Environment and Natural Resources Trust Fund
2016 Request for Proposals (RFP)

Project Title: **ENRTF ID:** **046-B**

Reducing Salt and Metal Removal Costs with Microbes

Category: B. Water Resources

Total Project Budget: $ 596,599

Proposed Project Time Period for the Funding Requested: 3 years, July 2016 to June 2019

Summary:

To use recently discovered microbes from Minnesotas Soudan Iron Mine to reduce the cost of removing salts and metals from subsurface and aquatic water resources

Name: Daniel Bond

Sponsoring Organization: U of MN

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Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

Cartoon showing how bacteria tolerant of harsh subsurface conditions can be used to power salt removal reactors

_____ Funding Priorities _____ Multiple Benefits _____ Outcomes _____ Knowledge Base

_____ Extent of Impact _____ Innovation _____ Scientific/Tech Basis _____ Urgency

_____ Capacity Readiness _____ Leverage _______ TOTAL ______%

PROJECT TITLE: Reducing salt and metal removal costs with microbes

I. PROJECT STATEMENT

We recently discovered microbes in Minnesota's Soudan Mine that could significantly reduce the cost of removing salts and metals from water. The Soudan Iron Mine contains exploratory boreholes where water saltier than seawater and high in heavy metals contains unique organisms that thrive under extreme conditions. Our previous LCCMR-supported project revealed that some of these microbes can generate electricity, while others can remove metals from contaminated waters. This discovery makes it possible to use biology to power a new class of water remediation technologies. Termed 'microbial desalination cells', our devices harness this rare group of bacteria to create a 'push' of charged electrons to help 'pull' charged salts across membranes. This has the potential to cut energy use in many aspects of water purification by 50%.

These microorganisms are able to address applications specific to the chemistry of Minnesota, where sulfates, chlorides, and other salts escape into stormwater and mining effluents. Other organisms can remove metals before they clog treatment plants and create toxicity issues. Our project combines these two novel biological abilities into a single solution.

II. PROJECT ACTIVITIES AND OUTCOMES

1. Microbe-powered desalination. Salt ions, from the sodium chloride in road salts to sulfates that can be discharged at mining sites, can be the most difficult contaminants to remove from water. Energy must be invested to push salts across membranes in reverse osmosis or desalination plants. Our team recently discovered microbes that have two unique skills: an ability to generate biological electricity, and the capacity to grow in extremely harsh environments. We will harness this rare combination in a new class of salt-removing reactors.

The 'microbial desalination cell' contains electrodes colonized by bacteria, who generate electricity inside the device. The electrical flow created by bacteria helps drive salts across membranes, leaving a concentrated brine for collection or sale. Bacteria can reduce the power needed for this kind of water purification by 50%. Because few electricity-producing microbes were known to tolerate extreme conditions, such reactors were not possible in industrial, mining and fracking waters.

With over 20,000 large-scale plants worldwide, and millions of on-site salt treatment plants operating at natural gas hydraulic fracturing and industrial sites, even minor improvements to desalination technology will have a significant impact on energy usage and water recovery.

We will deliver microbes from extreme environments that are highly salt- and metal-resistant, yet still able to catalyze electrical reactions. These bacteria will be able to power model microbial desalination reactors under harsh conditions to reduce the overall cost of salt removal from waters. **Budget: $387,732**

Outcomes:	**Completion Date**
1. Demonstrate electricity production by bacteria obtained from underground brines, measure fastest rates to identify best candidates for use in desalination	June 30, 2017
2. Operate model microbial desalination cells to measure reduction in energy use	June 30, 2018
3. Demonstrate microbial desalination cell to treat a real-world mining effluent	June 30, 2019

2. Removing metals with novel microbes. Metals, from iron to mercury, present special toxicity and solubility issues, and can clog devices designed for water purification. In our previous work, we found microorganisms thriving in areas heavily contaminated with copper, cobalt, iron and manganese that can act as natural sponges, adsorbing metals from the water. Additionally, some fungi accelerate removal of metals from water, causing rapid formation of particles that are easily removed. Together, these can make "upstream" bio-filter zones to treat metal-rich waters. This passive technology will greatly improve the operation of 'downstream' membrane-based microbial salt removal, and could be deployed in areas of high metal contamination. **Budget: $198,866**

