Environment and Natural Resources Trust Fund

M.L. 2026 Draft Work Plan

General Information

ID Number: 2026-055

Staff Lead: Noah Fribley

Date this document submitted to LCCMR: December 18, 2025

Project Title: Eliminating Phenolic Compounds from Water Using Enzyme Filter

Project Budget: $300,000

Project Manager Information

Name: Hua Zhao

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Project Reporting

Reporting Schedule: April 1 / October 1 of each year.

Project Completion: June 30, 2029

Final Report Due Date: August 14, 2029

Legal Information

Legal Citation:

Appropriation Language:

Appropriation End Date: June 30, 2029

Narrative

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

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 low concentrations could impart unpleasant tastes and odors to drinking water when it reacts with chlorine to form chlorophenols (taste thresholds 0.1–2 ppb and odor thresholds 10-300 ppb).

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 “enzyme-membrane 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" hydrophobic 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.

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

Activities and Milestones

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

Activity Budget: $96,395

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., phenol, 2,6-dimethoxyphenol, guaiacol, 4-chlorophenol, and bisphenol A) 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, 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: $100,904

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 enzyme's active sites. 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. Before IL coating, laccase will be immobilized on solid carriers such as silica sol-gel matrices, chitosan, and polystyrene beads by using glutaraldehyde as the cross-linking agent. 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, 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.

Activity Milestones:

Description	Approximate Completion Date
Construct laccases coated with functionalized ionic liquids	December 31, 2027
Evaluate the activity of immobilized laccases coated with functionalized ionic liquids	June 30, 2028
Conduct spectroscopic studies of enzymes coated with ionic liquids	June 30, 2028
Prepare a manuscript for peer-reviewed publication	June 30, 2028

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

Activity Budget: $102,701

Activity Description:
We will design an enzymatic membrane reactor (EMR) with a continuous stirred-tank reactor (CSTR) and an external crossflow microfiltration (MF) membrane. Immobilized laccases coated with ionic liquids from Objective 2 will be used in the CSTR. We will use α-alumina tubular, polyacrylic acid-functionalized PVDF microfiltration, and hollow-fiber-nylon microfiltration (MF) membranes. To address the potential pitfalls of MF membranes, two types of ultrafiltration (UF) ceramic membranes will be considered.

We will evaluate different phenolic compounds (phenol, 2,6-dimethoxyphenol, guaiacol, 4-chlorophenol, and bisphenol A) and analyze samples at various locations (CSTR feed, within CSTR, CSTR exit, and membrane permeate) by the HPLC or colorimetric method. In addition to different enzyme preparations and substrates, we will evaluate various reaction conditions such as pH (5–8), temperature (20–40 °C), enzyme stability, and residence time (as controlled by the flow rate).

We will collect water samples from lakes and rivers where phenolic compounds are detected, and run these water samples through the enzymatic membrane reactor to determine the effectiveness and efficiency of our system. We will evaluate the impact of natural organic matter (NOM) from natural water samples on enzymatic oxidation. We will conduct Techno-Economic Analysis (TEA) for EMR at various scales.

Activity Milestones:

Description	Approximate Completion Date
Construct laccase membrane reactor	September 30, 2028
Conduct the oxidation of phenolic compounds in CSTR catalyzed by immobilized and coated laccase	December 31, 2028
Evaluate enzymatic membrane reactor for eliminating phenolic compounds from water samples	June 30, 2029
Prepare a manuscript for peer-reviewed publication	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

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 the PI and students, and will be submitted for publication in peer-reviewed journals. Research results will be shared and discussed with the water 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 water pollution, biocatalysis, and green chemistry will be disseminated through our seminar series and our group website, and 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 collaborator Dr. Gary Baker will be funded by different sources. Results generated will enable a fundamental understanding of interactions between enzymes and phenolic compounds, and a practical aspect of how to integrate an enzymatic reactor with a membrane filter 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.

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
One undergraduate student ($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		$10,481
Researcher at 0.2 FTE (salary and fringe benefits)		Design research experiments and mentor graduate and undergraduate students			26.8%	0.6		$42,222
							Sub Total	**$232,206**
Contracts and Services								
Sample analysis	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		$3,000
							Sub Total	**$3,000**

Equipment, Tools, and Supplies								
	Tools and Supplies	Laboratory solvents including acetone, methanol, HPLC-grade water, and NMR solvents (such as CDCl3), etc.	These solvents are used to conduct the enzymatic oxidation reaction, and analyze the reaction products.					$15,000
	Tools and Supplies	Chemicals, enzymes and reagents	These are key phenolic compounds, enzymes (e.g., various laccases) and reagents (e.g., Karl Fisher titration reagent) needed to convert phenolic compounds.					$25,000
	Tools and Supplies	Laboratory consumables	Laboratory consumables such as reaction vials, HPLC vials, and cuvettes are needed to conduct the proposed experiments.					$11,794
	Tools and Supplies	General laboratory supplies	General supplies such as gloves, pipette and tips, and paper towels are needed for the daily operation and safety in the laboratory.					$6,000
							Sub Total	**$57,794**
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	**$300,000**

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	Supporting my collaborator Dr. Gary Baker's spectroscopic studies	Potential	$5,000
			Non State Sub Total	**$5,000**
			Funds Total	**$5,000**

