

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

Project Title:

ENRTF ID: 055-B

Novel Algae Bioreactors for Nitrogen and Phosphorus Removal

Category: B. Water Resources

Total Project Budget: \$ 350,000

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

Summary:

Novel algae bioreactors will be developed to reduce nitrogen and phosphorus concentrations in agricultural runoff water. The reactors will be installed and operated in the fields to improve water quality.

Name: Satoshi Ishii

Sponsoring Organization: U of MN

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Web Address _____

Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

Novel algal bioreactors can improve water quality by reducing N and P concentrations

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



PROJECT TITLE: Novel Algae Bioreactors for Nitrogen and Phosphorus Removal

I. PROJECT STATEMENT

Nitrogen (N) and phosphorus (P) leaching from agricultural fields can cause eutrophication in rivers and lakes. In addition, agricultural runoff water (e.g., water through tile drainage) from the upper Midwest States, including Minnesota, is considered a major cause of the dead zone in the Gulf of Mexico. Efforts have been made to reduce N and P leaching from agricultural fields, but it is still difficult to control N and P leaching. In this project, we propose to develop novel bioreactors to simultaneously reduce N and P that are leached from agricultural fields, by using algae.

Algae grow fast in response to N and P in water, and accumulate these nutrients in their biomass. Therefore, we can reduce N and P concentrations in agricultural runoff water by collecting and removing algal biomass from the water. In this project, we will develop novel algae bioreactors based on the microbial granular sludge technology to separate algae from water. Microbial granules are densely-packed, self-organized microbial structures, which have been used for wastewater treatment. These granules sink when they become large enough, leaving clean water in the upper part of the reactor. In general, granular-sludge based bioreactors have high nutrient removal efficiencies, small footprint, and small energy input compared with other N- and P-removal systems. We previously developed a granule bioreactor to treat N from wastewater (Song, Ishii, et al., 2013). We also observed the growth of algae on the surface of the granules when we operated this same reactor under light conditions. In addition, we previously identified the occurrence of nitrification and denitrification (i.e., microbial N removal processes) in naturally-occurring algal granules on a glacier (Segawa, Ishii, et al., 2014). These results suggest that it is possible to develop algal granules in reactor conditions to remove N and P from water; however, nobody has developed such an algal granule bioreactor so far.

The overall goal of this research is to improve water quality by reducing N and P concentrations in agricultural runoff water. To achieve this goal, we will first develop laboratory-scale algal bioreactors (activity 1). We will optimize the reactor operation conditions to enhance granular formation and N and P removal. Once we develop algal granule bioreactors, we will apply these reactors to treat actual agricultural runoff water to examine the feasibility of this approach (activity 2 and 3).

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Develop laboratory-scale N- and P-removing algal granule bioreactors **Budget: \$130,000**

Three laboratory-scale bioreactors will be designed and operated to develop the most efficient N- and P-removing algal granules. Lake sediment will be used as a source of algae and microbes. Synthetic agricultural runoff water with known amounts of N and P will be used in Activity 1 for the stable operation of the reactors.

Size and density of the granules, as well as N and P removal rates will be measured to evaluate the overall efficiency of the reactors. The reactor operation conditions will be optimized to maximize the granule size and N and P removal efficiencies. Our target N removal efficiency is >90%. The microbial community in the granules will be identified by using next generation sequencing technology.

Outcome	Completion Date
1. Development of N- and P-removing algal granules	June 30, 2017
2. Identification of the algal and microbial community structures in the algal granules	Dec. 31, 2017
3. N and P removal efficiency of >90% in the algal granule bioreactor fed with synthetic agricultural runoff water	Dec. 31, 2017



Environment and Natural Resources Trust Fund (ENRTF)

2016 Main Proposal

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Activity 2: Apply algal granule bioreactors to clean agricultural runoff water

Budget: \$110,000

Once we develop algal granule bioreactors for N and P removal, we will feed actual agricultural runoff water to the reactors. We will collect several agricultural runoff water samples with various N and P concentrations from different locations across the state. Stable operation of the reactors will be evaluated by the granule size and the N and P removal efficiency. If necessary, reactor operation conditions will be optimized.

