidases (i.e., lignin peroxidase and manganese peroxidase) for breaking large PFAS molecules into smaller fragments. We will examine each of these enzymes for their capability in degrading simple and common PFAS first [e.g., perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA)], and then pesticide PFAS (i.e., broflanilide, pyrifluquinazon, and noviflumuron). We will evaluate different reaction conditions such as temperature, pH value, and substrate concentration, etc. All PFAS samples will be analyzed by an external testing laboratory for fees.

We will further use spectroscopic tools including infrared, fluorescence emission spectra, and far-UV circular dichroism (CD) spectroscopy to probe enzyme structural changes. Dr. Gary Baker at University of Missouri will assist with the analysis and interpretation of these spectroscopic studies (no cost to this project). Dr. Tao Wei at University of South Carolina will conduct the Molecular Dynamics (MD) simulations of enzyme-PFAS interactions (no cost to this project). This will enable a molecular-level understanding of how enzymes interact with PFAS molecules.

Activity Milestones:

Description	Approximate Completion Date
Determine enzyme activity for PFOS and PFOA degradation	December 31, 2025
Determine enzyme activity for pesticide PFAS degradation	June 30, 2026
Conduct spectroscopic and computational studies of enzyme-PFAS interactions	June 30, 2026

Activity 2: Design enzyme cocktails for synergistic biodegradation of pesticide-type PFAS

Activity Budget: $100,072

Activity Description:

We will prepare enzyme cocktails by combining one metalloenzyme with one peroxidase, resulting in four types of cocktails in total. We will vary the metalloenzyme/peroxidase ratio in each combination to achieve the optimum synergistic effects on the enzymatic degradation of PFOS, PFOA and pesticide PFAS. For each enzyme cocktail, we will evaluate different reaction conditions such as temperature, pH value, and substrate concentration, etc.

We will also use spectroscopic tools including infrared, fluorescence emission spectra, and far-UV circular dichroism (CD) spectroscopy to probe enzyme cocktails' structural changes. Dr. Gary Baker at University of Missouri will assist with the analysis and interpretation of these spectroscopic studies (no cost to this project). Dr. Tao Wei at University of South Carolina will conduct the Molecular Dynamics (MD) simulations of enzyme cocktail-PFAS interactions (no cost to this project). This will enable a molecular-level understanding of how enzyme cocktails interact with PFAS molecules.

Activity Milestones:

Description	Approximate Completion Date
Evaluate each enzyme cocktail on PFOS and PFOA degradation	December 31, 2026
Evaluate each enzyme cocktail on pesticide PFAS degradation	June 30, 2027
Conduct spectroscopic and computational studies of enzyme cocktail-PFAS interactions	June 30, 2027

Activity 3: Design an enzymatic biofilter system for continuous elimination of PFAS from water at a pilot scale

Activity Budget: $102,661

Activity Description:
We will immobilize selected enzymes and enzyme cocktails on solid carriers such as silica sol-gel matrices, chitosan, and polystyrene beads, and compare the residual enzyme activity and stability after the immobilization with corresponding free enzymes. We will design a reactive biofilter by packing immobilized enzymes in a disk (to become a continuous flow reactor). We will examine the adsorption and enzymatic conversion of PFOS, PFOA and pesticide PFAS in the biofilter. Furthermore, we will scale up the biofilter to handle a water flow rate of 1 gallon per hour. We will collect water samples near farmlands where pesticide PFAS have been applied, and run these water samples through the enzymatic biofilters to determine the effectiveness and efficiency of our PFAS-removing system.

Activity Milestones:

Description	Approximate Completion Date
Immobilize enzymes and enzyme cocktails on solid carriers and evaluate their activities	September 30, 2027
Design enzymatic biofilters by packing immobilized enzymes in a disk	December 31, 2027
Evaluate enzymatic biofilters for removing and degrading PFAS from water samples near farmlands	June 30, 2028

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Gary A. Baker	University of Missouri	Assist with the analysis and interpretation of spectroscopic studies of enzymes and enzyme cocktails	No
Tao Wei	University of South Carolina	Conduct the Molecular Dynamics (MD) simulations of enzymes and their cocktails interacting with PFAS molecules	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 work be funded?

Hua Zhao and his students will lead research design and experiments to implement proposed activities. Their collaborators on this project will be funded by different sources for their efforts. Results generated in this project will enable a fundamental understanding of interactions between enzymes and PFAS, and a practical aspect of how enzymes can be used as a biofilter to eliminate PFAS. The preliminary data generated by this study will allow us to acquire a larger NSF or USDA grant that involves multiple institutions to tackle a much bigger scale of PFAS removal and elimination with a focus on agricultural applications.

