



Environment and Natural Resources Trust Fund (ENRTF) M.L. 2015 Work Plan

Date of Report: October 15, 2014

Date of Next Status Update Report: January 1, 2016

Date of Work Plan Approval:

Project Completion Date: June 30, 2018

Does this submission include an amendment request? ___

PROJECT TITLE: A novel biofilm technology for water nutrient removal

Project Manager: Bo Hu

Organization: University of Minnesota

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Location: The experiment will be primarily done at Biological Agricultural Engineering Building (BAE) 320, 1390 Eckles Ave, St Paul, MN, 55108. The impact of the project will be statewide

Total ENRTF Project Budget:

ENRTF Appropriation: \$ 281,000

Amount Spent: \$0

Balance: \$ 281,000

Legal Citation: M.L. 2015, Chp. xx, Sec. xx, Subd. xx

Appropriation Language:

I. PROJECT TITLE: A novel biofilm technology for water nutrient removal

II. PROJECT STATEMENT:

Non-point source pollution is the leading cause of water quality impairment in Minnesota. Excessive loading of nutrient from stormwater runoff at urban and agricultural landscapes results in eutrophication and encourages the growth of invasive species. We propose a novel biofilm technology to remove and possibly recover nutrients such as nitrogen and phosphorus from water and the process will also remove pollutants such as heavy metal ions (lead, zinc, copper, arsenic etc.) and pesticides. This new technology will be developed based on the concept of a “simulated lichen biofilm”, mimicking the natural symbiotic lichen ecosystem, for efficiently removing and recovering nutrients and pollutants, by introducing a supporting matrix, binding filamentous fungal strains and microalgae. The project will develop this technology through the lab experiments and evaluate its effectiveness on the Sarita wetland close to UMN St Paul campus.

Conventional practices use sedimentation methods to remove suspended pollutants; whereas dissolved nutrients (phosphorous and nitrogen), organics, pesticides and heavy metals (lead, zinc, copper, arsenic) require more complicated approaches to remove them from the runoff. Lichen, a natural ecosystem with phototrophic algae and heterotrophic fungi symbiotically growing on the solid surface of rock or roof, is not readily applied in engineering field due to their low growth rate. A concept of “simulated lichen system” is recently developed by our UMN research group that we can select different desired microalgae and fungal combinations that will be growing on the surface of some specific polymers to form the biofilm. We are proposing to study this concept and apply to remove and even possibly recover nutrients from water. Microalgae are naturally growing on the surface of the nutrient-rich water; however, biological treatment of polluted waters using microalgae is limited by problems associated with the settling and separation of algae downstream of the treatment site. The proposed methodology using bioaugment filamentous fungi in lichen biofilms overcomes this limitation, by efficiently retaining algae and recovering the nutrients and heavy metals with possible recycling of useful nutrients. The simulate lichen biofilm will also grow much faster than aquatic plants for removing nutrients.

Our preliminary research has shown that filamentous fungi and fresh water microalgae can naturally be grown attached on some specific bio-based polymers to form the “simulated lichen biofilm”. Both types of cells can accumulate phosphorous, nitrogen and toxic heavy metals, and pollutants will be removed by removing this biomass from water. The development of a stable lichen biofilm will have multiple benefits over the current available technologies. This composite will have the capacity to remove the pollutants in a wide concentration range, and to possibly recycle valuable and non-renewable nitrogen and phosphorous. The technology can be incorporated into current practices for protecting our water bodies. A successful on-site demonstration of the prototype would transform the storm water runoff treatment, in polluted lakes, pond, and lagoon.

The project will develop this technology through the lab experiments with the samples from urban runoff sites at Twin Cities Metro, the swine manure wastewater lagoon at Waseca and a heavily polluted lake at Albert Lee and evaluate its effectiveness on the Sarita wetland close to UMN St Paul campus. We hypothesis that the lichen biofilm technology will significant improve the nutrient removal and recuperation with improved bioremediation potential. The beneficial interaction between the algae and fungi in the system will possibly enhance productivity and efficiently utilize the resources. Also understanding of the fundamental aspects of algae-to-fungi interactions underlying the observed phenomena is critical to expand the process to industrial scale and for other algae-related processes.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of January 1, 2016:

Project Status as of July 1 2016:

Project Status as of January 1, 2017:

Project Status as of July 1, 2017:

Project Status as of January 1, 2018:

Overall Project Outcomes and Results:

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Develop the biofilm technology through lab experiments

Description: The purpose of this task is to develop a lichen type biofilm with the algae and fungal type strains, and the native species found in the local wetlands. Effect of culture conditions on biofilm formation, cell distribution and the nutrient recuperation and pollutant removal capacity of the developed technology will be studied.

