National Institute of
Environmental Health Sciences
Superfund Research Program

National Institute of Environmental Health Sciences Superfund Research Program R01 Satellite Meeting

Optimizing Natural Systems for Remediation: Utilizing Innovative Materials Science Approaches to Enhance Bioremediation

Sponsored by the Superfund Research Program

Monday, December 4, 2023

8:30 a.m. – 2:30 p.m. MST

Superfund Research Program Annual Meeting
Individual Research Grants (R01)

All times Mountain Standard Time

Agenda

NIEHS Optimizing Natural Systems for Remediation:
Utilizing Innovative Materials Science Approaches to Enhance Bioremediation

Unless otherwise indicated, the R01 Program Session will take place in the Weavers Room.

Monday, December 4, 2023

8:30 – 9:00 a.m. **Poster Set-Up** – East Atrium Area

9:00 – 9:15 a.m. **Welcome and Brief Programmatic Updates – Heather Henry**, Program Officer, NIEHS

9:15 – 11:30 a.m. **Flash Talks**

University of Massachusetts – A Novel Strategy for Arsenic Phytoremediation

Yale University – Understanding and Enhancing PFAS Phytoremediation Mechanisms Using Novel Nanomaterials

Texas A&M AgriLife Research – Efficient Bioremediation of Environmentally Persistent Contaminants With Nanomaterial-Fungus Framework (NFF)

University of California, Riverside – Synergistic Material-Microbe Interface Toward Faster, Deeper, and Air-Tolerant Reductive Dehalogenation

Princeton University – Enhancing Transport and Delivery of Ferrihydrite Nanoparticles via Polymer Encapsulation in PFAS-Contaminated Sediments to Simulate PFAS Defluorination by Acidimicrobium sp. Strain A6

Break (15 min.)

SUNY at Buffalo – Model-Aided Design and Integration of Functionalized Hybrid Nanomaterials for Enhanced Bioremediation of Per- and Polyfluoroalkyl Substances (PFAS)

Oregon State University – Development of Passive and Sustainable Cometabolic Systems to Treat Complex Contaminant Mixtures by Encapsulating Microbial Cultures and Slow-Release Substrates in Hydrogels

Florida State University – Enhancing Bioremediation of Groundwater Co-Contaminated by Chlorinated Volatile Organic Compounds and 1,4-Dioxane Using Novel Macrocyclic Materials

University of Iowa – Elucidating Mechanisms for Enhanced Anaerobic Bioremediation in the Presence of Carbonaceous Materials Using an Integrated Material Science and Molecular Microbial Ecology Approach

University of Maryland, Baltimore County – Leveraging the Chemo-Physical Interaction of Halorespiring Bacteria With Solid Surfaces to Enhance Halogenated Organic Compounds Bioremediation

11:30 a.m. – 12:15 p.m. **Lunch on Your Own — Sawmill Market**

12:15 – 12:45 p.m. **Panel on Roadmap to Application: Case Studies on Regulatory, Scale-Up, and Commercialization Considerations**

12:45 – 1:45 p.m. **Roundtable Discussion: "Combining Bioremediation and Materials Science"**
Moderators: Susie Dai, Texas A&M University; **Lew Semprini**, Oregon State University; **Om Parkash Dhankher**, University of Massachusetts Amherst; **Upal Ghosh**, University of Maryland, Baltimore County

1:45 – 2:00 p.m. **Roundtable Report Back**

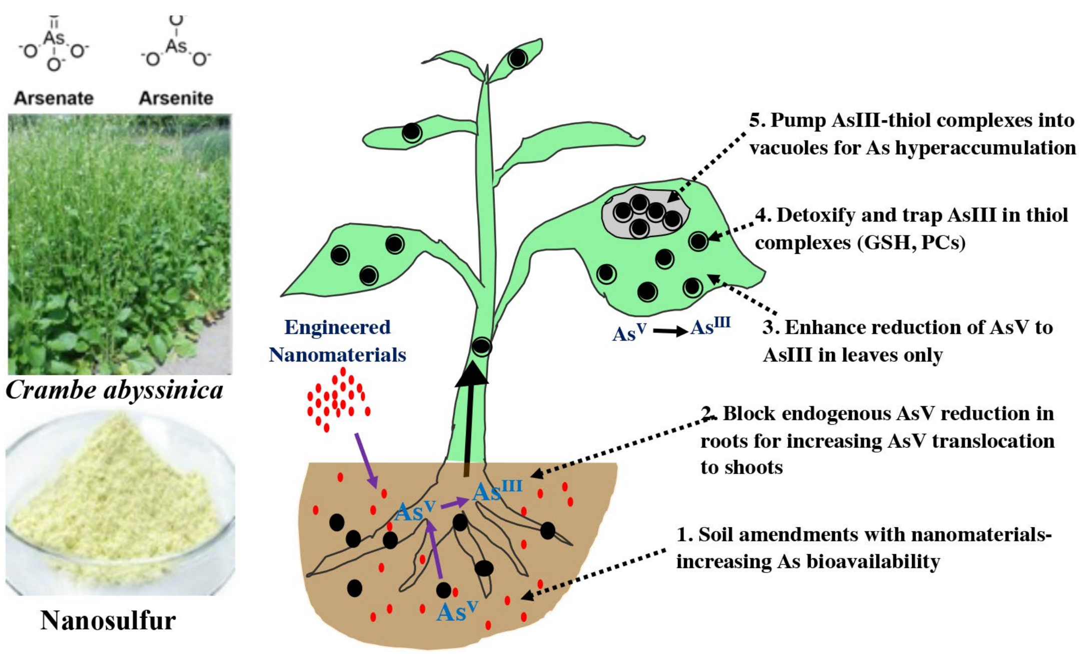
2:00 – 2:25 p.m. **Next Steps Discussion**
Moderator: Heather Henry, NIEHS

2:25 – 2:30 p.m. **Group Photo** — Juniper Garden (tentative)

Grant Information: Institution, Principal Investigator(s), Contact Information, Grant Number	**University of Massachusetts** **Project:** A Novel Strategy for Arsenic Phytoremediation **Project Leaders**: Om Parkash Dhankher, Venkataraman Dhandapani, Jason C. White, Baoshan Xing **Grant Number:** R01ES032686 **Funding Period:** 2021-2025
Technology	Developing a genetics-based phytoremediation strategy for arsenic uptake, translocation, detoxification, and hyperaccumulation into the fast-growing, high biomass, non-food oilseed crop *Crambe abyssinica*.
Innovation	**Materials:** Nanosulfur will be utilized to modulate the bioavailability and phytoextraction of arsenic from soil and to increase the storage capacity via enhanced sulfur assimilation. **Biological:** We are engineering a fast-growing, high biomass, non-food crop *Crambe abyssinica* to remediate arsenic-contaminated soil. **Why is this technology/approach different than what is already in the market?** We are using a gene pyramiding approach to co-express several genes that control the transport, oxidation state, and binding of As for efficient extraction and hyperaccumulation into above-ground plant tissues of *Crambe abyssinica*. Phytoremediation is a cost-effective and ecologically friendly alternative to physical remediation methods.
Contaminant and Media	**Contaminants:** Arsenic **Media:** Soil, sediment, and maybe water
Expansion Potential	**Looking Forward: What other contaminants/media would work for your technology?** Toxic metals: Pb, Cd, Hg, Cr **Combined Remedy: Would this technology work well with other treatment approaches?** Yes, this approach could be combined with biofuels production on contaminated sites.
Sites/Samples	We are using artificially contaminated soils, but will use the real-world samples, like field soils contaminated with As and other toxic metals.

Continued

Technology Readiness Level	TRL 3 — Experimental proof of concept TRL 4 — Technology validated in laboratory
Update of Progress	• We are co-expressing four genes that control the transport, oxidation state, and binding of As for efficient extraction and hyperaccumulation into above-ground plant tissues. All four genes are cloned and transformed into *Crambe* either single or stacked genes. • We have already developed transgenic *Crambe* plants for several gene constructs. • Analysis of double transgenic lines coexpressing arsenate reductase and glutathione biosynthesis pathway gene showed that double transgenic plant had significantly increased arsenate (AsV) tolerance as these plants attained almost three-fold higher biomass compared to wild type controls plants. These plants accumulated 2-fold more arsenic in the shoot tissues. Analysis of plants expressing other genes is in progress. • Analysis of transgenic plants for other genes is in progress. • We have also optimized the nanosulfur concentration on arsenic mobility from soil and subsequent uptake and accumulation in *Crambe* grown in soil supplemented with both arsenate and nanosulfur. • Additionally, for the Diversity Supplement award, we are modulating the expression of arsenate reductases for increasing tolerance and reducing As accumulation in rice for food safety.



