



Environment and Natural Resources Trust Fund

M.L. 2025 Approved Work Plan

General Information

ID Number: 2025-136

Staff Lead: Noah Fribley

Date this document submitted to LCCMR: June 5, 2025

Project Title: Terminating PFAS-Type Pesticides via Enzyme Cocktails

Project Budget: \$297,000

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

Web Address: <https://cfans.umn.edu/>

Project Reporting

Date Work Plan Approved by LCCMR: June 24, 2025

Reporting Schedule: March 1 / September 1 of each year.

Project Completion: June 30, 2028

Final Report Due Date: August 14, 2028

Legal Information

Legal Citation: M.L. 2025, First Special Session, Chp. 1, Art. 2, Sec. 2, Subd. 04k

Appropriation Language: \$297,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to evaluate the ability of selected enzymes and combinations of enzymes to biodegrade per- and polyfluoroalkyl substances (PFAS) found in pesticides and to design a pilot-scale biofilter for effective elimination of PFAS from water.

Appropriation End Date: June 30, 2028

Narrative

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.

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.

Project Location

What is the best scale for describing where your work will take place?

Region(s): Metro

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

Activity Budget: \$97,268

Activity Description:

We will investigate three types of enzymes: (1) two metalloenzymes (i.e., cytochromes P450 and cysteine dioxygenase) for breaking C–F bonds, (2) laccase from *Pleurotus ostreatus* and laccase from *Trametes Versicolor* for partial defluorination, and (3) peroxidases (i.e., hydrogen peroxidase, lignin peroxidase and manganese peroxidase) for breaking large PFAS molecules into fragments. We will examine each of these enzymes for degrading simple and common PFAS first [e.g., perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA)], and then fluorinated pesticides (i.e., bifenthrin, fluopyram, acifluorfen, broflanilide, pyrifluquinazon, and noviflumuron). We will evaluate different reaction conditions such as temperature, pH value, metal ions, NADPH or hydrogen peroxide concentration, and substrate concentration, etc. The fluoride concentration will be determined by a fluoride meter. The substrate will be monitored by HPLC. PFAS degradation samples will be analyzed by an external testing laboratory.

We will use infrared, fluorescence emission spectra, and far-UV circular dichroism (CD) spectroscopy tools to probe enzyme structural changes. Dr. Gary Baker at University of Missouri will support 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).

Activity Milestones:

| Description | Approximate Completion Date |
|--|-----------------------------|
| Examine enzymatic degradation of PFOS and PFOA by each of two metalloenzymes and two peroxidases | June 30, 2027 |
| Evaluate enzymatic degradation of fluorinated pesticides by each of metalloenzymes and peroxidases | June 30, 2027 |
| Conduct spectroscopic and computational studies of enzyme-PFAS interactions | June 30, 2027 |

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

Activity Budget: \$99,072

Activity Description:

We will prepare enzyme cocktails by combining metalloenzyme, laccase, and/or peroxidase, resulting in different types of cocktails. We will vary the enzyme ratio in each combination to achieve the optimum synergistic effects on the enzymatic degradation of PFOS, PFOA and fluorinated pesticides (i.e., bifenthrin, fluopyram, acifluorfen, broflanilide, pyrifluquinazon, and noviflumuron). For each enzyme cocktail, we will evaluate different reaction conditions such as temperature, pH value, metal ions, NADPH or hydrogen peroxide concentration, and substrate concentration, etc. The fluoride concentration will be determined by an ion meter with fluoride probe. The substrate will be monitored by HPLC. PFAS degradation samples will be analyzed by an external testing laboratory.

