Final Abstract

Final Report Approved on February 6, 2025

M.L. 2022 Project Abstract

For the Period Ending June 30, 2024

Project Title: PFAS Fungal-Wood Chip Filtering System

Project Manager: Jiwei Zhang

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Funding Source:

Fiscal Year:

Legal Citation: M.L. 2022, Chp. 94, Sec. 2, Subd. 08f

Appropriation Amount: $189,000

Amount Spent: $188,475

Amount Remaining: $525

Sound bite of Project Outcomes and Results

We developed a set of toolkits and knowledge towards inventing the uses of fungal species as a lost-cost filtering system for remediating per and poly-fluoroalkyl substances (PFASs). Combining Catalytic Microwave-Assisted Pyrolysis (CMAP) to mineralize organic fluorine, we attempted to build a treatment-train method for PFAS deconstruction from impacted environments.

Overall Project Outcome and Results

Large-scale manufacturing and disposal of fluorinated chemicals have led to global pollution by per and poly-fluoroalkyl substances (PFASs) that will require novel remediation techniques and investigation for their environmental fates. This project investigated the fugal roles in responding to and transforming these persistent fluorocarbons in the purpose of developing a low-cost bioremedial method of PFAS chemicals. To do this, we built a set of cheap, in-house tools to facilitate the chemical analysis of PFAS and related degradation products, which filled the tool gaps required for studying fungal PFAS degradation. With this, we screened the defluorination capacities of a large number of fungal species, which revealed a unique class of fungi that cause “white-rot” type of wood decay has developed an inherent defense mechanism for fluoride and fluorocarbon chemicals, and identified a dehalogenated PFCA structure that provokes C-F

cleavage. RNA-seqs were then conducted to further understand the fundamental mechanisms used by fungi to respond and degrade PFAS chemicals. In addition to screening the fungal capacities of degrading PFAS, we have also optimized the PFAS-sequestering abilities of a mycelium-wood filtering system, using long-carbon legacy compound PFOA and short-carbon chain PFAS crotonic PFCA as the representatives. Our research found the 50-80% removal rates of the test PFASs at the level of 1 µM (ppm) by the fungal filtration in sorption tube tests. Using CMAP for pyrolysis of the sequestered PFOA in mycelium/wood absorbent resulted in the deconstruction of 87% and 99% PFAS in bio-oil at temperatures of 400°C and 500°C, respectively. Overall, our research set off a foundation for testing the field uses of fungal-filtering system for PFAS remediation in the impacted landfill, water, and navy site and other environments.

Project Results Use and Dissemination

Discovering fungal removal of PFAS is novel to the research field, which will not only benefit us in understanding the fungal roles in recycling fluorinated carbons in nature, but it will also allow the development of alternative PFAS remediation techniques. This project generates 4 publications to disseminate these research findings, with 2 are currently under review and 2 are in preparation. The project team also reached out to industry partners, K-12, and governmental stakeholders, and gave five professional presentations to make the research publically accessible. While disseminating the results, we've seriously acknowledged the relevant financial support of the trust funds.

Environment and Natural Resources Trust Fund

M.L. 2022 Approved Final Report

General Information

Date: February 7, 2025

ID Number: 2022-188

Staff Lead: Noah Fribley

Project Title: PFAS Fungal-Wood Chip Filtering System

Project Budget: $189,000

Project Manager Information

Name: Jiwei Zhang

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Project Reporting

Final Report Approved: February 6, 2025

Reporting Status: Project Completed

Date of Last Action: February 6, 2025

Project Completion: June 30, 2024

Legal Information

Legal Citation: M.L. 2022, Chp. 94, Sec. 2, Subd. 08f

Appropriation Language: $189,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to identify, develop, and field-test various types of waste wood chips and fungi to sequester and degrade PFAS leachate from contaminated waste sites. This appropriation is subject to Minnesota Statutes, section 116P.10.

Appropriation End Date: June 30, 2025

Narrative

Project Summary: Develop and implement a fungal filtering system that combines the benefits of both waste wood chips and soil fungi to sequester and degrade PFAS leachate from contaminated waste sites.

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

Per- and Polyfluoroalkyl Substances (PFAS) are a large group of emerging environmental contaminants that are globally ubiquitous. These "forever chemicals" are highly recalcitrant and possess the capability to persist in the environment and accumulate in living organisms. PFAS exposure is increasingly being linked to a variety of adverse health outcomes. Regarding this, strategies to remediate PFAS polluted environments are urgently needed.
Not only the water systems but also soils have been found to be large reservoirs of PFAS chemicals, even in locations distant from any known source. The ubiquitous presence of PFAS in soils around the world is concerning and will likely have long-term ramifications on attempts to remediate contaminated waters. In Minnesota, MPCA (Minnesota Pollution Control Agency) and MDH (Minnesota Department of Health) have discovered PFAS pollution throughout the state since the early 2000s. Among these sites, landfills represent a large repository for PFAS, with 98 out of 101 MPCA tested landfill sites found PFAS and alarmingly 59 sites had detectable levels exceeding health guidelines. A cost-effective means to intercept leachate from landfills and remove PFAS pollutants, before they can escape to groundwater, will be critical for conserving Minnesota's environment.