Developing a genetics-based strategy for arsenic phytoremediation in Crambe abyssinica*, a non-food industrial oilseed crop.*

Yale

Grant Information: Institution, Principal Investigator(s), Contact Information, Grant Number	**Yale University** **Project:** Understanding and Enhancing PFAS Phytoremediation Mechanisms Using Novel Nanomaterials **Project Leaders**: Vasilis Vasiliou, Christy Haynes (University of Minnesota), Jason White (Connecticut Agricultural Experiment Station) **Funding Period:** 2021-2025
Technology	We will design and synthesize novel nanomaterials, such as ultraporous mesostructured silica nanoparticles (UMNs) and carbon dots with a tunable surface chemistry that have increased affinity for recalcitrant per- and polyfluoroalkyl substances (PFAS). These two nanomaterials are known to be accumulated by plants and effectively enhance PFAS uptake and translocation from water and soil into plants as a novel phytoremediation strategy.
Innovation	**Materials:** We have developed carbon dots (CDs) and ultraporous mesostructured silica nanoparticles (UMNs) with an affinity for a range of specific PFAS using sustainable precursors. CDs are synthesized with amine-rich precursors, such as chitosan and polyethyleneimine (PEI), to target a range of surface charges and nanoparticle size with high PFAS affinity and enhanced potential for plant uptake. Similarly, the UMNs are modified with different functional groups to target specific PFAS to significantly promote phytoextraction by plants. **Biological:** We are exploring the use of hemp *(Cannabis sativa)* and zucchini *(Cucurbita pepo)* as phytoremediation species. **Why is this technology/approach different than what is already in the market?** PFAS PFAS are a large and complex group of highly toxic organic pollutants that are resistant to degradation in the environment; some analytes are more mobile and can contaminant groundwater, whereas others are less mobile but exceedingly persistent in soil. Therefore, novel and effective remediation/cleanup technologies of PFAS are urgently needed. We are focused on a sustainable remediation approach known as phytoremediation (i.e., using plants to extract recalcitrant PFAS from soil). We hypothesize that novel nanomaterials such as UMNs and CDs can be used to enhance the mobility and accumulation of PFAS into plants. The luminescent properties of these novel materials will allow us to visually track both PFAS sorption to the particles, as well as movement of the nanoparticle-PFAS complex into and throughout the plants, thus providing critical mechanistic information about our phytoremediation system. The nanomaterials developed in this project will advance phytoremediation as an economical and sustainable technique for removing a wide range of PFAS from soil. In addition, findings from this project will result in a better understanding of how novel nanomaterials (NNMs) can be used to mobilize contaminants in plant-soil systems. This information can be translated to optimize phytoremediation processes with other plant species, contaminant classes, and nanomaterials.

Continued

Contaminant and Media	**Contaminants:** Examples include groundwater, drinking water, soil, and sediment. **Media:** The contaminants we are targeting are the 25 PFAS that are most prevalent in soil and water.
Expansion Potential	**Looking Forward: What other contaminants/media would work for your technology?** Our technology could be potentially adapted to other hydrophobic organic contaminants, such as pharmaceutical and personal care products, pesticides, and micro-nanoplastics, as well as toxic elements in soil that may not be prone to plant uptake, such as Pb. It is the ability to tune the surface chemistry of our nanomaterials that enables this platform to be applicable to a wide range of other environmental contaminants. Nano-enhanced phytoremediation strategies could be designed for soil, groundwater, and aquatic systems. For our current PFAS work, we are focused on phytoextraction; once the contaminant is out of the environment and in the plant tissues, disposal and other remediation technologies become easier. We are currently working in conjunction with two other technologies. **Combined Remedy: Would this technology work well with other treatment approaches?** We are working on the combination approaches, mainly: 1) phytoremediation and hydrothermal liquefaction (HTL) and 2) phytoremediation and biodegradation.

Novel sustainable nanomaterials, such as carbon dots and ultra mesoporous silica, are being designed to bind to recalcitrant PFAS molecules in hydroponic and soil systems to promote plant uptake as a nanotechnology-enabled phytoremediation strategy.

Continued

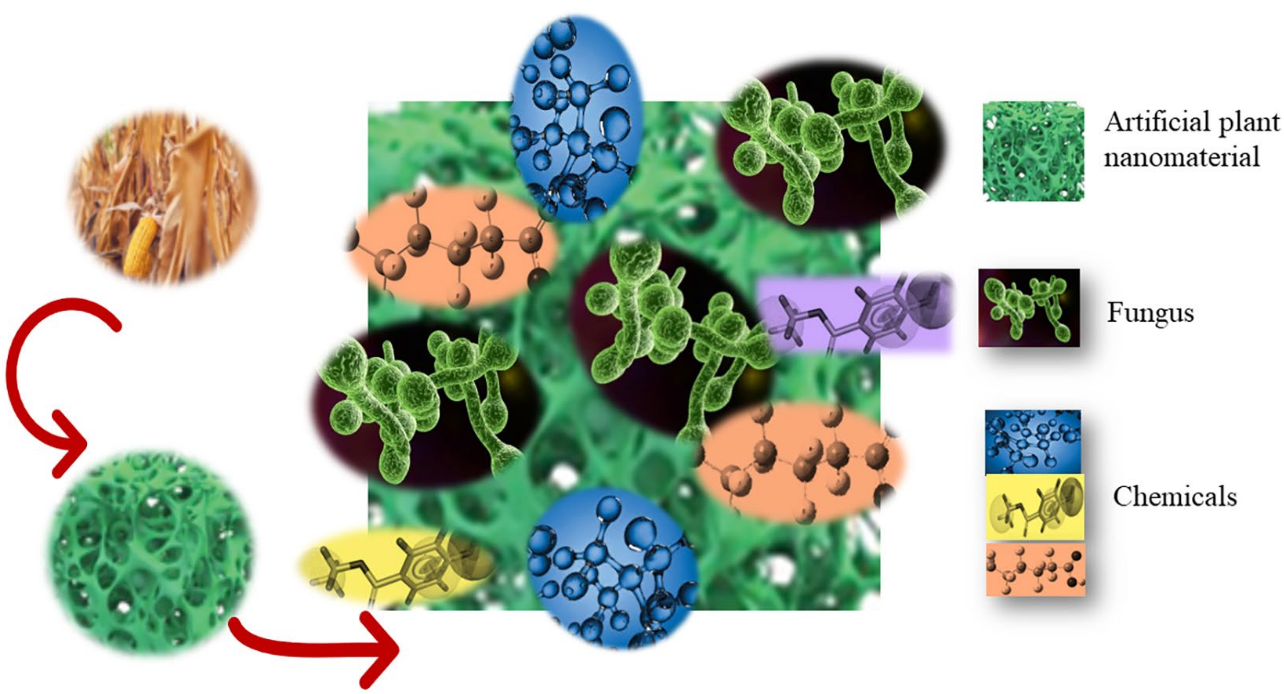
Yale

Sites/Samples	We don't have any record of sites in our databases. Loring Air Force Base, Maine.
Technology Readiness Level	TRL 3 — Experimental proof of concept (We are in the process of moving to TRL 4 and will do so within the next six to nine months.)
Update of Progress	UMNs: PFAS uptake experiments with UMNs show that tuning the surface charge of UMNs has a larger effect on PFAS removal efficiency in abiotic systems than tuning the hydrophobicity, and that positively charged UMNs have higher PFAS removal efficiency than the negatively charged UMNs (with percent recovery of 20% compared to nearly 100%). When incubated with multiple PFAS, UMNs show greater removal efficiency for longer chain and more hydrophobic PFAS. Plant uptake experiments with UMNs show a strong trend of increasing bioconcentration factors of PFOA with the treatment of UMNs, although our first experiment in this space did not achieve statistically significant enhancement. Further investigation of UMN effects on PFAS uptake by plants is currently underway. CDs: We are developing various carbon dot nanoparticles (CDs) to enhance plant uptake of PFAS in phytoremediation systems. CDs are synthesized to promote efficient PFAS loading and complexation; the CDs will then act as a carrier for PFAS into plant above-ground tissue. To promote electrostatic interactions between CDs and anionic PFAS, we have synthesized CDs with a positive surface charge using amine-rich precursors, such as chitosan and polyethyleneimine (PEI). We have developed 19F NMR techniques to probe the interactions occurring between CDs and PFAS that allow us to establish binding isotherms, extract Kd values, and thus rank affinity across different CDs and PFAS species. Results to date indicate that PEI-based CDs have the highest affinity for PFAS out of all the CD formulations considered; however, all show PFAS affinity. NMR spectral analysis indicates slow chemical exchange between PFAS and CDs, indicative of a high affinity interaction. The presence of PFAS does not induce any changes to the inherent CD fluorescent properties, which allows us to track CD localization within plants. Additionally, the capacity of CDs to promote PFAS uptake in plants has been studied hydroponically by exposing the plant to PFAS mixture with and without CDs.

Grant Information: Institution, Principal Investigator(s), Contact Information, Grant Number	**Texas A&M AgriLife Research** **Project:** Efficient Bioremediation of Environmentally Persistent Contaminants With Nanomaterial-Fungus Framework (NFF) **Project Leaders**: Susie Dai, Joshua S. Yuan (Texas A&M University), Gregory V. Lowry (Carnegie Mellon University) **Funding Period:** 2021-2025
Technology	The project develops a biomimetic sorbent derived from plant cell walls, which can adsorb persistent organic pollutants, such as PFAS, and present the contaminants-enriched biomass to fungus for bioremediation. The technology can potentially eliminate the treatment train approach and consolidate the pollutant removal and degradation sequentially in the same space.
Innovation	**Materials:** The bioremediation system utilizes 1) a material derived from lignocellulosic biomass and functionally modified to adsorb contaminants such as PFAS. **Biological:** The fungus utilizing the biomass and the organic contaminants as the carbon source. **Why is this technology/approach different than what is already in the market?** Our design uniquely differs from the other state-of-the-art, in that our sorbent is more than a media but an interactive component in a sustainable bioremediation system.
Contaminant and Media	**Contaminants and Media:** The technology targets water and media that can be treated by fungus.
Expansion Potential	**Looking Forward: What other contaminants/media would work for your technology?** The technology synergistically renders innovative sorbents for contaminant enrichment and fungus species that can efficiently degrade persistent contaminants. **Combined Remedy: Would this technology work well with other treatment approaches?** The two outcomes can be applied in a combined fashion or separately.
Sites/Samples	We will sample an Air Force base site in San Antonio, Texas. We will take water and soil samples. The water sample for treatment and the soil sample for isolating microbes that can potentially be useful for PFAS.