This project will show how novel fungi discovered in the most contaminated areas of mines can remove metals that would normally clog desalination membranes and create environmental toxicity issues

Outcomes:	**Completion Date**
1. Demonstrate new fungi can precipitate metals from high iron and copper waters	June 30, 2017
2. Operate laboratory scale reactors to recover metals from model waste	June 30, 2018
3. Use metal removal fungi upstream of microbial desalination cell in Activity 1	June 30, 2019

III. PROJECT STRATEGY

A. Project Team/Partners

Team Leader: Dr. Daniel Bond (UMN) is an Associate Professor of Microbiology and the BioTechnology Institute. He discovered microbial electricity production and will direct construction of microbial desalination reactors.

Dr. Jeff Gralnick (UMN) Associate Professor in the Department of Microbiology and the BioTechnology Institute is an expert in electron transfer by bacteria, and led the LCCMR project that discovered the bacteria tolerant of extreme conditions used in this proposal.

Dr. Brandy Toner (UMN) Associate Professor in the Soil, Water and Climate Department is an expert in geomicrobiology and toxic metals, and responsible for all mineralogical and metal analyses.

Dr. Robert Blanchette (UMN) is an expert in fungal biology, and discovered fungi able to adsorb metals in our previous LCCMR project.

Dr. Cara Santelli (UMN) is an expert in fungi active in mining and metal-impacted sites. She will be responsible for new fungal discovery and reactors operated upstream of microbial desalination cells.

Jim Essig (DNR Park Manager of Soudan Mine State Park) is an additional partner (not funded by ENRTF) include who will help coordinate research activities.

B. Project Impact and Long-Term Strategy

The proposed work is based on a discovery from our current LCCMR program, which first discovered a range of life in the abandoned mine, then explored the unique microbiology of the Soudan Mine in search of new drugs, bacteria, and remediation strategies (LCCMR 2010-2013 and 2013-2016). We are now prepared to demonstrate that some of these microbes can be used in industrial and environmental settings. This funding is essential to the scale-up and demonstrations that protect the Intellectual Property of the microbial component, while we will obtain federal funding (National Science Foundation, Department of Energy, United States Department of Agriculture) to support work understanding the biology enabling this technology. We will share our reactors and organisms with collaborators at NRRI and the University of Minnesota-Duluth for implementation studies related to bioremediation.

C. Timeline Requirements

Three years will be sufficient to accomplish the objectives of the two scientific areas, allowing early work to focus on obtaining unique microbial communities, with a second phase dedicated to building demonstration devices for removing salts and metals.