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 for PFOS and PFOA degradation | June 30, 2027 |
| Evaluate each enzyme cocktail for fluorinated pesticides 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: \$100,660

Activity Description:

We will immobilize selected enzymes and cocktails on solid carriers (e.g., silica sol-gel matrices, chitosan, and polystyrene beads) using glutaraldehyde as the cross-linking agent, and compare the residual enzyme activity and stability after the immobilization with corresponding free enzymes via common assay methods. We will compare thermal stability and resistance to pH changes and organic solvents of enzymes before and after enzyme immobilization. We will design a cylinder-shape or disk-shape plastic filter using 3-D printing and then pack the immobilized enzymes in the filter (to become a continuous flow reactor). We will determine the adsorption and enzymatic conversion of PFOS, PFOA and fluorinated pesticides in the biofilter by measuring the substrate concentration by HPLC and fluoride ion concentration by an ion meter with fluoride probe for both inlet and outlet streams of the biofilter. PFAS degradation samples will be sent to an external testing laboratory for analysis. 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 fluorinated pesticides (including PFAS) have been applied, and run these water samples through the enzymatic biofilter to determine the effectiveness and efficiency of our PFAS-eliminating 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 plastic disk or cylinder | December 31, 2027 |
| Evaluate enzymatic biofilters for eliminating fluorinated pesticides (including 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 |

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.

Following the ENRTF Acknowledgment Guidelines, the Environment and Natural Resources Trust Fund will be acknowledged through use of the trust fund logo or attribution language on our research website, project presentations, project print and electronic media, journal publications, and other communications. Major research findings will be presented twice every year at state, regional and/or national conferences by PI and students, and will be submitted for publication in peer-reviewed journals. Research results will be shared and discussed with PFAS research and development community in Minnesota through public forums, journal clubs, and seminars. Data collected and methods developed as part of the student research projects will be archived and be available upon request. Chemical and enzyme samples will be preserved in the laboratories under appropriate storage conditions as needed. General information relevant to PFAS, biocatalysis, and green chemistry will be disseminated through our seminar series and our group website. In addition, the general information will also be distributed to the public through the American Chemical Society (ACS) Minnesota Local Section outreach program, department student chapter (named "Food and Bioproducts Engineering Organization") events, and ACS green chemistry week activities.

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.

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 | | | | | | | | |
| PFAS sample analysis (30-40 samples each year) by an external testing laboratory (about \$300-400 per sample) | Service Contract | To determine the effectiveness of PFAS biodegradation by enzymes (the analysis requires consumable reagents, the use of instrument, and labor cost). | | | | 0.3 | | \$36,000 |
| | | | | | | | Sub Total | \$36,000 |

| Equipment, Tools, and Supplies | | | | | | | | |
|---------------------------------------|---|---|--|--|--|--|------------------|-----------------|
| | Tools and Supplies | Funds (\$20,000 in Year 1, \$20,365 in Year 2, and \$19,730 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. | | | | | \$59,730 |
| | | | | | | | Sub Total | \$59,730 |
| 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 \$1,000 per year for one paper | | | | | \$3,000 |
| | | | | | | | Sub Total | \$3,000 |

| | | | | | | | | |
|-------------------|--|--|--|--|--|--|----------------|-----------|
| Other Expenses | | | | | | | | |
| | | | | | | | Sub Total | - |
| | | | | | | | Grand Total | \$297,000 |

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 | | | | |
| In-Kind | University of Missouri | Support my collaborator Dr. Gary Baker's spectroscopic studies | Potential | \$5,000 |
| In-Kind | University of South Carolina | Support my collaborator Dr. Tao Wei's MD simulations studies | Potential | \$5,000 |
| | | | Non State Sub Total | \$10,000 |
| | | | Funds Total | \$10,000 |

Total Project Cost: \$307,000

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 |
| 2025-136 Research Addendum revised_final | 9f6dfa1e-1fb.docx |

