Project Title:

ENRTF ID: 062-B

Modular Biological Phosphorus Recapture for Field Application

Category: B. Water Resources

Total Project Budget: $ 618,565

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

Summary:

This project aims to remove excess phosphorus from impaired waters through the use of an algal biofilm bioreactor, and to utilize the resulting algal cells as a suitable compost.

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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:

This visual shows conceptual drawings of the current prototype inverted drum bioreactor system for phosphate removal from waterways.

_____ Funding Priorities _____ Multiple Benefits _____ Outcomes _____ Knowledge Base

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

_____ Capacity Readiness _____ Leverage _______ TOTAL ______%

PROJECT TITLE: Modular Biological Phosphorus Recapture for Field Application

I. PROJECT STATEMENT

WHY – Excessive application of phosphorus and nitrogen from commercial fertilizers or other industrial sources results in an imbalance in natural ecosystems, which can lead to eutrophication of our lakes and waterways. Phosphorus is an especially difficult contaminant, in part because certain cyanobacteria – a type of blue-green algae – require predominantly only this nutrient and sunlight to grow. These cyanobacteria can fix both carbon and nitrogen from the air. Additionally, certain strains of cyanobacteria produce toxins or can provide nutrients to other problematic species. In this manner, excessive amounts of phosphorus lead to impaired water quality, unsightly lakes and streams, odors and other public and ecological health problems.

GOALS – The goal of this project is to recapture phosphorus from impaired waters. In contrast to certain strains of cyanobacteria that are detrimental to the health of aquatic ecosystems, there are also naturally occurring strains that are not as problematic. These alternative strains include the model cyanobacterium *Anabaena variabilis*, which does not produce toxins. The only macronutrient required for growth is phosphate, which it rapidly accumulates from its surroundings. If the strain were grown in a manner allowing it to be easily separated from treated water, then it will act like a phosphorus sponge, lowering the levels of phosphorus present in any body of water. The separated cells may then be applied as a suitable soil supplement, where it will act as a biological fertilizer, similar to compost. This goal will be achieved by growing the target cyanobacterium in a specific reactor as a biofilm. Excess cell mass will be removed by continually harvesting a portion of the biofilm using established designs commonly utilized in other industries. The only alteration is that the design must include features that provide light to the biofilm.

OUTCOMES – The final outcome will be a natural, sustainable and autonomous modular system that can demonstrate successful operation to lower phosphorus levels in targeted water systems. This would include experiments designed to mimic standing water such as is found in lakes or continuous flows system similar to agricultural runoff zones, once initial designs have been proven effective in a controlled greenhouse space.

HOW – Our laboratory has worked with various algal growth systems for the past 10 years on projects that include bioreactor design and automation at both laboratory and commercial scale. A key facet of our work includes outdoor testing facilities and greenhouse systems where large-scale cultures can be managed for extended periods of time. To achieve the goals of this project, we will construct a prototype bioreactor with a simple design that is ideal for growing biofilms of *Anabaena variabilis*. A primary feature of the design is inclusion of all necessary components to operate the system autonomously in various environments. The system will include a small solar panel system to provide the energy needed to rotate the drum reactor system used to grow the biofilm, and will be constructed in a manner that is easily adapted to operation within a standing water body or by diverting the flow of a small stream or runoff ditch through the system.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Construction of Medium-Scale Prototype Bioreactor **Budget: $343,565**

The aim of this first activity is to construct a working prototype inverted-drum-biofilm reactor (see graphical image for a simple schematic and description). The reactor will be constructed in an iterative fashion focusing first on ideal growth of the phosphate accumulating cyanobacterium, and later adding modules to improve automation and allow it to function autonomously based on system performance. Operation in greenhouse facilities on the St. Paul campus at the University of Minnesota will allow us to optimize the system in a controlled environment while monitoring and adding additional modifications. This is important to the eventual goal of operating the complete system outdoors in impaired waters.