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.

Using woodchips which have been proven to filter other pollutants such as nitrogen and metals from water combined with wood decay fungi that have demonstrated good results in removing contaminants.
We seek to develop a mycoremediation system as an alternative method that integrates fungi with low-cost forest residues to filter and degrade PFAS. Sequestration and degradation of PFAS by the "fungal-woodchips" filtering system will be estimated after fungal acclimation in cultures spiked with both wood substrate and PFAS compounds. Fungal pathways involved in PFAS degradation will be investigated through isotopic metabolites mapping, thus enabling us to dissect the fundamental bioprocesses and guide their industrial applications. PFAS that is sequestered by the filtering system will be sent to the microwave-assisted gasification system for further destruction ("pyrolysis"), eliminating any remaining pollutants. A potential biochar byproduct from pyrolysis can be recycled as the PFAS absorption media, further improving the values of the "fungal-woodchips" filtering system.

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?

Development of a cost-effective method to intercept PFAS before it enters groundwater and degrades it in-situ using wood-decay fungi to begin controlling PFAS pollution from the array of contaminated sites throughout Minnesota. Establishing a platform for the further study of PFAS bioremediation to inform long-term efforts to remove PFAS from soil and water, and providing insights into fungal metabolic capabilities that can be applied to improve not just PFAS remediation but a variety of other applications.

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: Test fungal capacities in sequestering and degrading PFAS

Activity Budget: $77,200

Activity Description:
The goal of activity one is to identify fungal species able to degrade PFAS. Pure cultures of wood decay fungi and fungal consortia in landfill leachate and sewage sludge will be challenged by spiking PFAS substance in woodchips media, acclimating their capacities of PFAS sequestration and degradation monitored by GC/LC-MS and ion-selective electrode (for F-), respectively. Up to two dozen wood-decay fungi will be inoculated onto liquid or solid growth media containing the perfluorooctanoic acid (PFOA), a representative PFAS, and wood chips to test fungal capacities to degrade PFAS via monitoring F- liberation (i.e., defluorination). The best performance species will be further optimized for the cultural conditions to improve the defluorination rate, PFOA degradation will be confirmed by LC-MS/MS, and PFOA sequestration will be tested by measuring the acetonitrile extracted residuals in wood-mycelium. A lab-scale continuous microwave-assisted gasification (CMAG) system will be used to convert the woodchips/fungal biomass from the above cultures, and the composition of the syngas and biochar will be analyzed to confirm the complete removal of PFAS.

Activity Milestones:

Description	Approximate Completion Date
Screen Fungal Defluorination Capacities	December 31, 2022
Optimize Cultural Conditions for PFAS Defluorination	April 30, 2023
Confirm the PFAS Degradation and Sequestration by LC-MS	June 30, 2023
Test the Combined Use of CMAG for PFAS removal	August 31, 2023

Activity 2: Investigate the fungal degradative pathways of PFAS

Activity Budget: $54,400

Activity Description:
In this activity, the best-performing wood decay fungi obtained from the defluorination screening process will be used to further study the degradative mechanisms. Fungal strains will be cultured in liquid or solid Highley's media spiked with C13-labeled PFOA, facilitating metabolite identification. PFAS carbons incorporated into degradation metabolites will be identified during cultivation stages using HPLC in tandem with hybrid quadrupole orbitrap high-resolution MS, thus reconstructing the degradative path. Ultra-high mass accuracy of the mass spectrometer will be employed to enable us to reveal the exact molecular formula of molecular ions. Reported PFAS metabolites throughout the literature will be used to enhance the annotation confidence of PFAS degradation products. Enzyme activities will be measured to map the metabolite pathway to understand the degradative mechanisms. We envision this objective will be “high-reward” and will provide first-ever insights on fungal pathways of PFAS degradation. This fundamental understanding will enable us to optimize the remediation method by such as targeting culturing conditions or further investigating gene pathways for genetic engineering.