Outcome	Completion Date
<i>1. Stable operation of algal granule bioreactors with agricultural runoff water</i>	June 30, 2018
<i>2. N and P removal efficiency of >90% in the algal granule bioreactors fed with actual agricultural runoff water</i>	Dec. 31, 2018

Activity 3: Install and operate algal granule bioreactors in the field

Budget: \$110,000

We will select a field site where high N and P leaching is observed, and install and operate algal granule bioreactors in the field. Stable operation of the reactors will be evaluated by the granule size and the N and P removal efficiencies. We will optimize reactor operation conditions based on the climate and hydrological conditions in the fields.

Outcome	Completion Date
<i>1. Installation and operation of algal granule bioreactors in the field</i>	June 30, 2019
<i>2. N and P removal efficiency of >90% in the algal granule bioreactors in the field conditions</i>	June 30, 2019

III. PROJECT STRATEGY

A. Project Team/Partners

Dr. Satoshi Ishii (Assistant Professor, BioTechnology Institute and Department of Soil, Water, and Climate, the University of Minnesota) will lead and manage the project. One post doctoral research associate and one graduate student will design and operate the reactors, measure N and P removal efficiencies, and perform microbiological analysis. Dr. Gary Feyereisen (Research Agricultural Engineer, USDA Agricultural Research Service, Soil & Water Management Research Unit) will assist with the field deployment, without funding support from ENRTF. Dr. Ishii will be responsible for the financial and all other aspects of this project.

B. Project Impact and Long-Term Strategy

The long-term goal of this research is to improve water quality by reducing N and P concentrations in agricultural runoff water in upper Midwest states. The proposed project is the initial step (phase 1) of a three-phase project to achieve this goal. In the phase 2 project, multiple algal granule bioreactors will be installed and operated in the fields in the upper Midwest states, and their N and P removal efficiencies will be evaluated. Ecological and economic impacts of the installation of algal granule bioreactors on the water quality and sustainable agriculture will be calculated and evaluated. In this phase, we expect to have more people (soil scientists, climatologists, agricultural engineers, farmers, ecologists, economists, etc.) in the project and the support from federal agencies such as the US Department of Agriculture and the National Science Foundation (NSF). In the third phase, we will develop low-cost bioreactors (<\$1,000) to promote the installation of the algae bioreactors in the fields.

C. Timeline Requirements

We expect to complete the proposed project in 36 months. In the first year, we will develop algal granules with synthetic agricultural runoff water, and evaluate the reactor performance (Activity 1). In the second year, we will start collecting agricultural runoff water to feed to the bioreactors (Activity 2). In the third year, we will install and operate reactors in the field (Activity 3).

2016 Detailed Project Budget

Project Title: Novel Algae Bioreactors for Nitrogen and Phosphorus Removal

IV. TOTAL ENRTF REQUEST BUDGET 3 years

<u>BUDGET ITEM</u>	<u>AMOUNT</u>
Personnel:	
Assistant Professor, Satoshi Ishii, project management (75% salary, 25% benefits); 5.56%FTE (one month summer salary) each of 3 years.	\$ 37,910
1 Post Doctoral Research Associate, reactor design and operation, data collection and analysis (82% salary, 18% benefits); 100% FTE for each of 3 years	\$ 145,680
1 Graduate Research Assistant, reactor operation, data collection and analysis (54% salary, 46% benefits); 50% FTE for each of 3 years	\$ 111,739
Professional/Technical/Service Contracts:	
University of Minnesota Genomics Center: Next generation sequencing (\$2900 per run x 2 runs)	\$ 5,800
University of Minnesota Soil and Water Testing Lab: (\$20 per sample x 45 samples per year x 3 year)	\$ 2,700
Equipment/Tools/Supplies:	
3 lab-scale bioreactors (\$4,000 each) and 3 on-site bioreactors including pumps and solar panels (\$7,000 each)	\$ 33,000
Lab and field supplies: chemicals, tools, bottles, gloves, reagents (\$3,857 per year x 3 years)	\$ 11,571
Travel:	
Car rental for field trips (\$200 per trip x 8 trips)	\$ 1,600
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND \$ REQUEST =	\$ 350,000

