Environment and Natural Resources Trust Fund

2027 Request for Proposal

General Information

Proposal ID: 2027-033

Proposal Title: Removing Toxic Polychlorinated Biphenyls (PCBs) from Minnesota Waters

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 develop an effective process for removing a contaminant, known as polychlorinated biphenyls (PCBs), from Minnesota waters by forming aggregates with lignin fragments when catalyzed by natural enzymes.

ENRTF Funds Requested: $403,000

Proposed Project Completion: June 30, 2030

LCCMR Funding Category: Water (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.

Polychlorinated biphenyls (PCBs) were heavily produced in the US between 1930s and 1970s for their wide applications. Their high chemical stability makes them resistant to natural degradation processes; therefore, PCBs still accumulate in surface water, air, soil, sediments, and living organisms in large quantities. PCBs are probable human carcinogens (causing cancers) and are associated with hypertension, diabetes, obesity, and other diseases in humans and animals.

The FDA sets a tolerance level of PCBs as 2 ppm in fish, 10 ppm in paper food packaging, and 0.2–3.0 ppm for all foods. The EPA limits PCBs to a maximum contaminant level (MCL) of 0.5 ppb (parts per billion) in drinking water. According to the Minnesota Department of Health, fish in Lake Superior and major rivers such as the Mississippi River contain PCBs. Minnesota Pollution Control Agency (MPCA) released the 2024 Minnesota Impaired Waters List, and found PCBs in fish tissue exceeding the standard (>0.22 mg/kg) from multiple lakes (e.g., Lake Superior) and rivers (e.g., St. Louis River). Conventional PCB removal methods (e.g., solvent extraction, landfarming, thermal desorption, and microbial degradation) all face different challenges such as costly, labor-intensive, and unsuitable for large-scale operations.

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.

In nature, degradation and humification are two opposite processes: to break down, or to form natural organic matter (NOM). Degradation breaks down macromolecules into smaller molecules leading to mineralization; on the contrary, humification converts small organic molecules to macromolecular/supramolecular structures (known as ‘humic substances’). Our proposed oxidative coupling of PCBs is a humification process catalyzed by metal oxides or oxidative enzymes, producing PCB polymers ("aggregates") that have little ecotoxicity and can be easily removed.

The PCB humification process often involves the use of a co-substrate to facilitate its cross-coupling reaction with PCBs. Most co-substrates used are phenolic compounds. However, phenolic compounds themselves are pollutants to water because they can potentially cause cancer, endocrine dysregulation, genetic malformations, and the destruction to immune system, tissue, central nervous system, and internal organs. In nature, phenolic compounds in water come from the degradation of natural substances (e.g., lignin, algae, fish, and dead animals). In particular, phenolic fragments from plant lignin degradation are effective co-substrate for PCB removal. Therefore, our goal is to use these natural phenolic fragments from lignin degradation as co-substrates to form aggregates with PCBs via enzymatic coupling, so that both PCBs and phenolic compounds are removed from Minnesota waters.

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 benign and effective method for capturing and elimination of toxic polychlorinated biphenyls (PCBs) (and phenolic compounds) from Minnesota rivers, lakes, and sediments (due to the high volume of industrial activities involving PCBs in the past). Our reactive filtration system will not only capture PCBs, but also convert them to environmentally benign components to avoid their accumulation and contamination in Minnesota’s water resources and aquatic animals (e.g., fish).

Activities and Milestones

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

Activity Budget: $129,910

Activity Description:
Although horseradish peroxidase is an effective enzyme for oxidizing PCBs, it requires hydrogen peroxide (H2O2) to complete the reaction, so it is difficult for large-scale implementation. Thus, it is advantageous to use laccase as an alternative enzyme because it only requires oxygen from air to oxidize PCBs. We will evaluate several types of commercial laccases (from Trametes versicolor, Agaricus bisporus, Aspergillus sp., and Rhus vernicifera). Enzyme activities 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 PCBs [i.e., 3-chlorobiphenyl (PCB-2), 2,2´-dichlorobiphenyl (PCB-4), 2,4,4'-trichlorobiphenyl (PCB-28), and 2,2',5,5'-tetrachlorobiphenyl (PCB-52)] as quantified by the HPLC method. In addition to different free enzymes and substrates, we will evaluate different reaction conditions such as pH, temperature, and reaction time. The resulting polymers will be analyzed by Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM).

We will use infrared, fluorescence emission, and far-UV circular dichroism (CD) spectroscopy tools to probe structural changes of free laccases under different 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, 2027
Evaluate the enzymatic oxidation of different PCB compounds using free laccases	June 30, 2028
Carry out spectroscopic studies of structural changes of free laccases under different reaction conditions	June 30, 2028

Activity 2: Conduct the enzymatic cross-coupling of PCBs with lignin fragments

Activity Budget: $135,051

Activity Description:
To improve the stability and reusability of enzymes, we will immobilize laccases on solid carriers (such as silica sol-gel matrices, chitosan, and polystyrene beads) using glutaraldehyde as the cross-linking agent. Immobilized enzymes will be characterized by MALDI-TOF MS experiments to confirm the protein peak and scanning electron micrographs (SEM) to determine their surface properties. We will evaluate the activities of immobilized laccases by the ABTS assay described in Activity #1, and compare them with free enzymes. Most active enzymes will be selected for the following studies.