Activity Milestones:

Description	Approximate Completion Date
Identify the PFAS Degradation Pathway Metabolites	September 30, 2023
Measure the Enzyme Activities During PFAS Degradation	October 31, 2023
Reconstruct the Degradative Path of PFAS	December 31, 2023

Activity 3: Monitor the performance of the "fungal-woodchips" filtering system on landfill leachate

Activity Budget: $57,400

Activity Description:

Working with collaborators from Wenck Environmental Consulting suitable landfill field sites near the Twin Cities will be identified. Lab-scale tests followed by field setup will be performed to remediate PFAS using the fungal-woodchip filtering system. The lab-scale reactors to test PFAS removal from the contaminated field samples will be first designed and tested. Landfill leachates containing PFAS compounds will be added to fungal pre-colonized woodchips in flask reactors for monitoring PFAS degradation using LC-MS/MS, and key factors will be optimized to improve the remediation efficiency. For field setup, Wenck will support the Zhang lab by providing access to the site and equipment necessary for the installation of the pilot filtering system. Landfill leachate will filter through the fungi-woodchip application and PFAS concentrations will be monitored over the course of the experiment. Long-term monitoring of the field setup will be leveraged by other funds. Pyrolysis via CMAG will be performed in tandem with the filtration system to further remove PFAS and recycle wood chips to produce biochar. Pyrolysis will be performed using the equipment and expertise of Dr. Ruan who has agreed to allow us the use of the well-established pyrolysis lab to produce biochar.

Activity Milestones:

Description	Approximate Completion Date
Consult with Wenck-Stantec For Field Test	September 30, 2022
Lab-scale Test and Optimization with Landfill Leachate	February 28, 2024
Set Up Field Test for the Long-term Monitoring of PFAS Leachate	May 31, 2024
Final Data Compilation and Project Write-up	June 30, 2024

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Roger Ruan	University of Minnesota	Co-PI on the project, working on using "pyrolysis" to heat treat the sequestered PFAS for the complete removal.	Yes
Ed A. Matthiesen	Wenck Environmental Consulting	Industrial Partner	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.
Our research findings will be widely disseminated through published journal articles, community outreach through the UMN Biotechnology Institute, and K-12 partners. Our work will also be shared with industry partners Wenck-Stantec and SKB environmental, where findings will be used to further the development of remediation methods for PFAS compounds. In addition to industry and community partners, the audience for our work will also include other researchers and institutions investigating the environmental effects of PFAS, PFAS degradation, and fungal metabolism. The insights into PFAS effects on fungi, fungal degradation pathways, and PFAS degradation byproducts will be useful to a wide range of fellow researchers and industry professionals.
In our dissemination efforts, we will acknowledge the financial support from the Minnesota environment and natural resources trust fund in project publications,
signage, and other public communications and outreach related to work completed using the appropriation. The acknowledgment will occur, as appropriate, through the use of the trust fund logo or the inclusion of language attributing support from the trust fund. We understand this is important to enable the public to know about the support of the Environment and Natural Resources Trust Fund and to make the funding uses transparent.

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?
The data gathered will be used to inform further research into the genes and metabolic pathways involved in PFAS degradation and to further improve the field application. Studies of the molecular mechanisms of PFAS degradation will be further investigated by applying for other related academic grants from such NSF.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount	$ Amount Spent	$ Amount Remaining
Personnel										
Assistant Professor		PI			37.1%	0.3		$32,605	-	-
Graduate Student Assistant		Assist PI in carrying out project			25.1%	2		$53,837	-	-
Professor/Director		Co-PI			36.5%	0.04		$9,995	-	-
Postdoc Researcher		Assist PI in carrying out project			25.4%	0.5		$45,200	-	-
Undergraduate Student Assistant		Assist PI to carry out project			0%	0.85		$24,000	-	-
							Sub Total	**$165,637**	**$165,119**	**$518**
Contracts and Services										
Lab services	Subaward	The UofMinnesota Center for Mass Spectrometry and Proteomics or the Bridget Ulrich lab at Natural Resources Research Institute (Duluth, MN) will be performing LC/MS to test PFAS degradation, includes cost of shipping samples to Duluth				1		$2,805	$2,798	$7
							Sub Total	**$2,805**	**$2,798**	**$7**
Equipment, Tools, and Supplies										
	Tools and Supplies	Laboratory supplies and tools in year 1, including incubator or small equipment (<5,000$), chemicals, enzymes, dishes, flasks, and other lab consumables related to performing fungal tests on PFAS degradation	Materials to setup and perform laboratory procedures for culturing, screening, laboratory scale testing, and analysis					$10,000	$10,000	-
	Tools and Supplies	Laboratory supplies in year 2, including chemicals, enzymes, dishes, flasks, and other lab consumables related to performing	Install lab and field applications and collect samples to facilitate					$10,023	$10,023	-

		both lab- and field-scale PFAS degradation	monitoring of PFAS degradation							
							Sub Total	**$20,023**	**$20,023**	**-**
Capital Expenditures										
							Sub Total	**-**	**-**	**-**
Acquisitions and Stewardship										
							Sub Total	**-**	**-**	**-**
Travel In Minnesota										
	Miles/ Meals/ Lodging	400 miles/yr, two people approx. 12 trips (up to six trips for installation and for monitoring)	Travel to and from field site for installation and monitoring (year 2)					$535	$535	-
							Sub Total	**$535**	**$535**	**-**
Travel Outside Minnesota										
							Sub Total	**-**	**-**	**-**
Printing and Publication										
							Sub Total	**-**	**-**	**-**
Other Expenses										
							Sub Total	**-**	**-**	**-**
							Grand Total	**$189,000**	**$188,475**	**$525**

