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

Project Title: **ENRTF ID: 057-B**

Innovative Methods for the Removal of Trace Phosphate

Category: B. Water Resources

Total Project Budget: $ 345,405

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

Summary:

Phosphate is an environmental pollutant, including at trace levels. Current methods for removal are limited, and we proposed an innovative technology to capture efficiently, cost-effectively trace P from waste waters.

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Sponsoring Organization: U of MN

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Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

we propose to specifically engineer and improve the existing phosphate uptake system using high-affinity phosphate-specific transport system of bacteria and introduce this capability to environmentally safe cyanobacteria, and thereby creating “phosphate-scavenging self-sustaining bug” that could subsequently be turned into a bioremediation reactor.

_____ Funding Priorities	_____ Multiple Benefits	_____ Outcomes	_____ Knowledge Base
_____ Extent of Impact	_____ Innovation	_____ Scientific/Tech Basis	_____ Urgency
_____ Capacity Readiness	_____ Leverage	_____ TOTAL	_____%

I. Project statement

Phosphorus (P) is a serious water pollutant for Minnesota and the Great Lake area. EPA has set the mandatory limit to be lower than 0.05 mg/L for streams to be discharged into lakes or reservoirs. However, even traces of phosphorus (above 0.02 mg/L) cause eutrophication with the growth of invasive species, leading to the deterioration of water quality and threatening the life of aquatic creatures. The problem has dramatic consequences: on August 2nd 2014, a poisonous algae bloom knocked out the water supply for ~ 500,000 residents in the Toledo area, Ohio. Currently, most of the conventional methods for phosphorus treatment only work in a specific concentration range to meet the EPA regulations and approaches for low concentration P treatment are not available. We propose to address this shortcoming of the conventional methods with a high-P specific and high throughput biological scavenging method to specifically remove the trace amount of the P from water. In Nature, the phosphate uptake system in prokaryote is extremely efficient. It has been naturally evolved for the cells to transport phosphorus from the environment, in many cases, the depleted area where the phosphorus concentration is extremely low, to inside cell membranes, due to the critical importance of phosphate in cellular processes. Our preliminary research to introduce this phosphorus scavenging system into *E.coli* approved the high specificity of the phosphorus assimilation and showed the potential for this system to be applied for phosphorus removal from water [Elias M et al., Nature. 2012 Nov 1; 491(7422):134-7]. While *E.coli* can be ideal genetic host to prove the concept, introducing them into the water may generate concerns over safety. **Therefore, we propose to specifically engineer and improve the existing phosphate uptake system of bacteria and introduce this capability to environmentally safe cyanobacteria, thereby creating "phosphate-scavenging self-sustaining bug"** that could subsequently be turned into a **bioremediation reactor to completely remove the trace amount of phosphorus in water**. While this addresses issues that many industrial methods cannot, our approach is also unique compared to most of the current biological solutions to phosphate remediation which focuses on ecological, or bioaugmentation approaches. This proposal therefore fits the funding priorities of LCCMR by creating innovative solutions to protect or restore freshwater and habitats. Specifically, we propose to engineer the bacterial phosphate transporter using state-of the art protein evolution methods (Activity 1), transfer the beneficial mutations into the cyanobacteria transporter and improve its phosphate uptake abilities (Activity 2). Bacteria and cyanobacteria carrying this improved phosphate transporter will be more efficient at extracting phosphate from their environment, and will be used to construct a laboratory-scale bioreactor to establish the proof of concept (Activity 3). Due to the high affinity of the engineered strains, the reactor can remove even the traces of P present in the waste stream and runoffs, with the removal levels go way beyond EPA allowable limits.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Molecular engineering of the bacterial phosphate scavenging system **Budget: $110,931**

The objective of this activity is to engineer the existing sophisticated bacterial transporter, dubbed Phosphate Specific Transporter (Pst), for higher phosphate uptake efficiency. Molecular engineering and evolution will be performed on the *Escherichia coli* transporter, offering a convenient and proficient genetic system and selection method. The identified key beneficial mutations on the Pst transporter will be subsequently transferred onto the cyanobacteria Pst transporter; and more specifically, onto the pstS protein that capture the phosphate from the environment and release it into the transmembrane transporter. The feasibility of this approach is backed up by our extensive experience in working with bacterial Pst transporters, including our recent success to crystallize, produce and mutate the *pstS from P. fluorescens, C. perfringens, and E. coli.*