Water samples will be collected from Sarita wetland which will be used to isolate the native microalgae / fungi for the biofilm formation. Three different combinations of algae-fungal strains will be tried for the lichen-type biofilm formation. Meanwhile, we will identify one to three microalgae/fungal strain combinations suitable for both biofilm formation and growth on the local water. The fungi and algae in different ratio of cells will be co-cultured in a minimal medium with different matrix materials for cell attachment. The effect of different matrix type (ecofriendly polymer-cotton composite matrix; recycled polymer materials; lignocellulose matrix), agitation intensity, temperature and nutrient concentration on the algae-fungi cell concentration in biofilm formation will be estimated. The attachment material will be evaluated based on the degree of cell attachment, material durability, and material cost and the recycling capacity.

The biomass distribution and the nutrient recovery efficiency of the biofilm with the medium containing the nutrient and pollutant concentration mimicking the various runoffs will be tested in shake flasks. The synthetic runoff will be designed based on the chemical and physicochemical characteristics of typical runoffs. 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 under study. Central composite design will be implemented to estimate a second-degree polynomial model, expressing the relationship between the test variables and the response variables (cell population distribution, removal efficiency) to predict the behavior of this synthetic ecosystem.

Summary Budget Information for Activity 1:

ENRTF Budget: \$106,822
Amount Spent: \$ 0
Balance: \$106,822

Outcome	Completion Date
1: Screening native microalgae and fungal species of the local wetlands to have better growth and nutrient removal performance in the regional climate.	Oct 2015
2: Identify algae/fungal combinations suitable for biofilm formation.	Jan 2016
3: Laboratory study of microalgae/fungal systems to remove nutrients.	Jun 2016

Activity Status as of January 1, 2016:

Activity Status as of July 1 2016:

Activity Status as of January 1, 2017:

Activity Status as of July 1, 2017:

Activity Status as of January 1, 2018:

Final Report Summary:

ACTIVITY 2: PILOT SCALE ACTIVITY – Prototype design and testing with the polluted water.

Description: The samples from two urban runoff sites at Twin Cities Metro, the swine manure wastewater lagoon at Waseca, a heavily polluted lake at Albert Lea and a possible site at Northern MN will be collected and the physicochemical characteristics of the samples will be quantified. The microbial composition analysis of the samples and the possible effect on the biofilm will also be evaluated.

Prototype development for this technology through the model wetland setup with the biofilm will be developed in our laboratory with three different configurations: (1) *Submerged paddle wheel design*: The paddle wheel will serve dual purpose, both for mixing and as a matrix for the attachment of fungal cultures for lichen biofilm formation that will be partially immersed inside the medium. The removable paddle wheel enables harvesting the biofilm in the attached fungal cultures. These reactors will be more suitable for cultivations if mixing plays an important role in the biofilm development. (2) *Floating cascade design*: Cascades of the attached biofilm will be used as a float on the polluted waters and the movement of the cascade of biofilm will be mechanized in the model wetland-reactor system. (3) *Flow through design*: is an enclosed system with biofilm where the water movement will be facilitated to flow through an attached lichen biofilm continuously. This design will have the biofilm entrapped in the enclosure and will arrest the movement of cells.

The pilot reactor will be operated with the samples from different source under conditions as optimized previously in the flask level experiments. The effect of different polluted samples on the biofilm composition and nutrient recovery will be tested. The time of harvest should also be evaluated, as the thickness of the biofilm will have a significant effect on the process. The biomass grown on the surface needs to reach optimum thickness; then it will be harvested by scraping off, and the polymer matrix can be returned for the next cycle of growth. Microscopic examination and thickness evaluation will also be performed. The ideal biofilm should be thick enough to facilitate the cell harvest, but thin enough to allow for transfer of nutrients, byproducts, and, sometimes, light. This thickness can be affected by many factors and may be controlled by the cultivation time.