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	$ Amount Spent	$ Amount Remaining
State						
			State Sub Total	**-**	**-**	**-**
Non-State						
In-Kind	Waived UMN overhead	Waived UMN overhead	Secured	$102,236	$102,236	-
			Non State Sub Total	**$102,236**	**$102,236**	**-**
			Funds Total	**$102,236**	**$102,236**	**-**

Attachments

Required Attachments

Visual Component

File: 145692a2-310.pdf

Alternate Text for Visual Component

The use of a combination of woodchips and wood-decay fungi to control PFAS leachate originating from contaminated waste sites. Rain or irrigation carries pollutants that can then contaminate areas outside the waste site eventually entering groundwater. This leachate is filtered through the fungal biofilter installed around the periphery...

Supplemental Attachments

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

Title	File
Wenck Letter of Support	d0d00070-f03.pdf
CMSP Letter of Support	b9d916f7-fdb.pdf
LCCMR Proposal	27b16835-b8a.pdf
Institutional Approval to submit	966a5844-045.pdf
Approved Research Addendum	b475654b-ce4.pdf
Background Check Certification Form_2022-05-23	5f13f7f2-e7a.pdf
Final Deliverables	aa67ede9-250.docx

Difference between Proposal and Work Plan

Describe changes from Proposal to Work Plan Stage

To whom it may concern:
The work plan has been revised and updated according to LCCMR comments.
Please help proceed with the research plan.
Thank you!
Regards,
Dr. Jiwei Zhang

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

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

N/A

Work Plan Amendments

Amendment ID	Request Type	Changes made on the following pages	Explanation & justification for Amendment Request (word limit 75)	Date Submitted	Approved	Date of LCCMR Action
1	Amendment Request	• Budget - Personnel	The first-year personnel budget is adjusted according to the real salary needs of a postdoc and two UG assistants. This needs to switch the budget of one graduate student's salary to support a 6-month postdoc and two 10-month undergraduate students.	September 7, 2022	Yes	September 7, 2022
2	Amendment Request	• Budget - Personnel • Budget - Professional / Technical Contracts • Budget - Capital, Equipment, Tools, and Supplies • Budget - Travel and Conferences • Budget - Non-ENRTF Funds Contributed	Due to the departure of one of the Co-Pi's personnel leaving the country, and under spending on some categories, re-budgeting for the last period of the project is requested. Adding funds to lead PI summer salary, as well as other changes. Previously thought we would be adding a contract position, but that did not occur. This caused delay in this submission.	May 28, 2024	Yes	May 28, 2024

Status Update Reporting

Final Status Update August 14, 2024

Date Submitted: February 5, 2025

Date Approved: February 6, 2025

Overall Update

This project aims to develop a treatment-train method for PFAS remediation. This includes a consolidated filtering system that combines mycelium-colonized wood substrate's PFAS-degrading/absorbing capacities to remove PFAS pollutants from environments and a following Catalytic Microwave-Assisted Pyrolysis (CMAP) to mineralize organic fluorine. To achieve this, we have built a set of in-house tools to advance the PFAS remediation research, which includes a high-throughput method for testing fungal resistance of PFAS chemicals, ISE (Ion Selective Electrode) for fungal defluorination (deF) test, sloid phase purification (SPE) procedure of PFAS from fungal cultures, PFAS quantification methods (LC-MS and NMR), and CMAP pyrolysis of PFAS. With these tools, we have discovered a particular type of wood decay fungi that can resist PFAS and F-, the structure-functional relationship of defluorinable PFAS, and the optimized conditions for fungal deF. We have tested the physiological responses of fungal cells to PFAS chemicals by RNA-seq. We have also optimized the PFAS absorbing abilities of mycelium-wood filters, using PFOA and crotonic PFCA as the representative PFAS compounds. With the obtained systematic understanding of the mycelium filtering system, we are setting up field experiments in the impacted landfill and navy site to further advance the fungal-based remedial technique for PFAS remediation.