Outcome	Completion Date
1. Molecular evolution of the *E. coli* Pst system for improved phosphate uptake	Jan 2017
2. Characterization of mutants parameters, and further improvement of the Pst	Jul 2017
3. Transfer to cyanobacteria Pst and characterization of the new system	Feb 2018

Activity 2: Lab scale evaluation of the developed cyanobacteria. **Budget: $115,079**

This activity involves the design of a lab scale reactor to evaluate the engineered cyanobacteria for P scavenging and the process parameters that affect the system performance. The engineered bio-scavenger

strain will be tested in shake-flask followed by bioreactor studies to evaluate the effectiveness of the system in onsite operation. Scavenging efficiency and kinetic design parameters at different scale and environmental conditions will be estimated for designing a suitable reactor for large scale operation. The lab-scale evaluation of the operating parameters will be carried out using a robust statistical methodology and mathematical models will be developed for the system. The treatment capacities of the systems will be estimated and the effect of system design parameters will be evaluated.

Outcome	Completion Date
1. Estimation of important parameters affecting the scavenging efficiency	April 2018
2. Lab scale reactor design and set-up with engineered cyanobacteria	Sep 2018
3. Lab scale reactor - Data collection and evaluation with synthetic and real P containing water	Jan 2019

Activity 3 Evaluation for specific P contained runoff water and cost estimation Budget: $119,395

A module type flow through reactor will be set up in the identified P containing stream, and the number of modules in sequence is based on the inlet P concentration and the scavenging efficiency of the strains. The module will be developed with the grown and induced recombinant cells for protein expression and tested with real wastewater, including agricultural runoff and streams with high phosphorus. Cost will be estimated onto a spreadsheet calculation model in order to determine the capital investment, the useful life; and operations and maintenance costs of the system, considering size the pollution level of different water body.

Outcome	Completion Date
1. Setting up P scavenging modules	May, 2019
2. Water sample collection and analysis for the P removal efficiency	June, 2019
3. Cost estimation of the process	June, 2019

III. PROJECT STRATEGY

A. Project Team/Partners

The team includes Professor Mikael Elias and his postdoc researcher (TBN) from the Department of Biochemistry, Molecular Biology & Biophysics, BioTechnology Institute, University of Minnesota; Professor Bo Hu and his post-doc researcher Dr. Aravindan Rajendran, from the Department of Bioproducts and Biosystems Engineering, University of Minnesota. Professor Mikael Elias is specialized in structural biology, biochemistry, molecular evolution, and has extensive experience on the bacterial phosphate uptake system (*Pst*). A postdoc (TBN) will execute the molecular evolution and characterization of the Pst system. Professor Hu is specialized in bioprocess development, and has extensively studied different approaches for P recovery and reuse from waste water and agricultural runoffs. Aravindan Rajendran, a post doc researcher, specialized in bioprocessing technologies and bioprocess modeling, will execute the research activities and provide technical expertise in the reactor studies and the onsite demonstration of the process.

B. Project Impact and Long-Term Strategy

The approach proposed in this proposal is unique and highly innovative. Current solutions for phosphate removal are limited and methods to remove traces of P are lacking. Outcomes of the project will provide a sustainable solution to capture P from runoffs and thereby protects rivers, lakes, vital landscape, terrestrial and aquatic life, as well as securing water supply for residents in Minnesota and in the Great Lake area. The interest for phosphate removal goes well beyond Minnesota, as 78% of coasts and estuaries in continental United States shows symptoms of eutrophication, with an estimated cost of $4.3 billion annually in 2009.

C. Timeline Requirements

The project will be completed in 3 years, with two years for lab-scale study and the remaining one year for on-site implementation and evaluation as well as cost estimation.

2016 Detailed Project Budget

Project Title: *Removal of Trace Amount of Phosphate by High Throughput Biological Phosphorus Scavenger*

INSTRUCTIONS AND TEMPLATE (1 PAGE LIMIT)

Attach budget, in MS-EXCEL format, to your "2016 LCCMR Proposal Submission Form".

(1-page limit, single-sided, 10 pt. font minimum. Retain bold text and DELETE all instructions typed in italics. ADD OR DELETE ROWS AS NECESSARY. If budget item row is not applicable put "N/A" or delete it. All of "Other Funds" section must be filled out.)