Continued

Technology Readiness Level	TRL 4 — Technology validated in laboratory
Update of Progress	The material exhibits record absorption capacity for the PFAS compounds, perfluorooctanoic acid (PFOA) at 3529 mg/g, and perfluorooctanesulfonic acid (PFOS) at 4151 mg/g. At the same time, the material sustains fungal growth and serves as the sole carbon source for the microbe. Publications from this project: • Yu J et al, 2023. Genomic diversity and phenotypic variation in fungal decomposers involved in bioremediation of persistent organic pollutants. J Fungi 9(4):418. • Li J et al, 2022. Sustainable environmental remediation via biomimetic multifunctional lignocellulosic nano-framework. Nat Commun 13(1):4368. • Zhang W et al, 2022. Design of biomass-based renewable materials for environmental remediation. Trends Biotechnol 40(12):1519-1534.

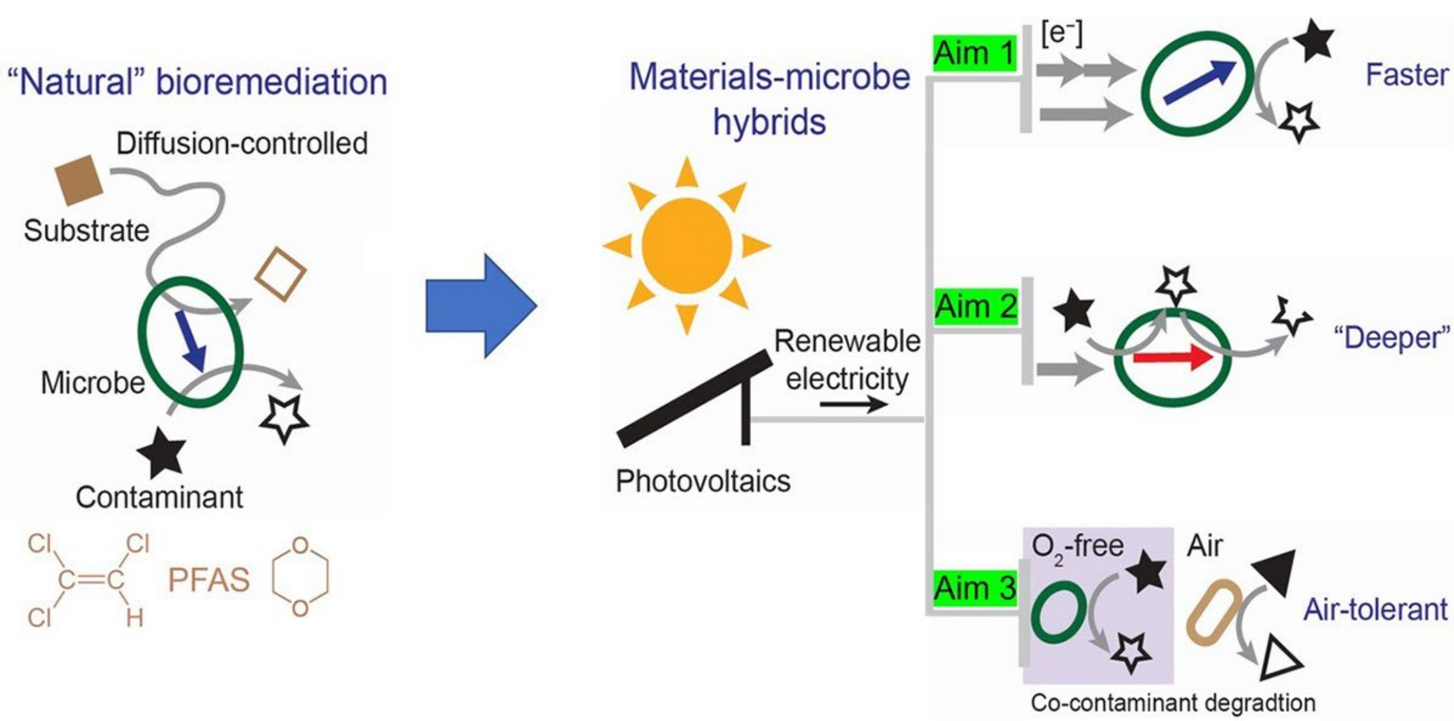


Illustrative presentation of the system design. PFAS are adsorbed into the biomimetic plant material. When the fungus consumes the plant material, it also eats the chemical that was adsorbed.

Grant Information: Institution, Principal Investigator(s), Contact Information, Grant Number	**University of California, Riverside** **Project:** Synergistic Material-Microbe Interface Toward Faster, Deeper, and Air-Tolerant Reductive Dehalogenation **Project Leaders**: Yujie Men, Chong Liu (University of California-Los Angeles) **Funding Period:** 2021-2025
Technology	Solar-powered material-microbe interface to accelerate the activity of bacteria used to clean up contaminants.
Innovation	**Materials:** Nanomaterials that are biocompatible and/or can provide low redox potential for reductive dehalogenation. **Biological:** Microcosms capable of degrading target contaminants and performing synergies in enhancing the degradation at the electricity-driven material-microbe interface. **Why is this technology/approach different than what is already in the market?** It incorporates new advances in nanomaterials science to optimize bioremediation leveraging solar power.
Contaminant and Media	**Contaminants:** Halogenated contaminants, 1,4-dioxane **Media:** Groundwater
Expansion Potential	**Looking Forward: What other contaminants/media would work for your technology?** Other contaminants in water environments that may undergo reduction reactions and have a high demand for reducing power (e.g., H_2). **Combined Remedy: Would this technology work well with other treatment approaches?** It may be combined with electrochemical and biological oxidation processes.
Sites/Samples	We have not reached the stage of testing field samples. The PI has been in contact with several impacted field sites in California. Groundwater samples will be taken and used in the bioelectrochemical system to test the matrix effect.

Continued

Technology Readiness Level	TRL 3 — Experimental proof of concept
Update of Progress	We are now using dual sets of 8-channel bioelectrochemical systems to investigate synergies at the material-microbe hybrid for deeper defluorination of more environmentally relevant PFAS structures by various defluorinating cultures (including a new defluorinating enrichment culture and one isolate from the enrichment). The bioelectrochemical system is also being optimized by providing organic growth substrates for the defluorinating microorganisms to sustain the defluorination activity for a longer time. Besides optimizing the bioelectrochemical system as a whole, we also made efforts to optimize conditions for the electrochemical and microbial parts individually. Microbially, we further examined biodefluorination pathways for another group of PFAS, ether PFAS, and isolated defluorinating microorganisms to be used in the bioelectrochemical system. Electrochemically, we tested the reductive defluorination activities of a number of bio-inspired electrocatalysts against legacy PFAS, such as PFOA, under controlled voltages.



PRINCETON UNIVERSITY

Grant Information: Institution, Principal Investigator(s), Contact Information, Grant Number	**Princeton University** **Project:** Enhancing Transport and Delivery of Ferrihydrite Nanoparticles via Polymer Encapsulation in PFAS-Contaminated Sediments to Simulate PFAS Defluorination by *Acidimicrobium* sp. Strain A6 **Project Leaders**: Peter Jaffe, Bruce E. Koel **Funding Period:** 2021-2025
Technology	Biostimulation of *Acidimicrobium* sp. sp. Strain A6 for the purpose of PFAS defluorination in soils/groundwater requires the supply of solid-phase ferric iron as electron acceptor, which is challenging. Here, we are developing a polymer encapsulated ferric iron phase that is transportable in a porous medium and bioavailable to *Acidimicrobium* sp. Strain A6.
Innovation	**Materials:** Ferric iron phases (ferrihydrite, goethite, hematite) encapsulated in polyacrylic acids (PAAs). **Biological: What is the biological component?** *Acidimicrobium* sp. Strain A6, an autotroph that used ammonium as electron donor and ferric iron as electron acceptor. The genome of this organism contains sequences for various dehalogenases, which seem to play a role in reductive PFAS defluorination. **Why is this technology/approach different than what is already in the market?** At this point there is no known method to biodegrade perfluoroalkyl acids (PFAAs), and biostimulation of *Acidimicrobium* sp. Strain A6 for this purpose is a promising technology for their degradation at selected environmental settings.
Contaminant and Media	**Contaminants: What contaminant(s) does your project target?** PFAS, including PFAAs such as PFOA and PFOS. **Media:** Porous media, including soils, sediments, and groundwater. At this point, we are focusing on acidic, iron-rich systems, which favor the presence of that *Acidimicrobium* sp. Strain A6.
Expansion Potential	**Looking Forward: What other contaminants/media would work for your technology?** We have shown that Acidimicrobium sp. Strain A6 is also capable of degrading *Acidimicrobium* sp. Strain A6 is also capable of degrading chlorinated organics, such as 1,2,3 TCP, although more study is needed. Hence, it might be possible to address sites that contain chlorinated and fluorinated compounds. **Combined Remedy: Would this technology work well with other treatment approaches?** Biological defluorination most certainly decreases the mass of contaminates; hence, even if biological methods might not be able to reach drinking water standards, combining it with methods such as sorption to activated carbon will make the sorbent last much longer.