Summary Budget Information for Activity 2:

ENRTF Budget: \$74,105
Amount Spent: \$ 0
Balance: \$74,105

Outcome	Completion Date
1: Water sample collection from multiple sites and analysis.	Sep 2016
2: Pilot reactor design construction for the application.	Jan 2017
3: Pilot study of microalgae/fungi systems to remove nutrients from different water.	Jun 2017

Activity Status as of January 1, 2016:

Activity Status as of July 1 2016:

Activity Status as of January 1, 2017:

Activity Status as of July 1, 2017:

Activity Status as of January 1, 2018:

Final Report Summary:

ACTIVITY 3: ONSITE EVALUATION - Floating island evaluation in Sarita wetland

Description: The Sarita wetland in University of Minnesota will be selected as a model system for tracking patterns in field parameters for the design of prototypes and implementing the scaled-up model for evaluating the effectiveness of lichen composites. A “floating island” system will be designed and constructed to use fungal and microalgae species instead of native plants. The treatment capacities of the systems will be estimated and effects of system design parameters will be evaluated. The kinetic and model parameters evaluated from the previous bench-top studies will be used to scale-up the process. Water quality characterization namely, dissolved oxygen levels at the site, salinity, and temperature effects, ammonia concentration, nitrate, nitrite, and phosphate concentration will be evaluated. Testing in the real wetland may be challenging with the unforeseen factors affecting the process, which will be realistically studied and mitigated. Water samples from different sampling point in Sarita after having the floating island design installed, will be collected at regular intervals and the physiochemical parameters will be checked for technology evaluation.

Cost estimation will be developed 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. The research team expects that the matrix can be reused for multiple times, which will be one of the key factors to control the overall process economic feasibility.

Summary Budget Information for Activity 3:

ENRTF Budget: \$100,073
Amount Spent: \$ 0
Balance: \$100,073

Outcome	Completion Date
1: Floating island design and construction.	Jan, 2018
2: Data collection and evaluation at Sarita.	May, 2018
3: Cost estimation	June, 2018

Activity Status as of January 1, 2016:

Activity Status as of July 1 2016:

Activity Status as of January 1, 2017:

Activity Status as of July 1, 2017:

Activity Status as of January 1, 2018:

Final Report Summary:

V. DISSEMINATION:

Description:

Part of the reactor design in the lab scale, if proved to be innovative, will be applied to the University Office for Technology Commercialization for filling the patent protection. We will publish two to three peer-reviewed manuscripts in the related journals to disseminate our results to the general public. We will also use the university extension website www.extension.umn.edu as well as PI’s academic website <http://bohu.cfans.umn.edu/> for dissemination of the research. If proved to techno-economically feasible, we will collaborate with UMN Office of Technology Commercialization office and actively look for commercial partners to explore the possibility for commercialization of this technology.

The primary target to disseminate our research results will be the scientific community, environmental companies as well as local community concerned with their pond health. Information obtained from the mechanism study will be directly applied to establish the cultural conditions for different strain combinations, and the lichen biofilm formation will be evaluated for the cell harvest and microalgae production. The synergistic interactions between different species will provide opportunity to extend the process to different applications. The floating island can also be re-designed based on our results to include the simulated lichen biofilm system to manage the nutrients more efficiently.

Any royalty, copyright, patent, and sales of products and assets resulting from this project will be subject to revenue sharing requirements with ENRTF according to Minnesota Statutes, section 116P.10.

Status as of January 1, 2016:

Status as of July 1 2016:

Status as of January 1, 2017:

Status as of July 1, 2017:

Status as of January 1, 2018:

Final Report Summary:

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	\$ Amount	Overview Explanation
Personnel:	\$ 216,180	<p><u>Bo Hu's salary:</u> One month of salary will be charged to the project for Dr. Bo Hu's summer time on managing the grant. His one month salary is \$8,956 in the first academic year and FTE with 3% salary increase for the following project years. The fringe benefit for Nine-Month B-term faculty is 33.9% based on the University regulation.</p> <p><u>Salary for Bo Hu's Postdoc Researcher:</u> One postdoc will be hired for this project for two and half years. The postdoc researcher will be paid with \$40,000 for the first year and FTE with 3% salary increase for the following project years. The rate of fringe benefits the postdoc researcher is 21.5% based on the University regulation. The postdoc researcher will work with Dr. Hu to design experiments and collect the research data on the lab scale study as well as pilot demonstration.</p> <p><u>Salary for Bruce Wilson's Postdoc Researcher:</u></p>

