Environment and Natural Resources Trust Fund

2026 Request for Proposal

General Information

Proposal ID: 2026-055

Proposal Title: Eliminating Phenolic Compounds from Water Using Enzyme Filter

Project Manager Information

Name: Hua Zhao

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

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Project Basic Information

Project Summary: This project will study the biodegradation of phenolic compounds in water by an enzyme (laccase), and design an enzyme membrane filter to capture and destroy phenolic compounds in Minnesota waters.

ENRTF Funds Requested: $390,000

Proposed Project Completion: June 30, 2029

LCCMR Funding Category: Water (B)

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

Narrative

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

Phenolic compounds in water come from the degradation of natural substances (e.g., lignin, algae, fish, and dead animals), discharges from industrial products, agricultural practices, and domestic and municipal waste and wastewater. Phenolic compounds can potentially cause cancer, endocrine dysregulation, genetic malformations, and the destruction to the immune system, tissue, central nervous system, and internal organs. Phenol at a low concentration of 5 parts per billion (ppb) could impart unpleasant tastes and odors to drinking water when it reacts with chlorine to form chlorophenols.

US EPA has a strict regulation on phenolic compounds in drinking water. According to a report by Minnesota Pollution Control Agency, in 2017, water samples were taken from 50 Minnesota lakes (randomly selected), and several phenolic compounds were among several high priority contaminants identified posing a great risk to aquatic ecosystems. Conventional water treatment plant (WTP) is not efficient in removing many organic micropollutants. Existing methods for removing phenolic pollutants include recovery, incineration, adsorption, biological treatment, and chemical oxidation. Although these methods are effective, they are often associated with serious issues, such as high cost, incomplete removal, the formation of hazardous byproducts, and/or low efficiency.

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.

An enzymatic method to phenolic compounds treatment represents a ‘greener’ bioremediation to this issue. In particular, the enzymatic oxidation of phenolic compounds can be catalyzed by a type of enzyme known as laccases, which has been demonstrated as an effective strategy. However, this method suffers from the leaching and loss of enzymes (for physical immobilization) or low enzyme activity (for chemical immobilization). Different membranes are capable of filtering off particles, pathogens, phenolic compounds, and other contaminants, but they cannot destroy these organic pollutants. Our goal is to combine membrane technology with enzymes to produce “membrane-enzyme biofilter” for effective removal and elimination of harmful phenolic compounds from water. To preserve the enzyme structure and activity, we will coat the immobilized laccase with enzyme-compatible "water-like" ionic liquids (ILs).

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 capture and elimination of toxic phenolic compounds from Minnesota rivers and lakes. Due to the high volume of agricultural, forestry, and industrial activities, Minnesota’s rivers, lakes and underground water face constant contaminations from phenolic compounds. Our reactive filtration system will not only capture phenolic substances, but also convert them to environmentally benign components to avoid their accumulation and contamination in Minnesota’s water resources.

Activities and Milestones

Activity 1: Evaluate different laccases for the oxidation of phenolic compounds

Activity Budget: $125,683

Activity Description:
Enzymes like horseradish peroxidase are effective for oxidizing phenolic compounds, but they require the use of hydrogen peroxide (H2O2) and its in situ regeneration, so it is challenging for large-scale water treatment. On the other hand, another type of enzyme known as laccase only requires oxygen to oxidize phenolic compounds. Thus, we will evaluate several types of commercial laccases (from Trametes versicolor, Agaricus bisporus, Aspergillus sp., and Rhus vernicifera). The enzyme activity will be screened by using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as the substrate and monitored at 420 nm with a UV–visible spectrophotometer. We will evaluate the oxidation of several model phenolic compounds (i.e., 2,6-dimethoxyphenol, guaiacol, and 4-chlorophenol) as quantified by the HPLC or colorimetric method. In addition to different free enzymes and substrates, we will evaluate different reaction conditions such as pH, temperature, and reaction time.