2016 Detailed Project Budget

Project Title: Reducing salt and metal removal costs with microbes

IV. TOTAL ENRTF REQUEST BUDGET: 3 **years**

BUDGET ITEM	AMOUNT
Personnel: Activity #1 will be staffed by 1 full time postdoctoral scientist and 1 graduate level student supported according to NIH guidelines current Graduate School rates, **Activity #2** will be staffed with 1 postdoctoral scientist and 1 undergraduate researcher. No faculty salaries, support or fringe is included.	
Postdoc #1 : Design, build, operate microbial desalanation cells. (83% salary, 17% benefits), 1 FTE	$ 157,093
Graduate Student #1 : Cultivate, test, and provide bacteria capable of electricity production (81% salary, 19% benefits) 1 FTE	$ 101,583
Postdoc #2 : cultivate and test metal-removing fungi and metal-removing reactors. (83% salary, 17% benefits), 1 FTE	$ 157,093
Undergraduate researcher : Assist with mine sampling and microbiology (15h/wk, $8/h)	$ 18,730
Equipment/Tools: Cost of equipment used in construciton of water treatment reactors, and specialized equipment for electrical culturing of bacteria. Once built, these reactors can be used throughout the project	
Machine shop charges for reactors and lab scale reactor temperature control and pumps ~$1500/each	$ 12,000
Power supplies, gas controllers, and monitoring equipment such as conductivity and pH probes	$ 14,000
Electrochemical workstations for controlling microbial electricity production (potentiostats), each channel requires ~$2725	$ 21,800
Supplies: Costs of specialized anaerobic gasses for culturing bacteria, DNA sequencing and synthesis, chemicals and reagents. Included here is also cost of membranes for desalination cells, cost of metal and water analysis, and consumables such as reference electrodes, catalysts and filters to support three active researchers in both the field and laboratory demonstration projects.	
Laboratory consumables; gloves, sterile pipet tips, tubes, syringes, stoppers, glassware for cultivating bacteria in the absence of oxygen, ~3200/y per active researcher based on historical average	$ 28,800
Molecular biology reagents: polymerase chain reaction enzymes, restriction enzymes, plasmid and PCR miniprep kits, cloning reagents, sequence verification, DNA synthesis, ~$3800/y per researcher	$ 34,200
Electrochemical consumables: reference electrodes, wire and electrodes, anaerobic grade gasses and catalysts to remove oxygen, membranes. ~$3500/y per researcher (2 researchers)	$ 21,000
DNA sequencing (Mayo clinic or UMN sequencing center, $1500/genome or metagenome sample) Imaging of electrodes ($40/h), software licenses ($300/y), offsite water analysis ($250/sample), columns and detectors for HPLC/GC/IC analysis ($500/y)	$ 19,800
Travel: Routine sampling and research trips to Soudan Mine (~4x/y), includes one night lodging and vehicle costs according to University of Minnesota reimbursement rates, includes travel to NRRI and UMD for interactions and 1 group conference/y for field-scale demonstraiton.	$ 7,500
Additional Budget Items: *Publications and dissemination of results in Open Access (non-restricted) journals*	$ 3,000
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 596,599

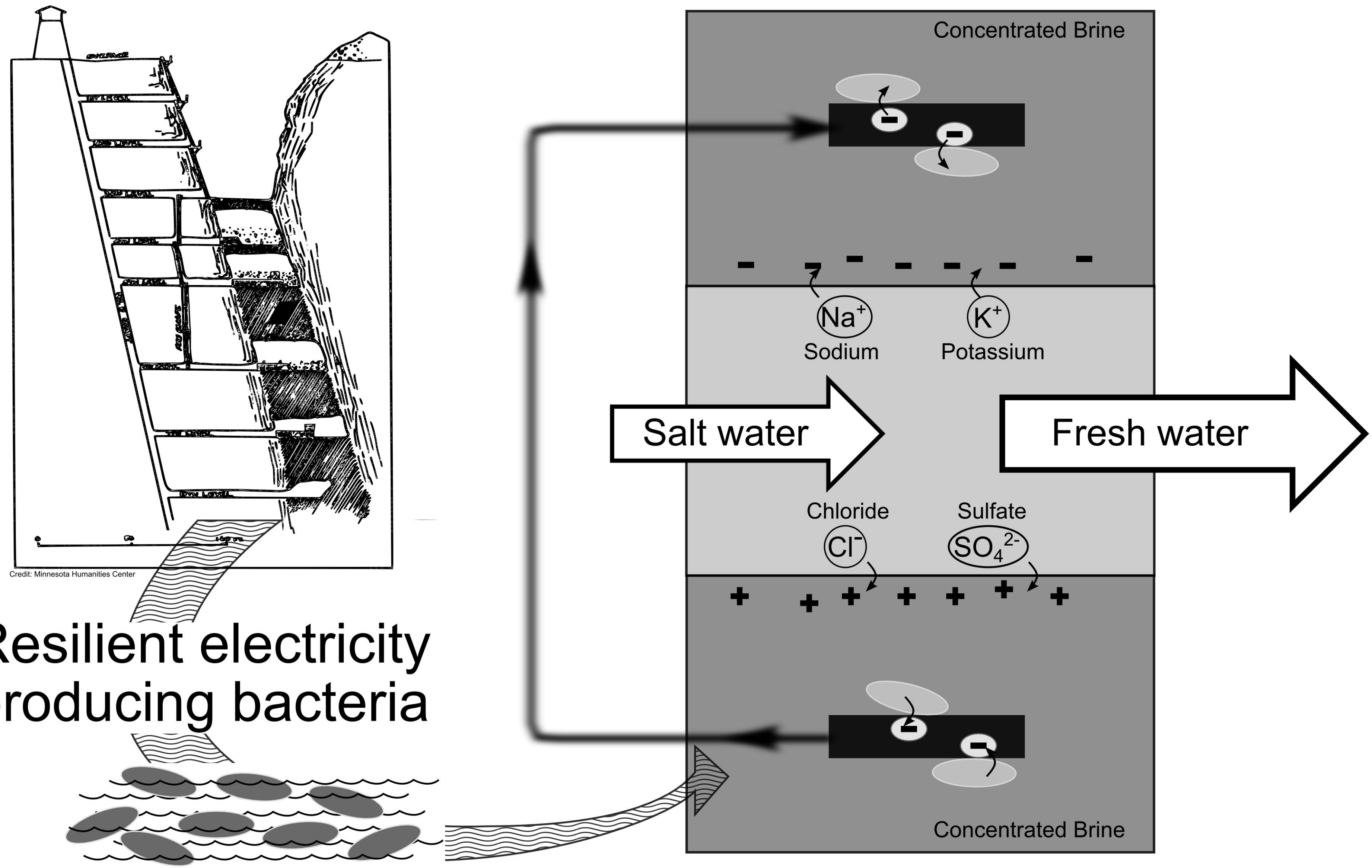
V. OTHER FUNDS *(This entire section must be filled out. Do not delete rows. Indicate "N/A" if row is not applicable.)*