Continued

Sites/Samples	We are testing the technology in laboratory column studies using PFAS impacted sediments from two Department of Defense (DoD) sites: The Naval Air Station Oceana, Virginia, and the Lakehurst Naval Air System Command, New Jersey. Sediments from both sites are acidic and iron-rich, and that *Acidimicrobium* sp. Strain A6 is naturally present at low numbers; hence, we are testing if we can stimulate its growth using the technology developed in this project.
Technology Readiness Level	TRL 4 — Technology validated in laboratory

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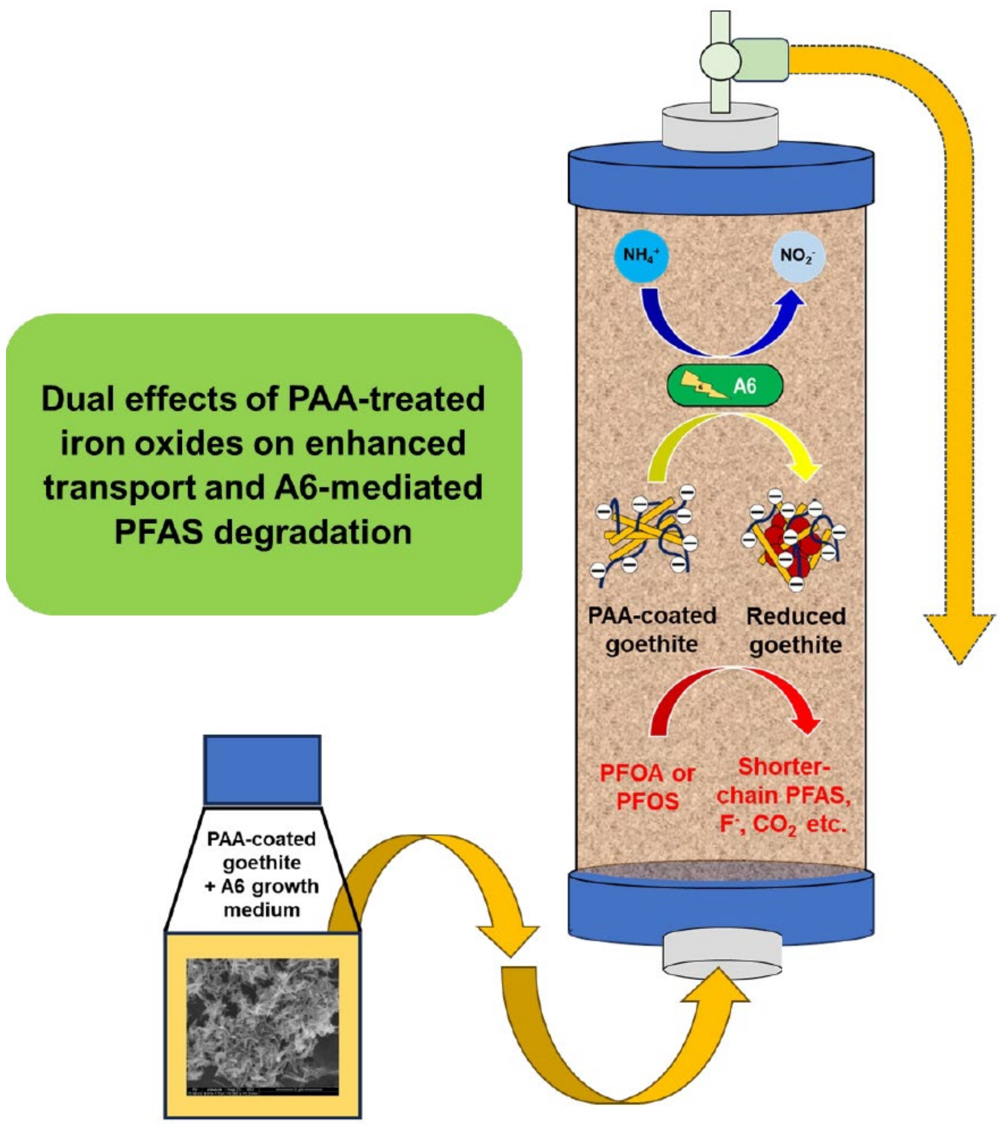

Supplying polyacrylic acid coated goethite to a soil column to stimulate PFAS defluorination by Acidimicrobium *sp. Strain A6.*

Update of Progress	The goal of this project was to (i) Determine if previous findings of PFOS, PFHxS, and PFOA degradation at the mg/l levels also occur at levels typically observed at PFAS impacted DoD sites; (ii) Gain further insights into the PFAA defluorination mechanism by *Acidimicrobiaceae* sp. A6 (A6); and (iii) determine if A6, which has been observed in many iron-rich acidic soils, is present in PFAS impacted soils with such characteristics at DoD sites, and if its activity can be stimulated. In terms of concentration effects, results have shown that the half-life of these PFAS is relatively constant over concentration ranges from 10 ppm to 1 ppb. Focusing on the defluorination mechanism, the production of H-PFOA is typically observed during incubations with PFOA. Preliminary results, needing additional confirmation, indicate that the missing fluorine is either from the alpha carbon and from the delta and/or epsilon carbon. If corroborated, this would explain the relative amounts of the shorter chain PFAAs that are being formed during the degradation of PFOA. To determine if A6 is present at different DoD sites that are iron-rich and acidic, PFAS impacted sediment samples were obtained from the Naval Auxiliary Landing Field Fentress, Virginia, (pH = 6.11); Naval Air Station Oceana, Virginia, (pH = 6.04); Lakehurst Naval Air System Command, New Jersey (pH = 6.57); and Joint Base Charleston–Air, South Carolina. Various incubations were performed, either by just adding DI water, or augmenting these sediments with either a medium containing NH_4^+ or a medium containing Fe(III) and NH_4^+. While A6 was either barely detectable or not detectable in the initial sediments, after 40 days of incubation, especially in the Fe(III) and NH_4^+ amended incubations, it became detectable, and the production of F- was observed. Incubations amended with PFOA or with PFOS did show a decrease in their concentrations during the incubations, as well as F- production. Based on these results, column experiments were conducted with sediments from Oceana and Lakehurst, which had the highest and lowest Fe(III) levels, respectively, of the sediments studies. The same treatment as for the batch incubations were applied, but Fe(III) was treated with polyacrylic acids to make it transportable in the soil columns. After Fe(III) breakthrough, columns were allowed to rest for 40 days, after which pumping was restarted. Effluent, especially for the columns with added Fe(III) showed significant decrease in NH_4^+ and F- production, as well as decrease in PFOS, indicating that biostimulation at these sites might be feasible, although the effect of many environmental factors (i.e., much lower soil/groundwater temperature than in the laboratory, different redox conditions, more complex microbial communities, etc.) would have to be investigated first.

University at Buffalo
The State University of New York

Grant Information: Institution, Principal Investigator(s), Contact Information, Grant Number	SUNY at Buffalo **Project:** Model-Aided Design and Integration of Functionalized Hybrid Nanomaterials for Enhanced Bioremediation of Per- and Polyfluoroalkyl Substances (PFAS) **Project Leaders**: Diana S. Aga, Ian Bradley, Nirupam Aich (University of Nebraska-Lincoln), Carla A. Ng (University of Pittsburgh) **Funding Period:** 2021-2025
Technology	Catalytic graphene oxide-metal nanohybrids are being designed and synthesized to transform persistent PFAS to more biodegradable forms that are then fed into bioreactors containing microbial cultures to complete PFAS degradation. Computational models and high-resolution mass spectrometry techniques are being developed to understand, predict, and optimize the biodegradation process.
Innovation	**Materials:** (i) Redox-active reduced graphene oxide nano zerovalent iron (rGO–nZVI); and (ii) photocatalytic rGO-titanium dioxide (TiO2) or rGO-TiO2 and rGO- TiO2-nZVI. **Biological:** Selection of microbial communities and assessment of their potential to degrade structurally variable PFAS followed by identification of community structure, functional enzymes, and pathways. **Why is this technology/approach different than what is already in the market?** The model-aided approach for nano-enhanced PFAS biodegradation will allow predictive screening of materials to identify the most promising degradation pathways for a wide range of PFAS structures without producing toxic byproducts.
Contaminant and Media	**Contaminants:** PFOA, PFOS, GenX, 6:2 FTS, PFHxS, PFBS, PFBA, and other emerging PFAS **Media:** Surface water, wastewater, drinking water
Expansion Potential	**Looking Forward: What other contaminants/media would work for your technology?** Our technology could be tuned to address any other persistent halogenated contaminant. **Combined Remedy: Would this technology work well with other treatment approaches?** This technology could be implemented as part of a treatment chain to address PFAS concentrates (e.g. from membrane filtration).
Sites/Samples	This project does not have a specific site for collecting real-world samples.