We will use infrared, fluorescence emission spectra, and far-UV circular dichroism (CD) spectroscopy tools to probe structural changes of free laccases under different reaction conditions. Dr. Gary Baker at the University of Missouri will support these spectroscopic studies.

Activity Milestones:

Description	Approximate Completion Date
Screen the activities of different laccases using ABTS assay	December 31, 2026
Evaluate the enzymatic oxidation of different phenolic compounds using free laccases	June 30, 2027
Conduct spectroscopic studies of structural changes of free laccases under different conditions	June 30, 2027

Activity 2: Construct immobilized laccases coated with "water-like" ionic liquids to improve the enzyme stability

Activity Budget: $130,545

Activity Description:
Ionic liquids (ILs) are neoteric solvents that are made of ions. ILs can coat enzymes with a thin layer of compatible ionic medium, preserving active conformations and facilitating a fast diffusion of substrates to the enzyme's active site. IL-coated enzymes are known for improved reactivity and stereoselectivity. Our laboratory prepared a series of "water-like" hydrophobic ILs that are highly compatible with enzymes. We will prepare "water-like" IL-coated laccases by: (1) lyophilization method; or (2) precipitation method. IL-coated enzymes will be characterized by MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) experiments to confirm the protein peak, scanning electron micrographs (SEM) to determine their surface properties, and optical microscopy to confirm the IL coating on solid carriers. By following the ABTS method described earlier, we will compare the enzyme activity and stability in aqueous solutions after IL coating on free enzymes.

We will also use spectroscopic tools including infrared, fluorescence emission spectra, and far-UV circular dichroism (CD) spectroscopy to probe enzyme coating on their structural changes. Dr. Gary Baker at the University of Missouri will assist with the analysis and interpretation of these spectroscopic studies. This will enable a molecular-level understanding of how enzyme coating impacts the enzyme activity.

Activity Milestones:

Description	Approximate Completion Date
Construct laccases coated with functionalized ionic liquids	December 31, 2027
Evaluate the activity of laccases coated with functionalized ionic liquids	June 30, 2028
Conduct spectroscopic studies of enzymes coated with ionic liquids	June 30, 2028

Activity 3: Design an enzyme-membrane filter system for continuous elimination of phenolic compounds from water

Activity Budget: $133,772

Activity Description:
We will consider α-alumina tubular membranes and polyacrylic acid-functionalized PVDF microfiltration membranes to construct the enzyme membrane filter using glutaraldehyde as the cross-linking agent. After the immobilization on membrane, we will determine the residual protein content in each supernatant by commercial Bradford reagent, or BAC (Bicinchoninic Acid) protein assay kit to estimate the amount of enzyme being immobilized. We will use the ABTS method to compare the enzyme activity in enzyme membrane filter with free laccases.

Following the method described earlier, we will coat the membrane-bound laccase with “water-like” ionic liquids. We will use the ABTS method to compare the enzyme activity in ionic liquid-coated enzyme membrane filter with free laccase.

By using the laccase membrane filter, we will evaluate the oxidation of model phenolic compounds (i.e., 2,6-dimethoxyphenol, guaiacol, and 4-chlorophenol) as quantified by the HPLC or colorimetric method. In addition to various free enzymes and substrates, we will evaluate different reaction conditions such as pH, temperature, and reaction time. We will collect water samples from lakers and rivers where phenolic compounds are detected, and run these water samples through the enzyme membrane biofilter to determine the effectiveness and efficiency of our system.

Activity Milestones:

Description	Approximate Completion Date
Construct laccase membrane reactor as a biofilter	September 30, 2028
Coat the membrane-bound laccase with “water-like” ionic liquids	December 31, 2028
Evaluate enzymatic biofilters for eliminating phenolic compounds	June 30, 2029

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	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 the research design and experiments to implement proposed activities. Their collaborator Dr. Gary Baker will be funded by different sources. Results generated in this project will enable a fundamental understanding of interactions between enzymes and phenolic compounds, and a practical aspect of how enzymes can be used as a biofilter to eliminate phenolic compounds. The preliminary data generated by this study will allow us to acquire a larger NSF or EPA grant that involves multiple institutions to tackle a bigger scale of phenolic compounds removal and elimination with a focus on surface water.