V. OTHER FUNDS

<u>SOURCE OF FUNDS</u>	<u>AMOUNT</u>	<u>Status</u>
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	\$ -	N/A
Funding History: N/A	\$ -	N/A
Remaining \$ From Current ENRTF Appropriation: N/A	\$ -	N/A

Our overall goal

Current situation

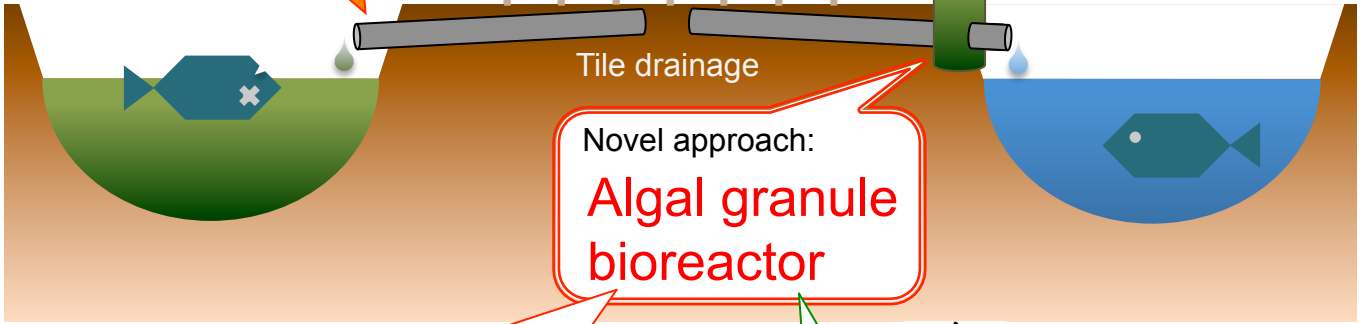
Eutrophication

N & P leaching

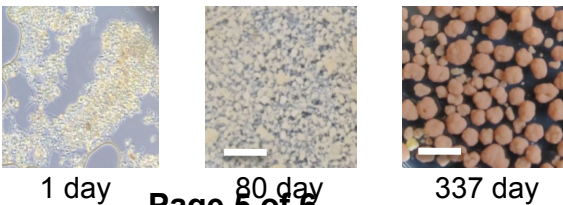
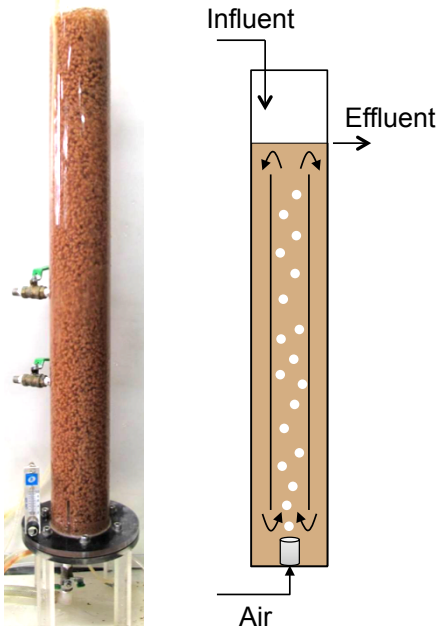
In the future

Clean water

N & P removal



Aerobic, nitrification-denitrification granule reactor for the N removal from wastewater (Song, Ishii, et al., 2013)

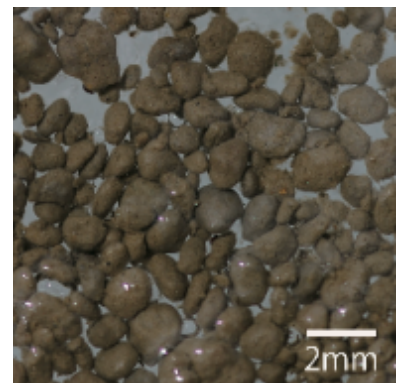


Development of the granular structures



We can develop N&P-removing algal granules in reactor conditions!