Continued

Technology Readiness Level	TRL 3 — Experimental proof of concept
Update of Progress	**Aga Laboratory for Environmental Research and Testing (ALERT)** (Trainees: John Michael Aguilar and Karla Rios) We have developed an LC-MS/MS method that can target potential transformation products of PFAS during bacterial degradation and nano-enabled oxidative degradation. To quantify ultra-short chain PFAS, we also developed other methods, such as supercritical fluid chromatography/mass spectrometry (SFC/MS) and fluorine nuclear magnetic resonance spectroscopy (F-NMR), in addition to LC/MS/MS. Using ion chromatography that measures the amounts of fluorine, acetate, and formate produced during degradation of PFAS, we showed that degradation of PFAS leads to nontoxic byproducts. **Aich Lab (#AichLENS)** (Trainee: Md Arafat Ali) We evaluated how, and to what extent, different environmental and operational parameters, such as initial PFAS concentration, oxidant (H2O2) dose, pH, ionic strength, and natural organic matter (NOM), could influence the removal and degradation of PFOS and PFOA by rGO-nZVI (manuscript under review). We also developed photocatalytic rGO-TiO2 nanohybrid and evaluated its efficacy and extent to which it could remove/degrade multiple PFAS, including PFOS, PFOA, GenX, and 6:2 FTS under UV irradiation. **Ng Lab** (Trainee: Melissa Marciesky) We have performed computational studies using density functional theory (DFT) in Gaussian 16 and calculated the bond dissociation energies (BDEs) of two current-use PFAS: perfluorobutanoic acid (PFBA) and perfluorobutanesulfonic acid (PFBS). We then created multiple PFAS-metal complexes in both gas and water phases and confirmed the lowest-energy conformations using multiple DFT methods and the MP2/Aug-cc-PVTZ level of theory. We then calculated homolytic and heterolytic BDEs for the carbon-fluorine and carbon-carbon bonds in PFBA and PFBS with a variety of metal ions, including Mg(II), Ag(II), Fe(II)/(III), Cu(II), and V(V).

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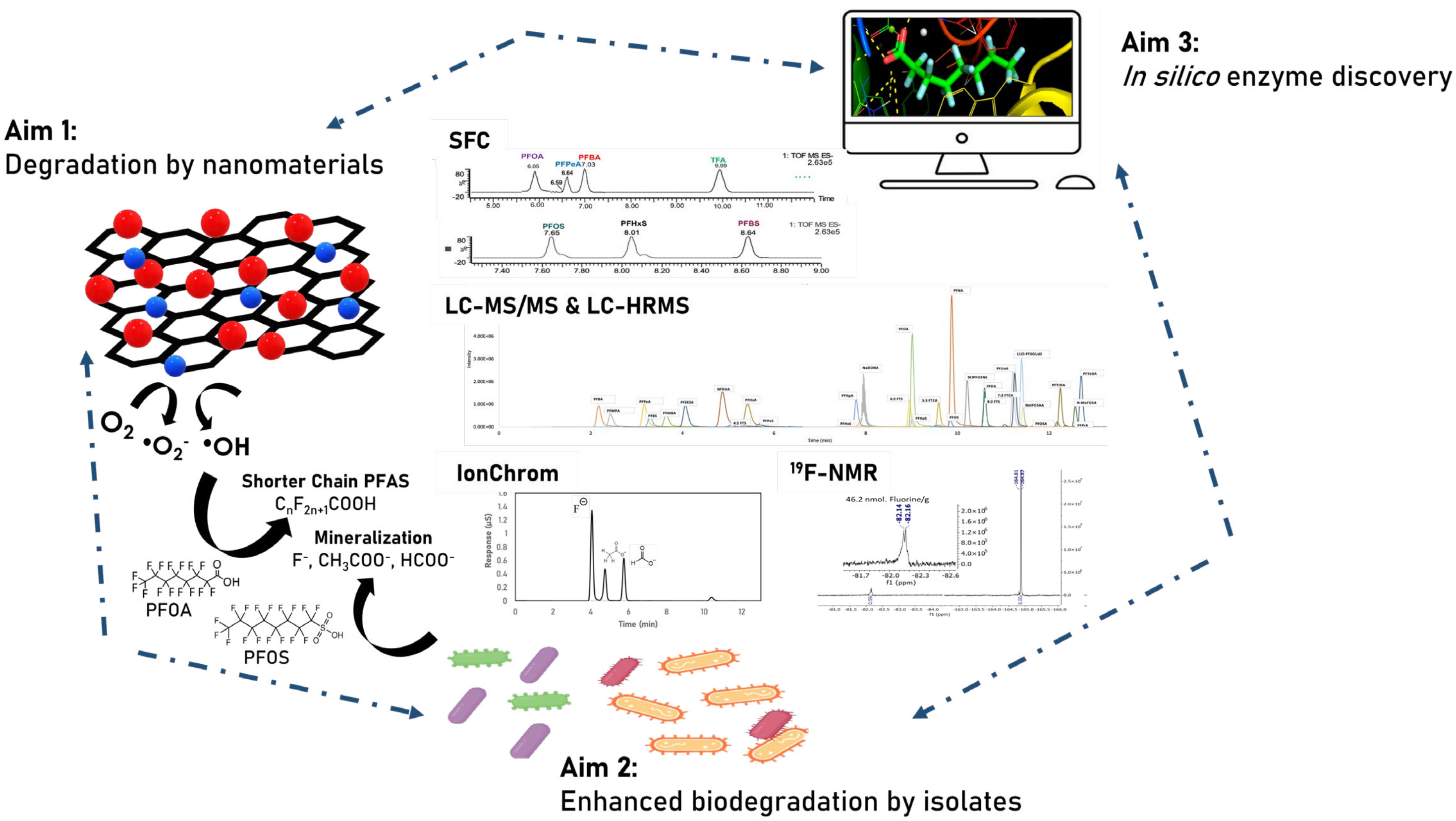
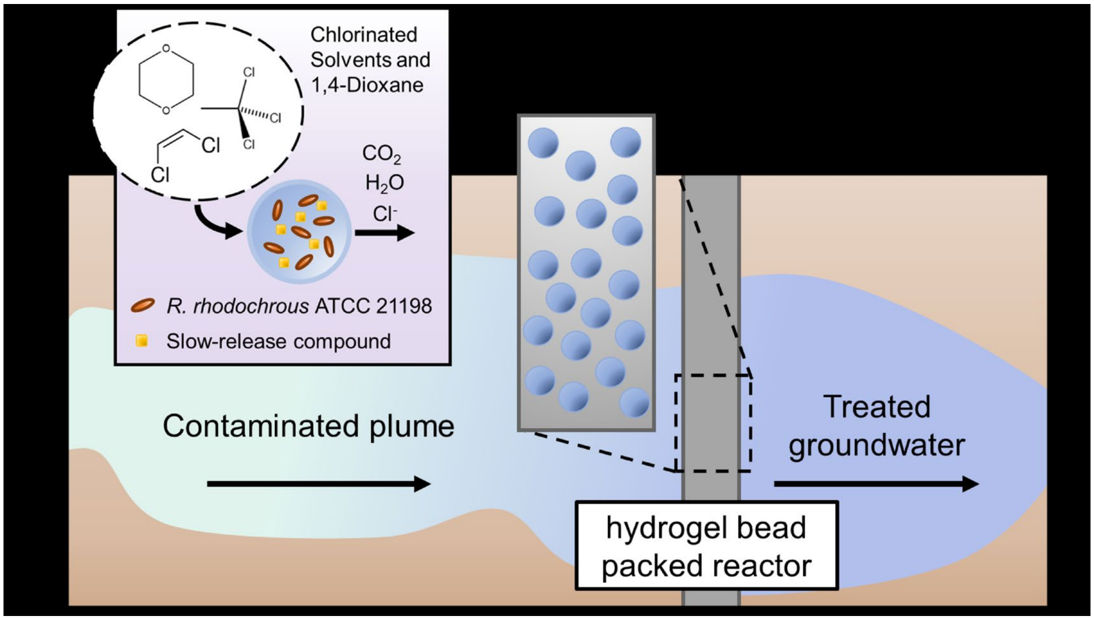

Aim 3:
In silico enzyme discovery
Aim 1:
Degradation by nanomaterials
SFC
LC-MS/MS & LC-HRMS
IonChrom
19F-NMR
46.2 nmol. Fluorine/g
O2
•O2-
•OH
Shorter Chain PFAS
CnF2n+1COOH
Mineralization
F-, CH3COO-, HCOO-
PFOA
PFOS
Time (min)
Response (μS)
Aim 2:
Enhanced biodegradation by isolates