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ To Be Applied To Project During Project Period: *N/A*	$ -	*N/A*
Other State $ To Be Applied To Project During Project Period: *N/A*	$ -	*N/A*
In-kind Services To Be Applied To Project During Project Period: N/A	$ -	*Indicate: Secured or Pending*
Funding History: The previous LCCMR allocation that led to this discovery is ENRTF ML 2013-03f "Harnessing Soudan Mine Microbes: Bioremediation, Bioenergy, and Biocontrol" $838,000. Team member Gralnick led ENRTF, ML-2010-"Science and Innovation from Soudan Underground Mine State Park", $545,000, which represented the earliest visits to the Soudan Mine.	$ 1,383,000	
Remaining $ From Current ENRTF Appropriation: *Current funds are obligated to finish by end of project*	$ -	*Legally Obligated*

Reducing the Cost of Salt Removal with Microbes

Soudan Mine

Microbial Desalination Cell



Attachment : Project Director Qualifications

Dr. Daniel R. Bond

Associate Professor of Microbiology, University of Minnesota BioTechnology Institute

Daniel Bond was lead author of the 2001 work that discovered bacteria could generate electricity on electrodes in ocean environments. Since coming to the University of Minnesota, his research group has pioneered the electrical and genetic techniques used to isolate, study and harness bacteria that transform metals. His laboratory houses over 50 independent workstations able to grow bacteria on electrodes, and multiple anaerobic chambers for growth of these unique organisms.

Dr. Bond was involved in prior research that found the Soudan Mine housed unique biological life. As part of LCCMR research in the past 3 years, his laboratory participated in isolation of the first electricity-producing bacterium from the mine, "*Desulfuromonas soudanensis*", and sequenced the DNA of multiple isolates involved in metal resistance and antibiotic production.

Dr. Bond is actively involved in research focusing on bacteria that change the solubility and toxicity of metals in the environment. His group uncovered the basis for how bacteria transform iron and manganese minerals and is currently developing techniques for sensing the activity of bacteria in bioremediation zones.

The team assembled for this project brings together scientists from departments encompassing Microbiology, Earth Science, and Soil, Water and Climate. The team has a solid record of collaboration and outreach supported by LCCMR, and we are excited to add a recently hired faculty member who is a leader in the field of metal remediation.