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.

The PI received a combined education in both chemistry (bachelor's degree) and chemical engineering (master's and doctoral degrees). He has over 20 years of experience in studying enzymatic reactions/processes using various enzymes and DNA-based hybrid catalysts. His research projects include biomass conversion to biofuels and biomaterials, biodiesel preparation, polyester synthesis, biosurfactants, asymmetric catalysis, desulfurization of liquid fuels, coal liquefaction, PFAS elimination, and carbon dioxide capture and utilization, etc. His research group developed new solvent systems (e.g., ionic liquids and deep eutectic solvents) that are highly compatible with enzymes; in particular, he designed so-called 'water-like' ionic liquids to activate and stabilize enzymes. His group provided some fundamental understanding of how ionic solvents interact with enzymes through different mechanisms such as ion specificity (Hofmeister's series), hydrogen bonding basicity, hydrophobicity, and polarity, etc. He has published about 100 journal papers and book chapters with high citations (h-index = 44 based on Google Scholar). His research has been supported by NSF, NIH, ACS-PRF, and LCCMR. He received the prestigious Henry Dreyfus Teacher-Scholar Award from Camille and Henry Dreyfus Foundation.

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, tuition and fringe benefits for three years)		Research design and conduct experiments as proposed			12.6%	1.5		$179,503
Two undergraduate students ($16.95 per hour, 10 hour per week, and 20 weeks each year; 3% increase in each year afterwards)		Receive research training and collect experimental data			0%	0.75		$20,961
Research associate at 0.5 FTE (salary and fringe benefits)		Design research experiments and mentor graduate and undergraduate students			20.6%	1.5		$122,266
							Sub Total	**$322,730**
Contracts and Services								
Sample analysis by DSC, TGA, XRD and SEM	Service Contract	Ionic liquid-coated enzymes will be characterized by MALDI-TOF MS experiments, scanning electron micrographs (SEM), and optical microscopy. Enzyme samples are analyzed by infrared, fluorescence emission spectra, and far-UV circular dichroism spectroscopy.				0.3		$9,000
							Sub Total	**$9,000**

Equipment, Tools, and Supplies								
	Tools and Supplies	Funds ($17,271 in Year 1, $17,000 in Year 2, and $17,000 in Year 3) are requested to purchase laboratory chemicals (phenolic compounds), reagents (acetone, methanol, and HPLC-grade water), enzymes (laccases), and reagents.	These chemicals and enzymes are needed to carry out the proposed experimental work.					$51,270
							Sub Total	**$51,270**
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 ($150), and meals and other costs ($400 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.					$3,000
							Sub Total	**$3,000**
Travel Outside Minnesota								
							Sub Total	**-**
Printing and Publication								
	Publication	Open-access journal publication cost	Publish research results in open-access journal, $2,000 per paper for two papers					$4,000
							Sub Total	**$4,000**
Other Expenses								

							Sub Total	**-**
							Grand Total	**$390,000**

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: $390,000

This amount accurately reflects total project cost?

Yes

Attachments

Required Attachments

Visual Component

File: dfe42b2f-18e.docx

Alternate Text for Visual Component

A membrane filter packed with enzymes is used to capture and convert phenolic compounds to nonhazardous insoluble products....

Supplemental Attachments

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

Title	File
Audit	dfdb1f4a-a9e.pdf
UMN Sponsored Projects Office Letter of Authorization to Submit	51a20be2-82c.pdf

Administrative Use

Does your project include restoration or acquisition of land rights?

No

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 proposal:

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