Oregon State University

Grant Information: Institution, Principal Investigator(s), Contact Information, Grant Number	**Oregon State University** **Project:** Development of Passive and Sustainable Cometabolic Systems to Treat Complex Contaminant Mixtures by Encapsulating Microbial Cultures and Slow-Release Substrates in Hydrogels **Project Leaders**: Lewis Semprini, Michael R. Hyman, Willie Ernest Rochefort **Funding Period:** 2021-2025
Technology	Passive and sustainable systems are being developed for the aerobic cometabolic in-situ treatment of emerging contaminants, such as 1,4-dioxane, that are mixed with chlorinated aliphatic hydrocarbons (CAHs). These passive systems are being created by co-encapsulating axenic bacterial cultures with a slow-release compound (SRC) in hydrogel beads.
Innovation	**Materials: What novel materials are you developing?** The hydrogel beans are being fabricated using polyvinyl alcohol and sodium alginate that are crosslinked with boric acid and calcium ion. **Biological: What is the biological component?** The hydrogel beads co-encapsulated the bacteria *Rhodococcus rhodochrous* ATCC 21198 and an SRC that produces an alcohol as a growth substrate upon hydrolysis with water. **Why is this technology/approach different than what is already in the market?** The innovation is that long-term cometabolic transformations can be achieved by the co-encapsulated hydrogel beads.
Contaminant and Media	**Contaminants:** We are focusing on the emerging contaminant 1,4-dioxane and broad range of chlorinated ethanes, such as 1,1,1-trichloroethane, and chlorinated ethenes, such as 1,2-cis- dichloroethene and vinyl chloride. **Media:** Focusing on in-situ treatment of contaminated groundwater.
Expansion Potential	**Looking Forward:** The process using *Rhodococcus rhodochrous* strain ATCC 21198 has potential to treat a broad range of other contaminants, including MTBE, BTEX, polycyclic aromatic hydrocarbons (PAHs), 1,2,3-trichloropropane, and NDMA. **Combined Remedy:** The hydrogels might also be used to treat contaminated sediments and soils, and potentially drinking water.
Sites/Samples	We are working with groundwater samples and sediments for a U.S. Navy site on the West Coast.
Technology Readiness Level	TRL 4 — Technology validated in laboratory

Continued

Update of Progress	Column Study of Aerobic Cometabolism of Chlorinated Solvents and 1,4-Dioxane With Co-Encapsulated Hydrogel Beads. Kaden Bennett, Kestrel Bailey, and Lewis Semprini (NIEHS SRP Annual Meeting, 2023) Chlorinated aliphatic hydrocarbons (CAHs) are a class of organic compounds that include several widely used industrial solvents. These chlorinated solvents have known toxic effects and are common groundwater contaminants across the United States. *Rhodococcus rhodochrous* 21198 (ATCC 21198) is a bacteria species that can dechlorinate CAHs. It is currently being studied in an engineered system for eventual application at contaminated groundwater sites. In this novel remediation approach, ATCC 21198 is encapsulated in hydrogel beads along with the slow-release substrate tetrabutyl-s-orthosilicate (T2BOS). T2BOS hydrolyzes to produce 2-butanol, a growth substrate for ATCC 21198. As ATCC 21198 metabolizes 2-butanol, a co-metabolic process occurs to dechlorinate certain CAHs. A packed column of hydrogel-encapsulated ATCC 21198 is being tested with a dilute groundwater media containing the CAHs 1,2-cis-dichloroethylene (cis-DCE) and 1,1,1-trichlorethane (1,1,1-TCA), as well as the common groundwater co-contaminant 1,4-dioxane (1,4-D). Hydrogen peroxide is being spiked into the column media to provide sufficient oxygen in the system. There are ongoing experiments to determine the system's oxygen requirements via in-situ dissolved oxygen measurements with a fluorescent probe. In the current system, over 99% remediation of 66 ppb cis-DCE, over 90% remediation of 200 ppb 1,1,1-TCA, and over 60% remediation of 1 ppm 1,4-D has been achieved with a hydraulic residence time of 25.8 hours.



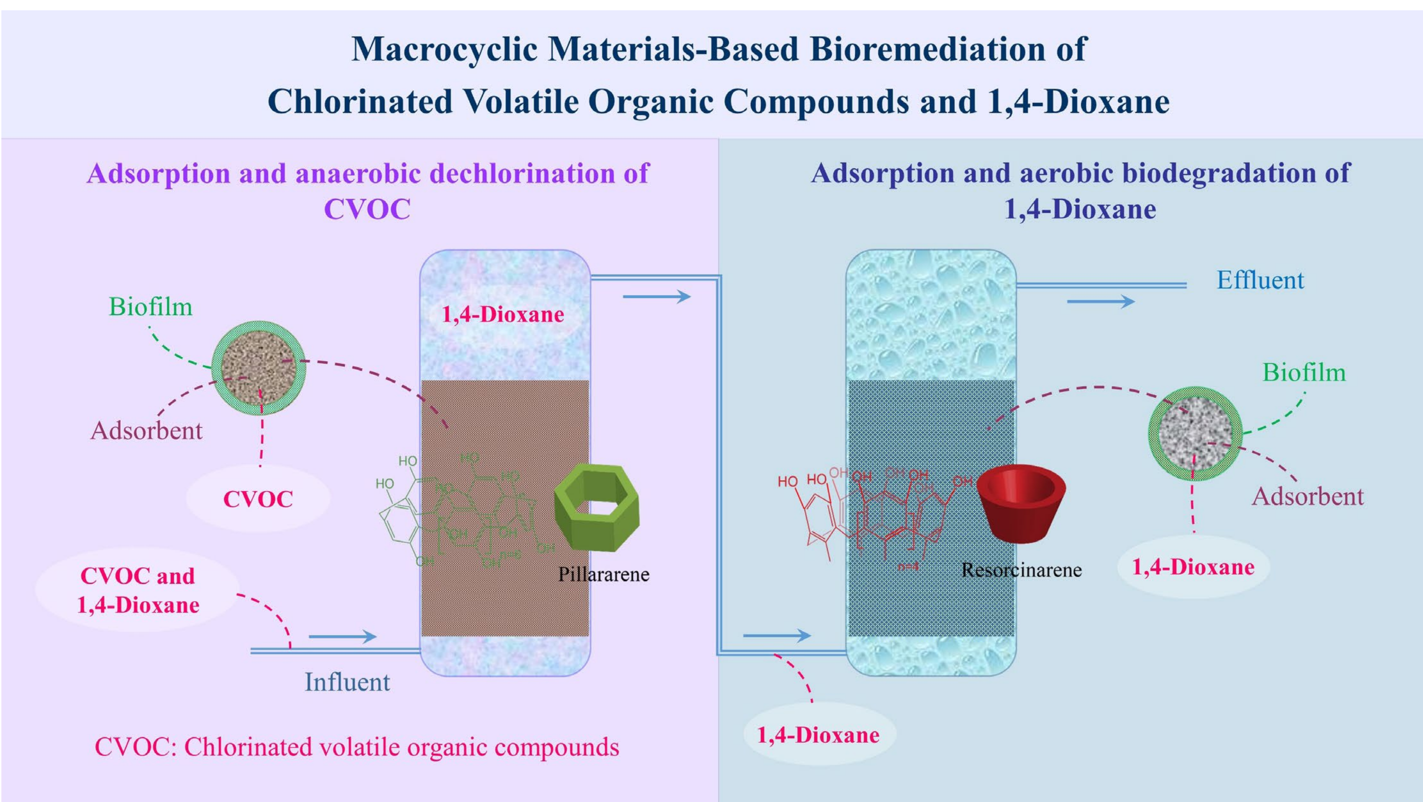
Co-encapsulated hydrogel beads for the long-term in-situ aerobic cometabolic treatment of 1,4-dioxane and chlorinated aliphatic hydrocarbons.

FLORIDA STATE UNIVERSITY

Grant Information: Institution, Principal Investigator(s), Contact Information, Grant Number	**Florida State University** **Project:** Enhancing Bioremediation of Groundwater Co-Contaminated by Chlorinated Volatile Organic Compounds and 1,4-Dioxane Using Novel Macrocyclic Materials **Project Leaders**: Youneng Tang, Yuexiao Shen (Texas Tech University) **Funding Period:** 2021-2025
Technology	First, a macrocyclic material selectively adsorbs chlorinated volatile organic compounds (CVOCs) and promotes dechlorinating biofilm on the material surface to anaerobically biodegrade CVOCs. After the CVOCs treatment, another macrocyclic material efficiently adsorbs 1,4-dioxane and sustains biofilm to aerobically metabolize 1,4-dioxane.
Innovation	**Materials:** Macrocyclic materials that selectively adsorb either CVOCs or 1,4-dioxane. **Biological:** 1,4-dioxane-degrading cultures that can metabolize 1,4-dioxane at environmentally relevant concentrations. **Why is this technology/approach different than what is already in the market?** The selective sorption enables separation of CVOCs from 1,4-dioxane, which further enables their biodegradation that requires different environmental conditions. The high efficiency of the 1,4-dioxane, which further enables their biodegradation that requires different environmental conditions. The high efficiency of the 1,4-dioxane-degrading cultures enables their application at environmentally relevant conditions.
Contaminant and Media	**Contaminants:** CVOCs and 1,4-dioxane **Media:** Groundwater
Expansion Potential	**Looking Forward: What other contaminants/media would work for your technology?** This remediation framework would also work for other mixed contaminants that require separation before biodegradation. However, the sorbents and microbial cultures should correspondingly change. **Combined Remedy: Would this technology work well with other treatment approaches?** We are evaluating the combination of advanced oxidation process (AOP) with sorption to chemically degrade CVOCs and 1,4-dioxane together.
Sites/Samples	Sites to be determined by Geosyntec Consultants Inc.