Project Manager and Organization Qualifications

Project Manager Name: Hua Zhao

Job Title: Professor and Department Head

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

Hua Zhao is a full professor and department head in the Department of Bioproducts and Biosystems Engineering at University of Minnesota. He has been working on biocatalysis and renewable fuels (such as cellulosic ethanol and biodiesel) for the past 20 years since he was a chemical engineering Ph.D. graduate student at New Jersey Institute of Technology and a postdoctoral researcher at Rutgers University. He received a combined training and preparations in chemical engineering, chemistry, and food science. He has led multiple research and student research training projects funded by National Science Foundation (NSF), National Institutes of Health (NIH), and American Chemical Society – Petroleum Research Fund (ACS-PRF). His current and past projects include enzymatic catalysis for the synthesis of biosurfactants, new asymmetric medicinal molecules, and biopolymers, as well as enzymatic coal treatment, desulfurization of liquid fuels, and DNA-based hybrid catalysis. He has established a broad range of collaborations with biocatalysis, protein engineering, biofuel, and computational chemistry communities that are essential to the success of this proposed project. In addition, he has mentored many undergraduate and graduate students especially those who are underrepresented in STEM, and has successfully prepared his students to advance in their careers through dedicated mentoring activities.

Organization: U of MN - College of Food, Agricultural and Natural Resource Sciences

Organization Description:

In the College of Food, Agricultural and Natural Resources Sciences (CFANS) at the University of Minnesota, we look at the bigger picture. When we envision a better tomorrow, it includes disease-resistant crops, products that protect our health, lakes free from invasive species, and so much more. We use science to find answers to Minnesota and the world's grand challenges and solve tomorrow's problems. Almost 93 percent of students who earn CFANS undergraduate degrees find jobs in their career field or enter graduate school within six months of graduation.

The Department of Bioproducts and Biosystems Engineering, in CFANS, discovers and teaches solutions for the sustainable use of renewable resources and the enhancement of the environment. We discover innovative solutions to address challenges in the sustainable production and consumption of food, feed, fiber, materials, and chemicals by integrating engineering, science, technology, and management into all degree programs.

We have a public impact through community engagement and extension efforts. We develop and deliver high quality, regionally and nationally-recognized research-based programs to meet current and emerging needs of industry and communities. We also have a long-standing tradition of close partnerships with alumni, industry professionals, organizations, government agencies, donors, and community members.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount
Personnel								
One graduate student (stipend and tuition for three years)		Research design and conduct experiments as proposed			25.1%	1.5		$173,638
Two undergraduate students ($6,000 for the first year for $15 per hour, 10 hour per week, and 10 weeks each year; 2% increase in each year afterwards)		Receive research training and collect experimental data			0%	0.75		$18,362
							Sub Total	**$192,000**
Contracts and Services								
							Sub Total	-
Equipment, Tools, and Supplies								
	Tools and Supplies	Funds ($20,000 in Year 1, $20,365 in Year 2, and $20,365 in Year 3) are requested to purchase laboratory chemicals (various PFAS and pesticides), reagents (acetone, methanol, and HPLC-grade water), enzymes (cytochromes P450, cysteine dioxygenase, lignin peroxidase, and manganese peroxidase), and reagents.	These chemicals and enzymes are needed to carry out the proposed experimental work.					$60,730
							Sub Total	**$60,730**

Capital Expenditures								
							Sub Total	-
Acquisitions and Stewardship								
							Sub Total	-
Travel In Minnesota								
	Conference Registration Miles/ Meals/ Lodging	One conference trip per year for PI and two students per year, $150 registration per person ($450 total per year), 200 miles per year ($120), lodging for 3 persons and 2 nights ($900), and meals ($620 for 3 persons, two days per year).	PI and two students each year will present and share research results in in-state conferences, and network with peers.					$6,270
							Sub Total	**$6,270**
Travel Outside Minnesota								
							Sub Total	-
Printing and Publication								
	Publication	Open-access journal publication cost	Publish research results in open-access journal, about $2,000 per year for one paper					$6,000
							Sub Total	**$6,000**
Other Expenses								
		PFAS sample analysis (30-40 samples each year) by an external testing laboratory (about $300-400 per sample)	To determine the effectiveness of PFAS biodegradation by enzymes					$36,000
							Sub Total	**$36,000**
							Grand Total	**$301,000**

Classified Staff or Generally Ineligible Expenses

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

Non ENRTF Funds

Category	Specific Source	Use	Status	Amount
State				
			State Sub Total	-
Non-State				
			Non State Sub Total	-
			Funds Total	-

Total Project Cost: $301,000

This amount accurately reflects total project cost?

Yes

Attachments

Required Attachments

Visual Component

File: 542bac69-50e.pdf

Alternate Text for Visual Component

A disk-shaped biofilter packed with enzymes is used to filter and convert PFAS-type pesticides such as Broflanilide into nonhazardous fluoride ions and other smaller fragments. This filter will be effective and efficient for terminating PFAS including fluorinated pesticides in water....

Supplemental Attachments

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

Title	File
Letter of approval to submit	3b967bbe-79c.pdf

Administrative Use

Does your project include restoration or acquisition of land rights?

No

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

Yes

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

Yes

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

No

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:

Wendy Moylan, University of Minnesota