Activity 1

We accomplished the wood decay fungal species screenings and monitored their tolerance capacities by cytotoxicity test on PFAS chemicals. White rot fungi were found to possess significant PFOA-tolerating abilities. We then selected representative wood-decay fungi to test their deF capacities and found they could deF the PFCA chemicals with the unsaturated carbon bond structures, which allowed us to reveal a “structure-functional” relationship of PFAS deF. Fungal deF rates were further optimized by changing the carbon nutrients and the solid-state fermentation (SSF), which demonstrated a 100% deF of crotonic PFCA at the original concentration of 100 µM. 19F-NMR tests validated the complete removal of the parent compound of crotonic PFCA by the fungal culture. To further study the PFAS degradation products, we used SPE to purify PFCA, and the purified samples were sent to Dr. Men's lab at UC Riverside to use HRMS/MS to quantify and characterize residual PFAS and its products. Using the mycelium-wood filters for the absorption test, we found 40-70% removal rates of PFOA and crotonic PFCA by the system. Specific absorbing kinetics were then studied. The absorbing capacities of the fungal system were also compared with that of the raw wood, biochar, and activated carbon absorbents.
(This activity marked as complete as of this status update)

Activity 2

We examined the potential fungal deF pathways with the defluorinable crotonic PFCA as the substrate. Firstly, we evaluated if the ligninolytic enzymes of T. versicolor – a fungus that can deF PFAS, including laccase, lignin peroxidase, and manganese peroxidase, can mediate the deF by creating the G. trabeum mutants that can heterologously express these enzymes. Unfortunately, no deF was found for all these mutants, which ruled out the deF functions of fungal ligninolytic oxidoreductases. We then measured the extracellular crude enzymes of T. versicolor for their deF activities with a cell-free system, and the results showed no deF again. These results suggested that T. versicolor might have adopted an intercellular mechanism to deF PFAS that is worth further exploitation. To rebuild the biochem degradation pathway, we prepared the fungal degraded PFAS samples (PFOA and Crotonic PFCA) using optimized SPE, and the samples were analyzed for the transformation products (TPs) by HRMS/MS. In addition, RNA-seqs were conducted to study the fungal gene pathways involved in degrading PFAS chemicals. Through these activities, we expect to advance

the fundamental understanding of PFAS-degrading pathways in fungal species and discover PFAS degradative enzymes, thus guiding the development of fungal methods for PFAS remediation.
(This activity marked as complete as of this status update)

Activity 3

We used a mock sample of mycelium/wood absorbent and PFOA for testing pyrolysis for deconstructing FPAS. Three streams were generated and monitored after the pyrolysis: bio-oil, biochar, and biogas. The results measured by LC-MS showed that the pyrolysis has degraded PFOA from the sample, reducing its concentration in the bio-oil by 87% and 99% at temperatures of 400°C and 500°C, respectively. The presence of fluorinated compounds in the gases was detected at minimal concentrations. A minor fraction of fluorinated compounds migrates to the bio-oil, with elevated temperatures facilitating a decrease in their levels in bio-oil and an increase in the gas phase. However, a less efficient extraction technique hampered the analysis of the biochar. Efforts are underway to refine these methods for bio-char analysis, aiming to establish a detailed mass balance of fluorine throughout the pyrolysis process of PFOA-enriched wood. In addition, field tests of PFAS absorption were initiated at the Naval Industrial Reserve Ordnance Plant (NIROP) sites in Fridley and South Andover/WDE landfills by coordinating with MPCA and EPA. With these, we are poised to develop a treatment train method that combines mycelium/wood filtration and pyrolysis for PFAS removal from the impacted areas such
(This activity marked as complete as of this status update)

Dissemination

The discovery of fungal deF mechanisms, per our knowledge, is novel to the research field. This finding will not only benefit us in understanding the fungal roles in recycling the organic F in nature, but it will also shed light on developing more degradable alternative PFAS materials. Understanding fungal degradation and absorption mechanisms on PFAS will help us develop the mycoremediation method. Till now, we've made efforts for dissemination: 1) We reached out to the industry partners Geosyntec Consultants, Inc., Wenck-Stantec, and SKB environmental and stakeholders at MPCA and EPA, collaborated on the field uses of our fungal methods; 2) We gave the five presentations speaking of PFAS mycoremediation at the professional meetings (e.g., annual meetings of 2022 ASM, 2023 society of industrial of microbiology and biotechnology, 2024 IonE); 3) We wrote and will be writing four manuscripts out this LCCMR supported project to report our discoveries of the fungal remedial methods, including one is under review at Nature-Communications Biology. For these disseminations, we've seriously acknowledged the relevant financial support of the trust funds.