To further improve the oxidative coupling of PCBs, we will select several lignin-derived compounds (such as syringaldehyde, catechol, p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol) as cross-coupling agents. This will facilitate the polymerization of PCBs by enzymatic cross-coupling with these agents. We will use selected free and immobilized laccases screened above to conduct the oxidative cross-coupling of model PCB compounds (described in Activity #1) as monitored by HPLC. We will examine different reaction conditions such as pH, temperature, and reaction time. The resulting polymers will be analyzed by Fourier Transform Infrared Spectroscopy (FT-IR) and scanning electron microscopy (SEM).

Activity Milestones:

Description	Approximate Completion Date
Immobilize laccases on solid support and evaluate their activities	December 31, 2028
Evaluate the cross-coupling of PCBs catalyzed by immobilized laccases	June 30, 2029
Prepare a manuscript for peer-reviewed publication	June 30, 2029

Activity 3: Design an enzymatic membrane reactor for continuous removal of PCBs from water

Activity Budget: $138,039

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 from Activity #2 will be used in the CSTR. We will evaluate α-alumina tubular, polyacrylic acid-functionalized PVDF microfiltration, and hollow-fiber-nylon microfiltration (MF) membranes in the membrane filter.

We will evaluate different PCB compounds described in Activity #1 and various cross-coupling agents described in Activity #2, and analyze samples at various membrane reactor locations by the HPLC 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). The resulting polymers will be analyzed by Fourier Transform Infrared Spectroscopy (FT-IR) and scanning electron microscopy (SEM).

We will collect water samples from lakes (e.g., Lake Superior), rivers (e.g., St. Louis River), and their sediments where PCB are detected, and run these samples through our EMR to determine the effectiveness and efficiency. We will examine the impact of natural organic matter (NOM) including lignin fragments from water samples on enzymatic oxidation. We will conduct Techno-Economic Analysis (TEA) for the membrane reactor at various scales.

Activity Milestones:

Description	Approximate Completion Date
Construct an enzymatic membrane reactor	September 30, 2029
Conduct the oxidative cross-coupling of PCBs in CSTR catalyzed by immobilized laccases without membrane filter	December 31, 2029
Evaluate the enzymatic membrane reactor for continuous removal of PCBs from Minnesota waters	June 30, 2030
Prepare a manuscript for peer-reviewed publication	June 30, 2030

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 a 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 UMN seminar series and our UMN 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?
Dr. Hua Zhao will lead research design and experiments to implement proposed activities. His collaborator Dr. Gary Baker will be funded by different sources. Results generated in this project will provide a fundamental understanding of interactions between enzymes and PCB compounds, and a practical aspect of how to integrate the enzymatic reactor with a membrane filter to eliminate PCB compounds. 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 large scale of PCB compounds removal with a focus on surface water and sediments.

Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Terminating PFAS-Type Pesticides via Enzyme Cocktails	M.L. 2025, First Special Session, Chp. 1, Art. 2, Sec. 2, Subd. 04k	$297,000

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 Professor of Bioproducts and Biosystems Engineering at University of Minnesota. He received his Ph.D.

degree in chemical engineering from New Jersey Institute of Technology (NJIT). He has more than 20 years of experience working with funded research projects focusing on environmental themes (such as desulfurization of liquid fuels, PFAS biodegradation, the removal of phenolic compounds, and carbon capture and utilization) and using enzymes as biocatalysts for these conversions. His research group specializes in developing different enzymatic systems for eliminating environmental contaminants and converting bioresources to valuable products. He has mentored many undergraduate and graduate (MS and Ph.D.) students on various research projects, and trained them on experimental skills, instrument operations, and problem-solving skills.

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			13.1%	1.5		$188,327
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%	0.27		$21,587
Researcher at 0.25 FTE (salary and fringe benefits) - appointment dependent on project funding		Design research experiments and mentor graduate and undergraduate students			26.8%	0.75		$93,341
							Sub Total	**$303,255**
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				0.6		$9,000

		spectroscopy. Small molecules are analyzed by GC-MS and NMR spectroscopy.						
							Sub Total	**$9,000**
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.					$40,000
	Tools and Supplies	Laboratory consumables	Laboratory consumables such as reaction vials, HPLC vials, and cuvettes are needed to conduct the proposed experiments.					$20,000
	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.					$8,745
							Sub Total	**$83,745**
Capital Equipment								
							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	**$403,000**

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: $408,000

This amount accurately reflects total project cost?

Yes

Attachments

Required Attachments

Visual Component

File: 1a1bbeff-17e.docx

Alternate Text for Visual Component

An enzyme membrane reactor is used to capture and convert PCBs along with phenolic compounds to form nonhazardous insoluble aggregates....

Supplemental Attachments

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

Title	File
Letter of Approval to Submit	0b6117de-f6b.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