Outcome	Completion Date
1. Complete Initial Prototype Construction for Growth Testing in a Greenhouse Space	*Dec 1st, 2016*

2. Incorporate Enhancements and Modifications to Complete Modular Autonomous Prototype for Environmental Testing	*June 1st, 2018*
3. Test the Potential of Anabaena variabilis Biofilms Obtained from Greenhouse Studies as a Suitable Soil Amendment and Fertilizer Alternative	*Aug 31st, 2018*

Activity 2: Laboratory and Field Studies of Bioreactor Performance **Budget: $275,000**

The aim of the second activity will be to determine potential parameters that should be modified to achieve the ultimate goal of the system, which is *rapid phosphate uptake and accumulation into biomass that can be easily collected for field application as a soil amendment*. An initial prototype (described in Activity 1) will be operated in a greenhouse setting at the University of Minnesota. Continuous operation will allow testing of both the engineering parameters and bioreactor robustness as well as performance criteria, including the ability of the reactor to rapidly clear phosphate from solution. Additionally, the collection of data will allow us to project the potential of individual systems which could then be either scaled up or replicated to meet the requirements for a specific application. The second component of this testing will be the outdoor performance criteria. Since this testing will be limited to certain times of the year, it will be done following the initial assessment in the greenhouse, which will allow us to establish benchmark parameters in a controlled environment.

Outcome	Completion Date
1. Complete Initial Laboratory (Greenhouse) Studies of Prototype 1 Performance	*Jan 31st, 2018*
2. Complete Outdoor Environmental Testing of Prototype 2 Performance	*May 31st, 2019*
3. Test the Potential of Field Study Grown Cyanobacterial Strains as a Suitable Soil Amendment and Fertilizer Alternative	*Jan 1st, 2019*

III. PROJECT STRATEGY

A. Project Team/Partners

The project will be overseen by Professor Brett Barney in the Department of Bioproducts and Biosystems Engineering at the University of Minnesota. Additional participants include several junior scientists, graduate students and postdocs who will perform the work and design of the systems.

B. Project Impact and Long-Term Strategy

Algae are often touted as a potential next-generation energy crop based on the potential to produce oils which can be converted to a product similar to what is obtained from soybean oil to make biodiesel. Algae have been used for many decades in a range of wastewater treatment systems, but have suffered in more recent years as it has been realized that unless these algae are harvested, their release to the environment will result in the eventual loss of phosphorus back to the ecosystem. The goal of this project is not to produce biodiesel. Our efforts are aimed at removing phosphorus in a manner that allows this valuable nutrient to be recaptured and applied as a soil implement (as current stockpiles of phosphorus are being increasingly viewed as finite). The goals and requested funding would cover the expenses of prototype design and testing. Success would then require additional phases of the project to scale up and make the system broadly available. We envision an eventual design that would utilize solar power to maneuver autonomous floating reactors across targeted lakes, and then would recall the reactors once biomass is maximized. The autonomous design would allow these to be operated with little effort or required energy inputs, and having a minimal impact on the remaining ecosystem. Additional designs could operate with small streams in the field by diverting water to a series of reactors.

C. Timeline Requirements

This project has a proposed timeline of three years. The duration of the project has been carefully considered based on past experience developing and then testing various bioreactor prototypes and performing full characterizations of the performance of these systems.

2016 Detailed Project Budget

Project Title: Modular Biological Phosphorus Recapture for Field Application

IV. TOTAL ENRTF REQUEST BUDGET *3* years

BUDGET ITEM	AMOUNT
Personnel:	
Brett Barney, Project Manager (75% salary, 25% benefits), Assistant Professor, 9 Month Appointment, Summer Salary; 10% FTE for 3 years	$ 49,361
Postdoctoral Microbiologist or Biological Engineer, Project Management (82% salary, 18% benefits); 75% FTE for 3 years	$ 115,515
Hanna Hondzo, Junior Scientist, Prototype Testing and Laboratory Experiment Data Analysis (78% Salary, 22% Benefits); 100% FTE for 3 years	130,367
Carolann Knutson, Graduate Research Assistant, Prototype Design and Testing (58% salary, 42% benefits); 50% FTE for 2 years each	$ 134,322
3 Undergraduate Technicians, Laboratory and Field Data Collection (93% salary, 7% benefits); 10% FTE for 3 years (generally rotating 1 year appointments)	$ 50,000
Contracts:	
Sequencing Analysis, Sequencing of Biofilms for Optimization and Characterization, Locally sourced through either the University of Minnesota Sequencing Center or Local Companies	$ 10,000
Equipment/Tools/Supplies:	
Laboratory Supplies: General Laboratory Chemicals, Media, Reagents and Kits for Performing Routine Molecular Biology, Analytical Reagents, DNA Synthesis of Primers, Liquid Nitrogen for Strain Storage (Based on historical costs of approximately $1250 a month)	$ 45,000
Ion Chromatography System: This piece of equipment is essential to routine monitoring of phosphorus and nitrogen compounds that accumulate in water. A similar system was utilized for many years at a previous institution, and would be instrumental to the success of this project. This piece of equipment will continue to be used through the duration of the project and through its useful life.	$ 25,000
Prototype Materials and Supplies: Various components to construct Medium-Scale Prototype Bioreactor system, including various control elements, solar panels and battery storage and communications equipment. Based on previous project costs and proposed costs from initial design.	$ 50,000
Publication Charges: Costs associated with the broad dissemination of research findings in journals that are largely accessible to the broader public.	$ 3,000
Travel:	
Travel to study sites within Minnesota for sample collection or to test prototype in various field settings.	$ 6,000
Additional Budget Items:	$ -
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 618,565