Status Update Reporting

Status Update March 1, 2024

Date Submitted: May 7, 2024

Date Approved: May 20, 2024

Overall Update

This project aims to develop an integrated method for a consolidated filtering system that combines the PFAS-degrading capacities of fungal species and PFAS absorption by wood substrate to remove PFAS pollutants from environments, followed by a Catalytic Microwave-Assisted Pyrolysis (CMAP) to completely mineralize organic fluorine. To achieve this, we have built a series of in-house toolkits that can allow the detection of fungal resistance of PFAS chemicals, defluorination (ISE: Ion Selective Electrode), sloid phase purification (SPE) of PFAS from wood cultures, PFAS quantification (LC-MS and NMR), characterization of transformative products of PFAS degradation (HR-MS), and CMAP mineralization of absorbed organic F. With these tools, we have discovered a particular type of wood decay fungi that can resist PFOA and F-, the deF of certain PFAS compounds, and the optimized conditions for fungal deF. We have also tested the PFAS-absorbing capacities of fungal-wood materials using FPOS and PFOA as the model compounds. Currently, we are testing the thermal degradation of the absorbed PFAS using mock samples to better monitor the conditions for pyrolysis. To understanding the systematic responses of fungal cells to PFAS chemicals, we have extracted the RNA samples for RNA-seq analysis as a following work.

Activity 1

We accomplished the fungal screenings on PFAS chemicals and tested their tolerance capacities. A group of wood decay fungi were discovered for their significant PFOA-tolerating abilities. We selected three representative wood-decay fungi to test their deF capacities and found all could deF the PFCA chemicals with the unsaturated carbon bond structures. Fungal deF rates were further optimized by changing the carbon nutrients and the solid-state fermentation (SSF). When a SSF condition with MC = 83% (w/w) was applied, 100% of the fluorine was removed from crotonic PFCA at the original concentration of 100 μM. To further confirm the PFAS degradation, we used SPE to purify PFCA after fungal treatment, and the purified samples have been sent to Dr. Men's lab at UC Riverside to use HRMS/MS to quantify residual PFASs. On the other hand, by an in-house 19F-NMR procedure to quantify PFAS, we found the absorptions of PFOA at the 20-30% absorption rates by the fungal-wood filtering materials. Specific absorbing kinetics were determined. Meanwhile, the biochar manufactured from Ruan's lab at UMN by pyrolyzing fungal-treated wood materials were tested for its absorbing capacities by NMR. Currently, we are using SEM and XDR to investigating the absorbing mechanisms by fungal-wood

Activity 2

We associated ligninolytic enzymes' activities to deF of PFAS and found that the solid-State culture optimization enhanced the deF of the "Crotonic PFCA" probably through maintaining ligninolytic enzymes activities. To validated this, we've created the fungal mutants expressing MnP and Laccase to test these ligninolytic enzymes' functions in degrading PFAS. To investigate the unknown deF enzymes and the systematic responses of fungal cells to organic F chemicals, we've prepared the total RNAs extracted from fungal cells treated with F-, PFOA/Octanoic Acid, and Crotonic PFCA/Crotonic Acid for transcriptomic analysis. We expect the RNA-seq will be done at the end of the June, 2024. On the other hand, we prepared the fungal degraded PFAS samples (PFOA and Crotonic PFCA) using optimized SPE, and the samples are now in UC Riverside at Dr. Meng's group, waiting in the queue to analyze the transformation products (TPs) by HRMS/MS. We expect to reconstruct the degradation pathways to dissect the fungal mechanisms used for PFAS degradation. By these activities, eventually we expect to generate a fundamental understanding of PFAS-degrading pathways in fungal species and discover PFAS degradative enzymes, thus guiding the development of fungal methods for PFAS remediation.

Activity 3

In the past period, we used a mock sample composed of wood materials and PFOA for pyrolysis treatment for removing FPAS. Three streams were generated after the pyrolysis of the mock wood: bio-oil, biochar, and biogas. The results measured by LC-MS showed that the pyrolysis has significantly contributed to PFOA decomposition within the sample, achieving a reduction in its concentration in the bio-oil by 87% and 99% at temperatures of 400°C and 500°C, respectively. The presence of fluorinated compounds in the gases was detected at minimal concentrations. A minor fraction of fluorinated compounds migrates to the bio-oil, with elevated temperatures facilitating a decrease in their levels in the bio-oil and an increase in the gas phase. However, our analysis of the biochar samples was hampered by less than ideal extraction techniques. Efforts are underway to refine these methods for bio-char analysis, aiming to establish a detailed mass balance of fluorine throughout the pyrolysis process of PFOA-enriched wood. We have also attempted landfill leachate samples for the treatments, as a step towards its real-world application, but the relevant experiments are still in process. We expect the optimized pyrolysis will benefit its combined uses for PFAS removal from fungal filtering

Dissemination

The discovery of fungal deF capacities, per our knowledge, is novel to the research field. This finding will not only benefit us for understanding the fungal roles in recycling the organic F in nature, but it will also shed lights on developing more degradable alternative PFAS materials. The understanding of fungal degradation and absorption mechanisms on PFAS will facilitate us developing the mycoremediation method. Till now, we've made efforts for dissemination: 1) We reached out to the industry partners Geosyntec Consultants, Inc., Wenck-Stantec, and SKB environmental, discussing the potential field uses of our research; 2) We gave the presentations speaking of PFAS mycoremediation at the ASM 2022 annual meeting, the Minnesota Mycological Society meeting in June to July 2022, and the 2023 annual meeting of the society of industrial of microbiology and biotechnology; 3) we wrote the manuscript to report our discoveries of the fungal degradative mechanism of PFAS, which is waiting for submission and peer-review. For these disseminations, we've seriously acknowledged the relevant financial support of the trust funds.