Naturally-occurring, nitrification-denitrification algal granules on a glacier (Segawa, Ishii, et al., 2014)



Previous achievements:

Segawa S, Ishii S, et al. 2014. The nitrogen cycle in cryoconites: naturally occurring nitrification-denitrification granules on a glacier. *Environ. Microbiol.* 16: 3250-3262.

Song, Y., S. Ishii, et al. 2013. Development and characterization of the partial nitrification aerobic granules in a sequencing batch airlift reactor. *Bioresour. Technol.* 139:285-291.

Project Manager Qualifications

Dr. Satoshi Ishii is Assistant Professor in the BioTechnology Institute (BTI) and the Department of Soil, Water, and Climate (SWC) at the University of Minnesota. He joined the BTI and SWC in April, 2015. He was hired as a part of the MnDRIVE (Minnesota's Discovery, Research and Innovation Economy) initiative, to advance industry and conserve our environment. Prior to this position, he was Assistant Professor in the Division of Environmental Engineering at Hokkaido University, Japan, for 4 years, and Research Assistant Professor at the Department of Applied Biological Chemistry at the University of Tokyo, Japan, for 3.5 years.

Dr. Ishii has over 10 years of research experiences on water quality and bioremediation of inorganic nutrients such as nitrogen (N) and phosphorus (P). He has used biotechnology, microbiology, molecular biology, analytical chemistry, and engineering approaches to solve environmental problems. In relation to the proposed project, he has developed aerobic nitrification-denitrification granules in a sequence batch airlift reactor to treat N in wastewater (Song, Ishii *et al.*, 2013; Ishii *et al.*, 2014). He also identified the occurrence of N cycle in naturally-occurring algal granules on a glacier (Segawa, Ishii *et al.*, 2014). These experiences will help develop algal granule bioreactors in this project.

Dr. Ishii's lab is located on 322 Snyder Hall (750 sq. ft). The Ishii's Lab is equipped with all the necessary items for the proposed research. In addition, his group has access to the growth chambers, which is necessary to cultivate algae. Next generation sequencing and analysis will be done in the University of Minnesota Genomics Center.

Selected publications related to the proposed research:

- Segawa, S., S. Ishii, N. Ohte, A. Akiyoshi, A. Yamada, F. Maruyama, Z. Li, Y. Hongoh, and N. Takeuchi. 2014. The nitrogen cycle in cryoconites: naturally occurring nitrification-denitrification granules on a glacier. *Environ. Microbiol.* 16:3250–3262.
- Ishii, S., Y. Song, L. Rathnayake, H. Satoh, A. Tumendelger, S. Toyoda, N. Yoshida, and S. Okabe. 2014. Identification of key N₂O production pathways in aerobic partial nitrifying granules. *Environ. Microbiol.* 16:3168–3180.
- Song, Y., S. Ishii, L. Rathnayake, T. Itoh, H. Satoh, and S. Okabe. 2013. Development and characterization of the partial nitrification aerobic granules in a sequencing batch airlift reactor. *Bioresour. Technol.* 139:285–291.

Organization Description

The University of Minnesota is the main research and graduate teaching institution in the state of Minnesota. The BioTechnology Institute provides advanced research, training, and university-industry interaction in biological process technology. In the Department of Soil, Water, and Climate, we seek to improve and protect the quality of soil, air, and water resources in natural and managed ecosystems, through research, reaching, and extension.