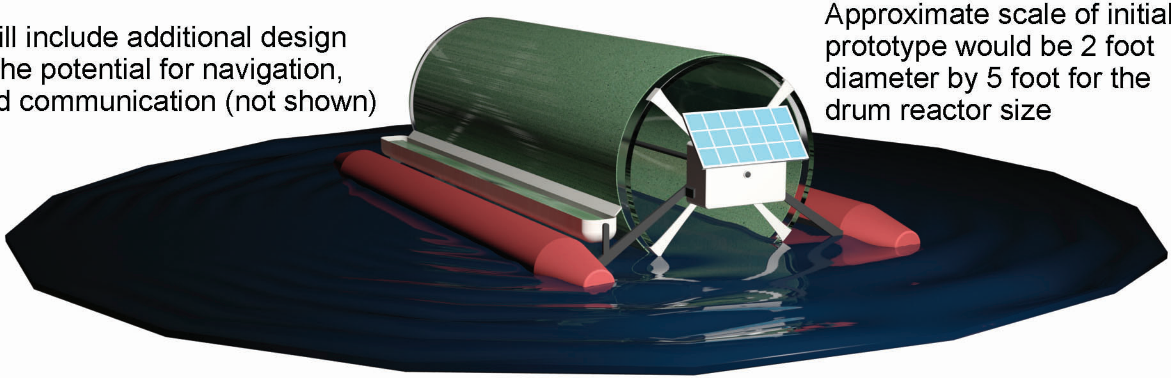
V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ To Be Applied To Project During Project Period:	N/A	
Other State $ To Be Applied To Project During Project Period:	N/A	
In-kind Services To Be Applied To Project During Project Period:	$ 280,891	
Funding History:	$ 650,000	
~$500,000 - Defense Advanced Research Projects Agency (DARPA) as part of a subcontract through General Atomics. This project was aimed at large scale production of algae for biodiesel production and involved the construction and operation of various bioreactors and open raceway ponds. The PI was a co-PI on this project while at Utah State University.	$ 500,000	
$150,000 - IREE Career Award: Microbial Communities for Enhanced Biofuel Feedstock Production; This proposal funded initial studies into beneficial nitrogen-fixing bacteria in combination with certain algae species.	$ 150,000	
Remaining $ From Current ENRTF Appropriation:	N/A	

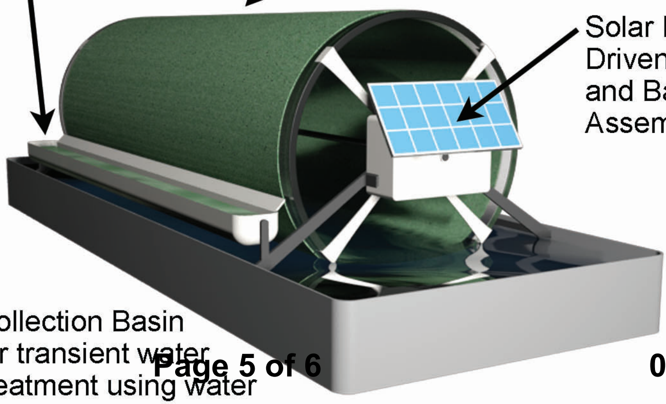
Modular Biological Phosphorus Recapture for Field Application