Status Update Reporting

Status Update September 1, 2023

Date Submitted: September 20, 2023

Date Approved: October 3, 2023

Overall Update

This project aims to develop an integrated method that combines fungal species and wood substrate for a filtering system to sequester and degrade PFAS pollutants from water and soil environments. To achieve this, we have built a series of in-house or collaborative toolkits that can allow the detection of fungal defluorination, measuring of fungal resistance to PFAS chemicals, extraction of PFAS chemicals from wood samples, quantification of PFAS removal, and the characterization of transformative products of PFAS degradation. Using these tools, we have discovered a particular type of wood decay fungi that can be tolerant to the legacy PFAS species PFOA, the fungal capacities of cleaving the C-F bonds for defluorination of certain PFAS compounds, and the optimized conditions for defluorination. We also have gained some preliminary understandings of the PFAS-absorbing capacities of our fungal-wood materials using two legacy PFAS model compounds - FPOS and PFOA. Thermal degradation tests of the absorbed PFAS were planned and will be conducted using mock samples to better monitor the conditions for pyrolysis. The relevant results were delivered as a poster presentation at the annual meeting of the Society for Industrial Microbiology and Biotechnology held from July 30 to August 2 in Minneapolis.

Activity 1

For "activity 1", we have accomplished the fungal screenings on various PFASs, testing their toleranting capacities. By this, we have discovered a unique fungal group expressing a significant PFOA tolerance. Following the screening, we selected three representative wood-decay fungi to test their defluorination capacities, and we found all of them could defluorinate the PFAS chemicals with the unsaturated carbon bond structures. The cultural conditions were further optimized for fungal defluorination by changing the carbon nutrients and the solid-state fermentation methods. To confirm the PFAS removal by the living fungal cultures, we have been collaborating with Dr. Men's lab at UC Riverside to use HRMS/MS to quantify residual PFAS. Leveraging the in-house equipment at UMN, we've also developed a 19F-NMR procedure to test the PFAS absorptions by the fungal-wood filtering materials, and the specific absorbing kinetics are currently being measured. Meanwhile, pyrolysis of wood materials was done by Ruan's lab at UMN for manufacturing biochar for testing its absorbing capacities by NMR. Finally, to integrate the thermal degradation approach, we designed experiments by using mock samples to test and optimize pyrolysis conditions. We expect the optimized pyrolysis will benefit its uses for PFAS removal from our fungal filtering system.

Activity 2

For "activity 2", we have prepared the fungal degraded PFAS samples using the solid phase extraction, and the samples are now in UC Riverside at Dr. Meng's group, waiting in the queue to analyze the transformation products (TPs). By characterizing these TPs, we will reconstruct the degradation pathways to dissect the fungal mechanisms used for PFAS degradation. Meanwhile, we are exploring the functional enzymes involved in the fungal defluorination of PFAS, and the preliminary results showed that certain enzymes were secreted by fungi for cleaving C-F bonds based on the cell-free defluorination tests using crude enzymes. Future works involve using proteomics and transcriptomics to isolate and validate the fungal defluorinating enzymes. By accomplishing these activities, we expect to generate a fundamental understanding of PFAS-degrading pathways in fungal species and discover PFAS degradative enzymes, thus guiding the development of fungal methods for PFAS remediation.

Activity 3

This objective includes activities that are mainly conducted at the later project stages. We have contacted Wenck Environmental Consulting, Co. and allocated suitable landfill field sites for collecting PFAS-contaminated samples.

Experiments to test the sorption capacity of fungal-modified wood have begun. In the following step, we will use the fungal filtering system to test the landfill samples for PFAS removals. This will be challenging due to the complex PFAS backgrounds in the real-world samples. Wood modifications will be applied to optimize the absorbing efficiencies of the filtering system. Partnering with the Ruan lab (Co-PI), we have also started to apply biochar as a filter amendment and compare the biochar characteristics derived from raw and fungal-modified wood. Overall, we are currently poised to begin testing the sorption/degradation capacity of a complete fungal-wood filter system and using results from previous experiments to optimize the filter system with stimulated capacities for remediating landfill leachate.