Difference between Proposal and Work Plan

Describe changes from Proposal to Work Plan Stage

- 1) In Activity 1, we included three top-sold fluorinated pesticides (bifenthrin, fluopyram, and acifluorfen) in the study: "pesticide PFAS (i.e., bifenthrin, fluopyram, acifluorfen, broflanilide, pyrifluquinazon, and noviflumuron)".
- 2) In Activity 1, we added "metal ions, NADPH or hydrogen peroxide concentration" as two additional conditions to be studied.
- 3) In Activity 1, we added "The fluoride concentration will be determined by an ion meter with fluoride probe" and "The substrate will be monitored by HPLC".
- 4) In Activity 1, the first milestone is modified to "Examine enzymatic degradation of PFOS and PFOA by each of two metalloenzymes and two peroxidases" from "Determine enzyme activity for PFOS and PFOA degradation".
- 5) In Activity 1, the second milestone is modified to "Evaluate enzymatic degradation of fluorinated pesticides by each of metalloenzymes and peroxidases" from "Determine enzyme activity for pesticide PFAS degradation".
- 6) In Activity 2, we changed "pesticide PFAS" to "fluorinated pesticides (i.e., bifenthrin, fluopyram, acifluorfen, broflanilide, pyrifluquinazon, and noviflumuron)".
- 7) In Activity 2, we added "metal ions, NADPH or hydrogen peroxide concentration" as two additional conditions to be studied.
- 8) In Activity 2, we added "The fluoride concentration will be determined by an ion meter with fluoride probe. The substrate will be monitored by HPLC. PFAS degradation samples will be analyzed by an external testing laboratory."
- 9) In Activity 2, the second milestone is modified to "Evaluate each enzyme cocktail for fluorinated pesticides degradation" from "Evaluate each enzyme cocktail on pesticide PFAS degradation"
- 10) In Activity 3, we added "using glutaraldehyde as the cross-linking agent" for the enzyme immobilization method.
- 11) In Activity 3, we added "via common assay methods for metalloenzymes with peroxidases" for the measuring the residual enzyme activities.
- 12) In Activity 3, we clarified the shape of biofilter by stating "a cylinder-shape or disk-shape plastic filter using 3-D printing".
- 13) In Activity 3, we specified the analysis method by stating "by measuring the substrate concentration by HPLC and fluoride ion concentration by an ion meter with fluoride probe for both inlet and outlet streams of the biofilter. PFAS degradation samples will be sent to an external testing laboratory for analysis".

- 14) In Activity 3, the second milestone is modified to "Design enzymatic biofilters by packing immobilized enzymes in a plastic disk or cylinder" from "Design enzymatic biofilters by packing immobilized enzymes in a disk".
- 15) In Activity 3, the third milestone is modified to "Evaluate enzymatic biofilters for eliminating fluorinated pesticides (including PFAS) from water samples near farmlands" from "Evaluate enzymatic biofilters for removing and degrading PFAS from water samples near farmlands".
- 16) The original budget is reduced by \$4,000 (\$3,000 in publication cost and \$1,000 in supplies) to meet the recommended budget (\$297,000).
- 17) Reclassified PFAS sample analysis as a service contract in the services and subawards subtab of the budget section.
- 18) Updated Activity 1 to reflect that we will investigate three types of enzymes, rather than two, as indicated in the revised research addendum, and made other necessary changes.
- 19) Updated Activity 2 to reflect revisions to the research addendum regarding the specifics of the enzyme cocktails to be mixed (from three different enzymes, rather than two).
- 20) Updated Activity 3 by adding the sentence, "We will compare thermal stability and resistance to pH changes and organic solvents of enzymes before and after enzyme immobilization" and other necessary changes.
- 21) Made changes to completion dates in Activities 1 and 2 to correspond with changes made to the timetable in the research addendum.
- 22) Included potential in-kind contributions from two collaborators as non-ENRTF funds contributed to this project in the budget section.
- 23) Included in the Dissemination section a statement "Following the ENRTF Acknowledgment Guidelines, the Environment and Natural Resources Trust Fund will be acknowledged through use of the trust fund logo or attribution language on our research website, project presentations, project print and electronic media, journal publications, and other communications."

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?

N/A

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?

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 project:

Wendy Moylan, University of Minnesota

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

Yes, I understand