Total Project Cost: $305,000

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
UMN Sponsored Projects Office Letter of Authorization to Submit	51a20be2-82c.pdf
Audit	dfdb1f4a-a9e.pdf
Zhao 2026-055 Research Addendum_Final	a444cc89-380.docx

Difference between Proposal and Work Plan

Describe changes from Proposal to Work Plan Stage

1) In Narrative section "Describe the opportunity or problem your proposal seeks to address", changing "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." to "Phenol at low concentrations could impart unpleasant tastes and odors to drinking water when it reacts with chlorine to form chlorophenols (taste thresholds 0.1–2 ppb and odor thresholds 10-300 ppb)."
2) In Narrative section "What is your proposed solution to the problem or opportunity discussed above?", changing "membrane-enzyme biofilter" to "enzyme-membrane biofilter".
3) In Narrative section "What is your proposed solution to the problem or opportunity discussed above?", changing ""water-like" ionic liquids" to ""water-like" hydrophobic ionic liquids".
4) In Activity 1, changing "model phenolic compounds (i.e., 2,6-dimethoxyphenol, guaiacol, and 4-chlorophenol)" to "model phenolic compounds (i.e., phenol, 2,6-dimethoxyphenol, guaiacol, 4-chlorophenol, and bisphenol A)".
5) In Activity 1, changing "fluorescence emission spectra" to "fluorescence emission".
6) In Activity 2, changing "fluorescence emission spectra" to fluorescence emission".
7) In Activity 3, changing "consider α-alumina tubular membranes and polyacrylic acid-functionalized PVDF microfiltration membranes" to "use α-alumina tubular, polyacrylic acid-functionalized PVDF microfiltration, and hollow-fiber-nylon membranes".
8) In Activity 3, changing "model phenolic compounds (i.e., 2,6-dimethoxyphenol, guaiacol, and 4-chlorophenol)" to "model phenolic compounds (i.e., phenol, 2,6-dimethoxyphenol, guaiacol, 4-chlorophenol, and bisphenol A)"
9) The original budget is reduced by $90,000 (decreasing $10,480 in undergraduate wages, $80,043 in researcher salary and fringe, and $6,000 in sample analysis; increasing $6,523 in laboratory supplies) to meet the recommended budget ($300,000).
10) Included potential in-kind contribution from the collaborator as non-ENRTF funds contributed to this project in the budget.
11) Added Dissemination Efforts.

12) Disaggregated the "tools and supplies" budget item into four sub-categories: (a) Laboratory solvents, (b) Chemicals,

enzymes and reagents, (c) Laboratory consumables, (d) General laboratory supplies. It is difficult to put quantities on these items, and they have different unit costs (e.g., enzymes are in small quantities like grams, but they are relatively costly).

Following the peer review process, some changes are made based on reviewers' comments:

13) The Project Summary is edited as "This project will study the biodegradation of phenolic compounds in water by an enzyme (laccase), and design an enzymatic membrane reactor/filter to capture and destroy phenolic compounds in Minnesota waters."
14) In Activity 2, deleted "IL-coated enzymes are known for improved reactivity and stereoselectivity", and also deleted "This will enable a molecular-level understanding of how enzyme coating impacts the enzyme activity". We added " Before IL coating, laccase will be immobilized on solid carriers such as silica sol-gel matrices, chitosan, and polystyrene beads by using glutaraldehyde as the cross-linking agent".
15) In Activity 3, following reviewers' comments, we re-design the enzymatic membrane reactor, thus we added "We will design an enzymatic membrane reactor (EMR) with a continuous stirred-tank reactor (CSTR) and an external crossflow microfiltration (MF) membrane. Immobilized laccases coated with ionic liquids from Objective 2 will be used in the CSTR", "We will evaluate different phenolic compounds (phenol, 2,6-dimethoxyphenol, guaiacol, 4-chlorophenol, and bisphenol A) and analyze samples at various locations (CSTR feed, within CSTR, CSTR exit, and membrane permeate) by the HPLC or colorimetric method. In addition to different enzymes preparations and substrates, we will evaluate various reaction conditions such as pH (5–8), temperature (20–40 °C), enzyme stability, and residence time (as controlled by the flow rate)", and "We will consider the impact of natural organic matter (NOM) from natural water samples on enzymatic oxidation. We will conduct Techno-Economic Analysis (TEA) for EMR at various scales". These new additions replaced other experimental sections in Activity 3.
16) In Activity 3, based on the above changes, we updated the milestones: (a) Construct laccase membrane reactor, (b) Conduct the oxidation of phenolic compounds in CSTR catalyzed by immobilized and coated laccase, and (c) Evaluate enzymatic membrane reactor for eliminating phenolic compounds.
17) In Long-Term Implementation and Funding, we updated "a practical aspect of how to integrate an enzymatic reactor with a membrane filter ...".

18) Added "Prepare a manuscript for peer-reviewed publication" as a milestone for Activities 2 and 3.
19) Removed the named entities from the sample analysis budget line from the entity description.

20) Based on the reviewer's feedback, I made a revision to research addendum and also aligned the work plan with these changes (see my comments above from #13 to #17 for these changes).

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