Dissemination

The discovery of fungal defluorination capacities, per our knowledge, is novel to the research field. It will significantly expand our understanding of fungal roles in recycling PFAS in nature and facilitate us in developing the mycoremediation method based on fungal processes. Till now, we've made efforts for dissemination: 1) We reached out to the industry partners Geosyntec Consultants, Inc., Wenck-Stantec, and SKB environmental, discussing the potential field uses of our research; 2) We gave the presentations speaking of PFAS mycoremediation at the ASM 2022 annual meeting, the Minnesota Mycological Society meeting in June to July 2022, and the 2023 annual meeting of the society of industrial of microbiology and biotechnology; 3) we wrote the manuscript to report our discoveries of the fungal degradative mechanism of PFAS, which is waiting for submission and peer-review. For these disseminations, we've seriously acknowledged the relevant financial support of the trust funds.

Status Update Reporting

Status Update March 1, 2023

Date Submitted: March 3, 2023

Date Approved: March 16, 2023

Overall Update

To accomplish our project goals, so far, we have: 1) Established a robust detection method that can be applied to quantify the PFAS degradation in fungal cultures accurately; 2) Screened a couple of fungal species that are capable of defluorinating representative PFAS compounds, the indicative of C-F degradation of the forever chemicals; 3) Initiated the test on using the fungal treated wood substrate to absorb PFAS compounds, in the purpose of developing the fungal-wood matrix filtering system; 4) Selected the candidate fungal species for further investigating the fundamental PFAS degradative pathways; 5) Initiated the trials aiming to combine the mycoremediation and pyrolysis approach to complete the PFAS removal.

Activity 1

Our main objective in this activity is to test fungal capacities in sequestering and degrading PFAS. We first established a method to detect the fluoride (F-) of PFAS degradation by fungal cultures. Three F- detection methods, including two colorimetric and one potentiometric method, have been trialed and compared to select an analytical method to accurately detect defluorination when applied to fungal culture. Colorimetric assays were found to be too susceptible to interferences particular to fungal culture systems, and therefore the potentiometric approach was selected. Using the selected F- analytical method, three fungal species have been screened for the capacity to degrade standard perfluorocarboxylic acids (PFCAs) compounds (n=8). Thus far, three of these common PFCAs have been determined to be degradable by each of the three fungi selected for culture via the detection of fluoride anion (F-), an indicative of fungal mediated defluorination of C-F bonds found in PFAS compounds. Ion-chromatography to confirm via a second method of fungal defluorination is in progress. A more in-depth analysis of fungal PFAS degradation using LC-MS to identify transformation byproducts other than F- is in preparation. Culture optimizations are in progress to further improve the PFAS degradation by the fungal species.

Activity 2

This objective is to investigate the fungal degradative pathways of PFAS that can provide fundamental insights and potentially be used to optimize the fungal remediation method of PFAS. Based on activity 1, we've selected Stereum hirsutum as the host fungal species to investigate its degradative pathways in degrading two representative PFAS compounds – GenX and Crotonic acid. To identify the degradation metabolites, we've also built a collaborative relationship with Yu-Jie Men's lab at the University of California, Riverside, to use her high-resolution MS. Future plans also include conducting transcriptomic experiments and enzyme assays to determine the involved genes and enzymes to characterize the fungal degradative system better. All of the required items have been well prepared to achieve the milestone goals in the next stage of this activity.

Activity 3

This objective includes activities mainly conducted at the later project stages. Nevertheless, we are making progress in this objective. We contacted Wenck Environmental Consulting, Co. and allocated suitable landfill field sites for collecting PFAS-contaminated samples. The construction of a fungal-wood filter system for PFAS requires the selection of an appropriate fungi and sorbent substrate. Currently, experiments to characterize the sorption capacity of wood and fungal-modified wood have begun. Partnering with the Ruan lab (Co-PI), initial wood samples have been pyrolyzed to produce biochar as a filter amendment and compare the biochar characteristics derived from raw and fungal-modified wood. Initial results indicated that the degree of fungal modification to the wood substrate was insufficient to alter the properties of the derived biochar. Fungal culture conditions were then optimized, and rates of fungal wood modification

were improved. The characterization of this improved biochar is underway. Sorption kinetic experiments have been initiated to determine the PFAS sequestration capacity of fungal-wood filter components wood, biochar, and fungi. Overall, we are currently poised to begin testing the sorption/degradation capacity of a complete fungal-wood filter system and using results from previous experiments to optimize the filter system with simulated leachate.

Dissemination

We made several efforts for dissemination: 1) We reached out to the industry partners Wenck-Stantec and SKB environmental, discussing the potential field uses of our research; 2) We gave the presentations speaking of PFAS mycoremediation at the ASM 2022 annual meeting and a Minnesota Mycological Society meeting in June to July 2022; 3) A paper reporting our discoveries of the fungal defluorination capacity is in preparation, which will acknowledgment the relevant financial support of the trust funds.