Conceptual Drawings showing the "Inverted Drum" bioreactor system that will grow a biofilm of the nitrogen-fixing and carbon-fixing cyanobacterium *Anabaena variabilis* to remove available phosphorus from impaired water systems. This cyanobacterium is indigenous to Northern waters and cosmopolitan in nature. It is nontoxic to the environment, requiring only simple salts (present in freshwater) and phosphate (which will be accumulated) to grow. The collected biomass is suitable for use as a fertilizer, similar to traditional compost, and is currently used in organic farming operations. The project will utilize native strains without any modification.

Floating Reactor will include additional design features including the potential for navigation, remote sensing and communication (not shown)



Approximate scale of initial prototype would be 2 foot diameter by 5 foot for the drum reactor size



Modular Design will allow the reactor to be applied to various environments by making minor modifications. Shown above is a floating reactor scheme. Combined with various sensors and "smart" technologies, this reactor could be directed to regions high in phosphorus, and would navigate back to a dock to empty excess biomass when the system reaches capacity. In place reactors (left) can be used to cycle water from small ponds or streams for temporary treatment, and could be set up in series.

The drawings shown are conceptual, and do not contain sufficient details to describe all of the key components and operational parameters. Similar reactor designs are found in wastewater treatment applications, and have been used successfully in the past.

Project Manager Qualifications

This research team is headed by Professor Brett Barney. Additional participants include a postdoctoral fellow, and a junior scientist who assists with laboratory and greenhouse studies. Additional graduate students and undergraduate students will be hired as part of this project and jointly advised. Dr. Barney is currently an Associate Professor in the Department of Bioproducts and Biosystems Engineering at the University of Minnesota. He is also a faculty member in the Biotechnology Institute at the University of Minnesota, and a member of the Microbial and Plant Genomics Institute at the University of Minnesota. He has served as a Laboratory Manager in multiple appointments, including both academic and industry settings for over 25 years. His extensive research experience includes enzymology, biological nitrogen fixation, hazardous waste site treatment methodologies, algal lipid production, genetic engineering and analytical approaches for the quantification of a range of chemicals.

Dr. Barney's laboratory has had an active algae research component for the past 10 years, working in both biofuels and nutrient requirements. Previous research funding has come from sources such as the National Science Foundation (NSF), the United States Department of Agriculture (USDA), the United States Department of Energy (DOE), the Defense Advanced Research Projects Agency (DARPA), Minnesota's Discover, Research and InnoVation Economy (MnDRIVE) and the Initiative for Renewable Energy and the Environment (IREE).

The Barney laboratory is housed on the second floor of the Cargill building for Microbial and Plant Genomics at the University of Minnesota. The Cargill building was designed with the intention to promote interdisciplinary collaborations and provide a shared lab space for each floor, which facilitates flexible group sizes. This large laboratory space is designed around a shared communal format, with various rooms available for utilization for specific experiments. The laboratory contains the primary equipment to perform this research project, including facilities to cultivate various bacteria, autoclaves, analytical instrumentation for analysis (gas chromatography, spectrophotometers, and balances), thermocyclers for PCR reactions, centrifuges, electrophoresis equipment and various incubators. Additional facilities include the Biotechnology Resource Center, the Genomic Sequencing Center and a broad range of additional analytical laboratories which are available as pay services.

Organization Description

Dr. Brett Barney has been a professor with the Department of Bioproducts and Biosystems Engineering at the University of Minnesota since 2009. The Bioproducts and Biosystems Engineering Department serves as a core department combining Agricultural Engineering, Biological Engineering and Environmental and Ecological Engineering. Numerous faculty members from the department have received support from the LCCMR program in the past. Our collaboration with Craig Sheaffer from the Department of Agronomy and Plant Genetics at the University of Minnesota brings expertise in plant systems that are important components of agriculture in Minnesota. The University of Minnesota provides a range of facilities and sufficient laboratory space to perform each of the activities described in this proposal. Additionally, controlled environments including greenhouse space sufficient for this work is conveniently located next door to Dr. Barney's laboratory space. UMN Sponsored Projects Administration (SPA) is the entity authorized by the Board of Regents to manage project agreements with LCCMR program.