IV. TOTAL ENRTF REQUEST BUDGET *[Insert # of years for project]* **years**

BUDGET ITEM *(See "Guidance on Allowable Expenses", p. 13)*	**AMOUNT**
Personnel: Mikael Elias, assistant professor, PI: 8% time; 66.3% salary; 33.7% benefits, 1 month/year . Prof. Elias is a new assistant professor (started 9/14) at the University of Minnesota. His one month salary is $8,888 in the first project year and FTE with 3% salary increase for the following project years.	$ 37,096
Bo Hu, assistant professor, co-PI, 4% time; 66.3% salary; 33.7% benefits, 2 weeks/year. Two weeks of salary will be charged to the project for Dr. Bo Hu's summer time on managing the grant. His one month salary is $4.252 in the first project year and FTE with 3% salary increase for the following project years.	$ 17,746
TBN, postdoc, 67% time, 77.6% salary; 22.4% benefits; 8 months/year. The postdoc researcher will work with Dr. Elias to design and engineer the Pst system, as well as assessing the ability of the engineered transporter to acquire phosphate.	$ 114,625
Aravindan Rajendran, postdoc, 33% time; 66.3% salary; 33.7% benefits, 4 months/year . The postdoc researcher will work with Dr. Hu to design experiments and collect the research data in the lab.	$ 52,587
TBN, graduate student, 25% time; 82.4% salary; 17.6% benefits, 3 months/year. This graduate student will be working on cell cultures with Dr. Hu.	$ 67,162
Professional/Technical/Service Contracts: *sequencing and gene synthesis*	$ 15,608
Equipment/Tools/Supplies: *Chemicals and supplies are requested to perform the molecular evolution of the P transprter and the development of the biological scavenger.*	$ 40,581
Acquisition (Fee Title or Permanent Easements): *In this column, indicate proposed number of acres and and name of organization or entity who will hold title.*	N/A
Travel: *Be specific. Generally, only in-state travel essential to completing project activities can be included.*	N/A
Additional Budget Items: *In this column, list any additional budget items that do not fit above categories. List by item(s) or item type(s) and explain how number was determined One row per type/category.*	N/A
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 345,405

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	*N/A*
Other State $ To Be Applied To Project During Project Period: *N/A*	N/A	*N/A*
In-kind Services To Be Applied To Project During Project Period: university indirect cost matching at 52% mtdc	$ 165,730	*secured*
Funding History: N/A	N/A	N/A
Remaining $ From Current ENRTF Appropriation: *N/A*	N/A	*N/A*

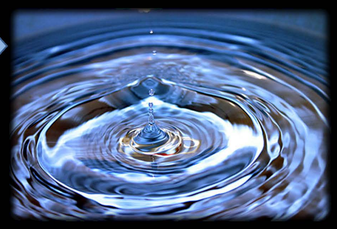
Activity 1: **Molecular engineering of the bacterial phosphate scavenging system**

Activity 2: **Lab scale modular Reactor Evaluation of the developed technology with cyanobacteria**

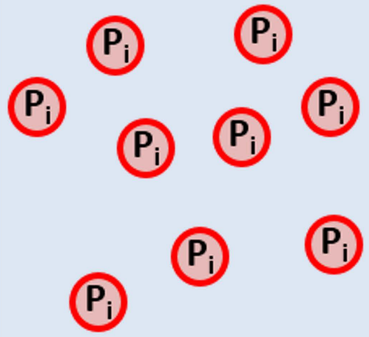
Activity 3: E**valuation for P contained water and cost estimation**

Reactor for the high-affinity bio-scavenger

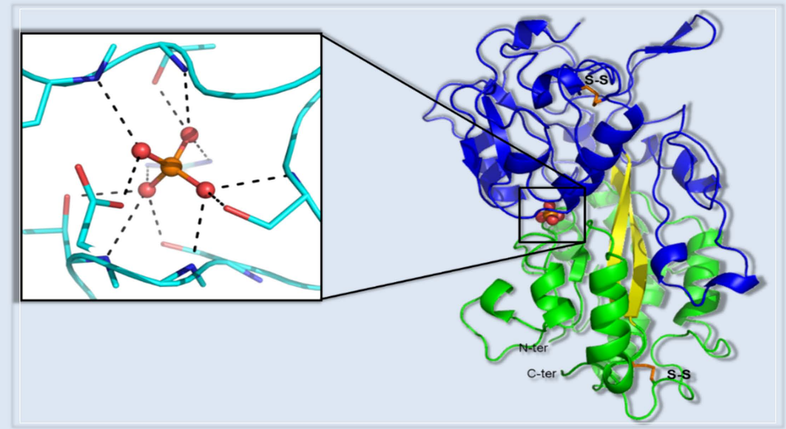
Clean water



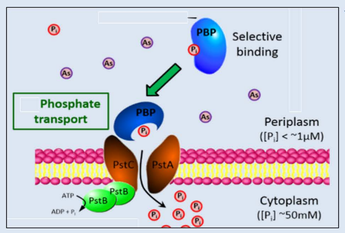
Biomass (P fertilizer)



High-affinity, High selectivity Phosphate-binding protein from bacteria. Crystal structure of the PBP from *P. fluorescens*. Phosphate anion is shown as red and orange balls and sticks.