Continued

Technology Readiness Level	TRL 4 — Technology validated in laboratory
Update of Progress	Based on atomistic modeling of macrocyclic sorbents and experimental screening of these sorbents, three sorbents that can selectively adsorb CVOCs have been found and synthesized. Five 1,4-dioxane-metabolizing pure cultures that could degrade 1,4-dioxane at low concentrations have been isolated and characterized in terms of taxonomy and biodegradation kinetics. The partial 16S rRNA gene sequences of the five pure strains are deposited at the National Center for Biotechnology Information (NCBI) GenBank database under accession numbers OP362562, OP362563, OP362564, OP362565, and OP362566, respectively. To study the interactions of the sorbents, microbial cultures, and contaminants, a mathematical model has been developed to simulate the simultaneous sorption and biodegradation of 1,4-dioxane. The model has been validated through experiments in continuously stirred tank reactors and data reported in the literature.



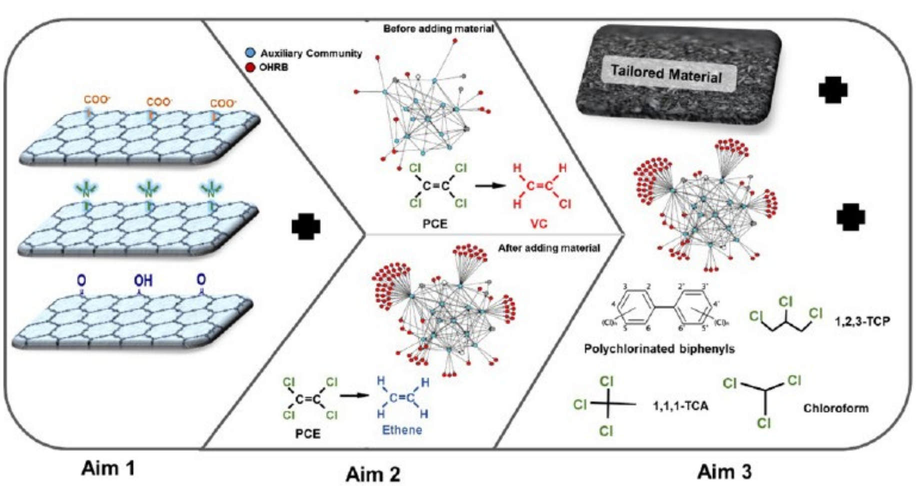
Macrocyclic materials-based bioremediation of chlorinated volatile organic compounds and 1,4-dioxane.

THE UNIVERSITY OF IOWA

Grant Information: Institution, Principal Investigator(s), Contact Information, Grant Number	**University of Iowa** **Project:** Elucidating Mechanisms for Enhanced Anaerobic Bioremediation in the Presence of Carbonaceous Materials Using an Integrated Material Science and Molecular Microbial Ecology Approach **Grant Number:** R01ES032671 **Project Leader:** Timothy E. Mattes **Email:** tim-mattes@uiowa.edu **Co-Investigator:** Wenqing Xu (Villanova University) **Funding Period:** 2021-2025
Technology	An emerging remediation strategy involving amendment of pyrogenic carbonaceous matter (PCM), such as activated carbon, to the subsurface could promote synergistic interactions among OHRB and the auxiliary microbial community and subsequently improve OHRB-driven bioremediation efficacy. Elucidating positive impacts between PCM and OHRB will allow for the development of tailored PCM that offers a potential solution to problems with organohalide respiring bacteria (OHRB)-driven bioremediation.
Innovation	**Materials: What novel materials are you developing?** We aim to develop tailored pyrogenic carbonaceous matter (PCM) with the aid of a tunable PCM-like polymer synthesis platform where material properties can be varied individually. **Biological: What is the biological component?** Organohalide-respiring bacteria (OHRB) and the supporting anaerobic microbial community. **Why is this technology/approach different than what is already in the market?** Colloidal activated carbon (CAC) is already available for injection into contaminated groundwater. However, the purpose of CAC is to sorb organic pollutants, such as chlorinated ethenes. The purpose of tailored PCM will be to promote/enhance bioremediation while providing the potential to sorb organic pollutants that inhibit OHRB.
Contaminant and Media	**Contaminants: What contaminant(s) does your project target?** Chlorinated ethenes **Media:** ***(e.g., groundwater, drinking water, soil, sediment)*** Contaminated groundwater, soil, and sediment

Continued

Expansion Potential	**Looking Forward: What other contaminants/media would work for your technology?** Our potential technology could be useful for bioremediation of other organic pollutants in groundwater and sediments, such as chlorinated ethanes and PCBs, and possibly emerging contaminants, such as chlorinated propanes. **Combined Remedy: Would this technology work well with other treatment approaches?** We expect that a tailored PCM amendment strategy would be compatible with other groundwater treatment approaches such as in-situ bioremediation.
Sites/Samples	We are not yet working on any sites or using real-world samples. We expect to begin working with environmental samples in the last year or two of this project.
Technology Readiness Level	TRL 2 — Technology concept formulated
Update of Progress	Tetrachloroethene (PCE), a halogenated pollutant commonly found at Superfund sites, can be detoxified (i.e., converted to ethene) by stimulating or adding specialized anaerobic dechlorinating microorganisms to contaminated groundwater. However, anaerobic dechlorinators sometimes have problems fully converting PCE to ethene, accumulating the lesser chlorinated products cis-dichloroethene (cDCE) and vinyl chloride. Pyrogenic carbonaceous materials (PCM) have promising applications in pollution remediation, including enhanced chlorinated ethene bioremediation processes at contaminated sites. Experiments were performed with a commercially available PCE-dechlorinating consortium experiencing "cDCE-stall" (i.e., converted PCE to cDCE and stopped). However, adding PCM (granular poplar biochar) to the stalled consortium stimulated complete dechlorination of PCE to ethene. Microbial community analyses showed that methane-producing and cDCE-dechlorinating microbes colonized the biochar surface. These findings are significant because a PCM amendment strategy during bioremediation could enhance important microbial interactions that ultimately improve bioremediation outcomes for chlorinated pollutants commonly found at Superfund sites.

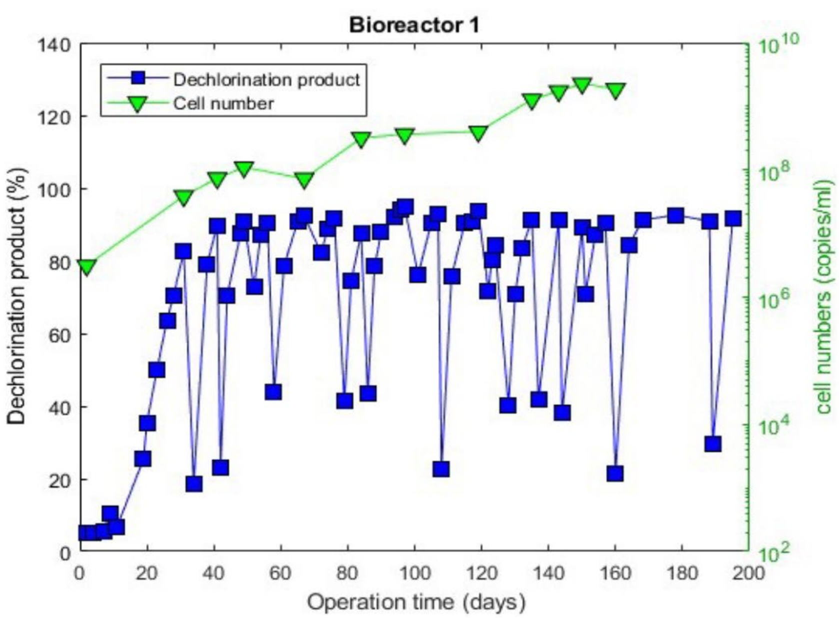


Technology development begins with a tunable platform for synthesizing PCM-like polymers where PCM surface properties can be varied individually (Aim 1). The effects of PCM surface properties on microbial interaction networks and subsequent performance of an organohalide-respiring mixed culture will be quantified (Aim 2). Tailored PCM for enhanced anaerobic bioremediation and contaminant mixture retention will be developed and its performance validated in microcosms (Aim 3).