ABC-type transport system that mediates phosphate uptake into bacterial cells



Polyphosphate accumulation

Bio-scavenging cyanobacteria

Management: The research team will include Dr. Bo Hu, and Dr. Mikael Elias. They will be jointly responsible for the completion of all of the research tasks.

Dr. Mikael Elias, PI, is a junior Assistant Professor at Department of Biochemistry, molecular biology and Biophysics. Elias has over 6 years of research experience on the bacterial phosphate uptake, producing 4 patents and >10 articles on this topic alone, including in prestigious journals (*JACS*, *Nature*) and extensive know-how in protein engineering where he pioneered methods, such as the use of ancestral mutation. He will invest most of his time on the project, and perform experiments and data analysis. Additionally, he reviews data and meet with the laboratory personnel on a daily basis to promote the projects. He also prepares the dissemination of results, such as publications. Mikael Elias has extensive experience with protein engineering methods, and X-ray crystallography. As the PI of the project, Dr. Elias will oversee the entire project, especially working on the genetic engineering of bacteria strains to enable phosphorus uptake. The postdoctoral researcher will conduct experiments, collect the research data, and help Dr. Elias to design the experiment plan, and draft the project reports.

Dr. Bo Hu is a junior Assistant Professor at Department of Bioproducts and Biosystems Engineering, University of Minnesota. With more than 10 years of active research experience specifically in biomass utilization, fermentative conversion, and waste management, he is leading projects to remove phosphorus from manure and from wastewater in the septic tank systems, projects to reveal the myth of recent swine manure foaming in Midwestern states, projects on synthetic ecology in lichen biofilm formation by co-culturing mixotrophic microalgae and filamentous fungi. His team is also developing several conversion platforms, such as lichen biofilm co-cultivation of fungi and microalgae, pelletized fungal fermentation, and solid and hemi-SolidSF of filamentous fungi, to produce bioproducts and biofuel from agricultural waste and residue, and to remove nutrients and pollutant from contaminated water. As the Co-PD of the project, Dr. Hu will work on the optimization of strain cultivation conditions, and on the reactor set-up. He will have one postdoc researcher and one graduate student to conduct experiments, and collect the research data.

Organization: the University of Minnesota has several missions: improve lives through research, education and outreach. The University possess extensive facilities that ensure high research performances. In particular, for this proposal:

X-ray crystallography facility: houses four complete macromolecular X-ray data collection beam lines with three RigakuMSC Micromax 007 X-ray generators, three R-axis IV++ image plate detectors, and a Saturn 944+ CCD Camera. Crystallization robots and crystal growth monitoring systems are also available.

Minnesota Supercomputing Institute (https://www.msi.umn.edu/services/informatics)

Biotechnology Resource Center: (http://www.bti.umn.edu/brc/index.html) A wide variety of bench-scale to pilot scale fermenters is available, up to 500L.

Elias Lab: 1,800 sq. ft. of renovated research space is dedicated to Dr. Elias. This space is located on the 1st floor of the GortnerLab Building, on the St Paul campus. Elias's office space is adjacent to the laboratory. The lab contains all of the necessary equipment for molecular biology, biochemistry, protein production and purification, enzyme kinetics, and crystallography. Numerous facilities are available, such as microplate readers, spectrophotometers, scintillation counters, fplc, liquid nitrogen storage, -80 freezers, incubators/shakers, autoclave, as well as 4 and -20 rooms.

Hu Lab: Dr. Hu's laboratory is located at BAE 320B, adjacent to Dr. Hu's office. The laboratory space is ~1400 square feet and is equipped with the two laminar flow hoods and one clean bench. Department of Bioproducts and Biosystems Engineering at the UMN houses the offices for the participating faculty and researchers and several laboratories suitable for bench scale algae/fungal screening, culture of various organisms in a wide range of formats, routine chemical and biological analysis, and pilot cultivation facility. Several laboratories are equipped with state of the arts instruments for chemical and physical analysis, laminar flow hoods, and biosafety cabinets. Dr. Bo Hu's lab has two walk-in cultural rooms with full range of temperature control, one walk-in refrigerator and one walk-in freezer.