Grant Information: Institution, Principal Investigator(s), Contact Information, Grant Number	**University of Maryland, Baltimore County** **Project:** Leveraging the Chemo-Physical Interaction of Halorespiring Bacteria With Solid Surfaces to Enhance Halogenated Organic Compounds Bioremediation **Project Leaders**: Upal Ghosh, Kevin R. Sowers, Amar Wadhawan (ARCADIS) **Grant:** R01ES032719 **Funding Period:** 2021-2025 **Contact:** ughosh@umbc.edu
Technology	The research team is developing carbon-based sorbent materials to enhance the ability of bacteria to break down mixtures of chlorinated organic contaminants, such as chloroethenes and polychlorinated biphenyls in groundwater and sediments.
Innovation	**Materials:** A set of BC materials are being synthesized to create a range of tailored properties such as specific surface area, pore size distribution, electron accepting capacity, electrical conductivity, and sorption capacity. **Biological:** A PCE and PCB dechlorinating bacteria, *Dehalobium chlorocoercia*, is being used to evaluate the interaction with tailored material surfaces. **Why is this technology/approach different than what is already in the market?** The research is first developing the fundamental understanding of the material surface and bacterial interaction to optimize the development of tailored materials that would enhance the biological process.
Contaminant and Media	**Contaminants:** We are targeting PCE and TCE in groundwater and PCBs in the sediment matrix. **Media:** Soil and sediment mix, groundwater
Expansion Potential	**Looking Forward: What other contaminants/media would work for your technology?** The technology can be translated to other chlorinated compounds, like DDT. **Combined Remedy: Would this technology work well with other treatment approaches?** This technology will work well with other in-situ remediation technologies.
Sites/Samples	For this R01 project, we are not working at a field site. However, we are using sediments from an intertidal marsh site located in Edgewood, Maryland, for laboratory experiments.

Continued

Technology Readiness Level	TRL 4 — Technology validated in laboratory
Update of Progress	In the last year, we have made progress on five fronts: 1) Successively maintaining two bioreactors with active DF-1 culture as a source for cell material for the kinetic studies; 2) Establishing methodologies for measuring biokinetics for surface-attached cells on fine-silica, graphite, and activated carbon surfaces; 3) Performing mass balance for chloroethenes and cell numbers using new methods developed in this project; 4) Optimizing growth medium for the optimal measurement of dechlorination kinetics; 5) Establishment of sediment microcosm experiments. We have completed the development of methodologies and optimization for the biokinetic measurement of microorganisms growing on solid surfaces incubated in the growth medium. Ongoing work is focused on using the developed method to acquire experimental biokinetic data and verify the impact of different material surfaces. Our current efforts on the sediment microcosm design will also focus on translating the biokinetic results from the liquid medium into the more complex sediment matrix.



Growth of Dehalobium chlorocoercia *in batch bioreactor through multiple cycles of PCE addition and purging of products.*

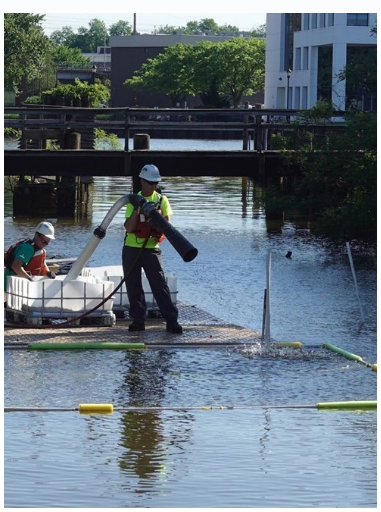
Grant Information: Institution, Principal Investigator(s), Contact Information, Grant Number	[2Witech Solutions LLC](#) **Project:** Fast Field Screening of PFAS in Non-Portable Waters **Project Leader**: [Qingwu Wang](#) **Funding Period:** Phase I: May 2023 - April 2024 R43ES035347
Technology	2Witech Solutions LLC develops and commercializes a portable electrochemical sensing device for quick field screening of trace PFAS in non-potable waters. The developed sensor will be examined for its applicability in real contaminated groundwater samples.
Innovation	The company uniquely combines its recent achievement in molecular-imprinting technology and electrochemical sensor development to produce an innovative PFAS sensor with advanced attributes unattainable before. The fabricated sensor will possess a dynamic range up to 200ppt levels of perfluorinated chemicals in water with a detection limit of 1ppt, response time within minutes, and selectivity of PFAS against interfering ions and organics, as well as recoverability for quick determination.
Contaminant and Media	PFAS in drinking water, groundwater, surface water, and wastewater
Expansion Potential	The sensor has been evaluated using PFAS-spiked synthetic wastewater samples, but has not validated with real-world samples yet.
Sites/Samples	For this R01 project, we are not working at a field site. However, we are using sediments from an intertidal marsh site located in Edgewood, Maryland, for laboratory experiments.
Technology Readiness Level	TRL 3

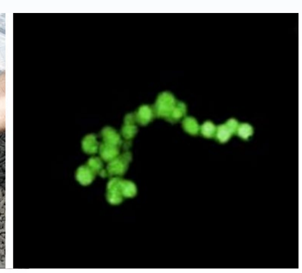
Portable PFAS Analyzer

A portable PFAS analyzer (left) and its laptop-based controller (right). The PFAS analyzer comprises an electrochemical workstation, a testing module, and a solution delivery module.

Grant Information: Institution, Principal Investigator(s), Contact Information, Grant Number	**Biomineral Systems LLC** **Project:** Green and Sustainable In Situ Remediation of Heavy Metals Contaminated Soils and Aqueous Systems **Project Leader**: Nadia Adam **Funding Period:** 2021-2025 R43ES035347
Technology	**Succinct 1-2 sentence description of the technology you have developed/are developing.** Novel, patent-pending, sustainable, highly reactive, cation and anion heavy metals binding metal phosphate-based nanoremediation technology. Technology has been validated for contaminated soils and groundwater in current superfund sites.
Innovation	**Why is this technology/approach different than what is already in the market?** Unlike current technologies, our nanoremediation approach provides in situ, permanent, and synchronous stabilization/ removal without bioaccumulation, toxicity, and cumbersome formulations at cost.
Contaminant and Media	**Contaminants: What contaminant(s) does your project target? In what media? (e.g., groundwater, drinking water, soil, sediment)** Contaminants targeted include all heavy metals and uranium in groundwater, drinking water, soils, and sediments.
Sites/Samples	**We don't have any record of sites in our databases. Are you working on any sites and/or using real world samples? Please include Site Name, City, State.** Soils from the Colorado River Indian Tribe Reservation in Parker, Arizona, and Ak-Chin Tribe in Maricopa, Arizona.
Technology Readiness Level	TRL 5 — Technology validated in relevant environment (industrially relevant environment in the case of key enabling technologies)

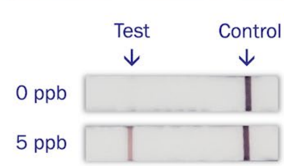
Grant Information: Institution, Principal Investigator(s), Contact Information, Grant Number	**RemBac Environmental LLC** **Project:** Development of an Innovative Approach for In Situ Treatment of PCB Impacted Sediments by Microbial Bioremediation **Project Leader**: Craig Bennett Amos, Upal Ghosh, Kevin Sowers Contact: ben@sedimite.com **Funding Period:** 2023-2025 R44ES032365
Technology	The proposed research will advance toward commercialization a novel in situ bioremediation technology that employs microbes and activated carbon to treat PCB impacted sediments.
Innovation	Current methods for treating PCB-contaminated sediments are expensive, energy-intensive, and disruptive to the ecosystem. Sustainable, minimally invasive, and relatively low-cost technology is critically needed to help reduce the vast inventory of PCB-contaminated sediments. Our innovative technology meets this need by employing naturally occurring PCB degrading microbes combined with activated carbon pellets as a delivery system for in situ sequestration and degradation of PCBs in sediments.
Contaminant and Media	We are targeting polychlorinated biphenyls (PCBs) in intertidal sediments.
Expansion Potential	The sensor has been evaluated using PFAS spiked synthetic wastewater samples, but has not validated with real world samples yet.
Sites/Samples	We proposed testing the technology at the New Bedford Harbor Superfund site located in Fairhaven, Massachusetts.
Technology Readiness Level	TRL 6 — Technology demonstrated in relevant environment (Industrially relevant environment in the case of key enabling technologies)
Update of Progress	The project was recently funded, and we are in the early stages of developing the work plan and seeking approvals from site stakeholders.





Application of bioamended SediMite™ that combines the advantages of adsorption by activated carbon and degradation by microbes for in situ cleanup of PCB-contaminated sediments.

Grant Information: Institution, Principal Investigator(s), Contact Information, Grant Number	**Stemloop Inc.** **Project:** A Paper-Based Synthetic Biology Platform for the On-Demand Testing of Water Quality **Project Leader**: **Khalid K. Alam, Ph.D.** **Funding Period:** Phase II: September 2022 – August 2024 R44ES031899
Technology	Stemloop is developing an inexpensive, easy-to-use, and rapid test for lead in drinking water.
Innovation	Stemloop's ROSALIND™ technology (*Nature Biotechnology* **38**, 1451–1459, 2020) rewires an ancient bacterial protein to sense lead and activate a genetic response in a "cell-free" biochemical reaction. The reaction is freez¸-dried for shelf-stable torage and distribution, is activated by adding water, and measured using a lateral flow device.
Contaminant and Media	Lead in drinking water
Sites/Samples	Stemloop is collecting and testing drinking water samples from systems across the United States, with a focus on Chicagoland and the surrounding Great Lakes region.
Technology Readiness Level	TRL 7 — System prototype demonstration in operational environment



uSense for Lead – an inexpensive, easy-to-use, and rapid test for lead in drinking water.

National Institute of Environmental Health Sciences
Superfund Research Program R01 Satellite Meeting

Optimizing Natural Systems for Remediation:
Utilizing Innovative Materials Science Approaches
to Enhance Bioremediation

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