		Dr. Bruce Wilson's postdoc research will be paid 100% for the third year to assist with the onsite evaluation. The annual salary for this postdoc will be \$45,000 and the fringe benefit rate is 21.5%.
Professional/Technical/Service Contracts:	\$	N/A
Equipment/Tools/Supplies:	\$23,545	<p>\$18,545 is budgeted to purchase necessary chemicals, tools, bottles, gloves etc. Specifically it is budgeted for \$6,000 for the first project year and FTE with 3% increase for the following project year. Regular chemical supplies are needed to measure nitrogen, phosphorus content of the wastewater. For instance, Hach Chemical TOTAL NITROGEN LOW RANGE 25/PK for \$79.00 (No.:TNT826), and Hach Chemical PHOSPHORUS TNTPLUS, UHR,25/PK (No.: NC9881792) for \$57.23. One batch cell culture. It is estimated that around 20 boxes of each test kits are needed for us to work on the lab and pilot scale study for the first two years. For the third year, around 10 boxes of test kits for nitrogen and phosphorus are needed. A 3700 Full-Size Portable Sampler will be needed in the third year to take samples at Sarita Wetland, and the cost of this sampler is around \$3K. The rest of the requested supply fund will be used to purchase other necessary chemicals, bottles and gloves etc.</p> <p>\$5,000 is budgeted at the end of the second year to construct the floating island for the onsite evaluation at Sarita Wetland. Large scale "floating islands" will be constructed containing the supporting wood structure for around 100 square foot area, the polymer matrix and necessary fungal attachment. The large scale fungal attachment will need to be cultured at Bioconversion Resource Center at UMN using their pilot scale equipment. The cost includes the material and labor for constructing the floating island as well as the equipment rental fees to be paid to the Bioresource Center.</p>
Capital Expenditures over \$5,000:	\$39730	\$30,000 is budgeted to purchase additional components for Dr. Hu's High Performance Liquid Chromatography (HPLC) for chemical analysis. Dr. Bo Hu's lab has already spent around \$32K for a modular model of the HPLC,

		including pump, manual injection, multi-wavelength detector and software. This is a request to add an additional modular reflect index detector, an autosampler and an add-on to convert multi-wavelength detector to Photodiode Array detector. \$9,730 is requested to purchase an automatic cell counter to measure the microalgae and fungi cell numbers in the lab experiments.
Travel Expenses in MN:	\$1,545	Travel to the agricultural lagoon at Waseca MN and other locations to obtain wastewaters. Two travels are planned per each project year and \$250 is budgeted per travel for the first year with 3% increase for the following years.
TOTAL ENRTF BUDGET:	\$281,000	The total cost for the first year is \$106,822, the second year \$74,105, and the third year \$100,073. The total project cost to ENRTF is \$281,000.

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than \$5,000: \$30,000 is budgeted to purchase additional components for Dr. Hu's HPLC for chemical analysis. The HPLC at the lab currently has the UV detector with manual injection. An addition to this HPLC is requested to include a Reflex Index detector, an auto-sampler and an addition to convert UV detector to PDA detector. These additions will expand the analysis range of the HPLC and significantly increase the measurement efficiency so that the equipment can better serve the project. It will continue to be used throughout its useful life in Bo Hu's research group for chemical measurement o improve the nutrient removal and wastewater treatment.

\$9,730 is requested to purchase an automatic cell counter. This equipment will enable us accurately and efficiently measuring the cell numbers in the culture therefore knowing the cell distributions between microalgae and fungal cells in the lichen biofilm. This capital equipment will will continue to be maintained and used throughout its useful life in the research group as well as the BBE department to analyze cell culture samples for environmental remediation types of research, even after the LCCMR project ends in 2018.

Number of Full-time Equivalent (FTE) Directly Funded with this ENRTF Appropriation: 3.74

Number of Full-time Equivalent (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: 0

B. Other Funds:

Source of Funds	\$ Amount Proposed	\$ Amount Spent	Use of Other Funds
Non-state	\$125,461	\$0	In-kind services during the project period will be provided even though UMN did not charge the indirect cost for this project. The total estimate fund \$125,461 is calculated based on the

			University F&A rate (52% of modified base).
State			
	\$	\$	
TOTAL OTHER FUNDS:	\$125,461	\$0	

VII. PROJECT STRATEGY:

A. Project Partners: The team includes Professor Bo Hu, Dr. Aravindan Rajendran, Professor Bruce Wilson and his postdoc researcher, all from the Department of Bioproducts and Biosystems Engineering, University of Minnesota. **Bo Hu**, an assistant professor, is specialized in bioprocess development, and has extensively studied the fungal/microalgae pelletization and co-culture. He will serve as the project director to manage the project, design the experiments and write the project reports. **Aravindan Rajendran**, a post doc researcher at Bo Hu’s research group specialized in bioprocessing technologies and bioprocess modeling, will execute the research activities and provide technical expertise. **Bruce Wilson**, a professor with 12 month appointment, will not receive any salary from this project. He has extensive experience in reducing nutrient loading from agricultural and urban watersheds. He will supervise his postdoc researcher to work with the onsite evaluations on Sarita wetland. Bruce Wilson’s postdoc researcher is to be determined.

B. Project Impact and Long-term Strategy:

The outcomes of the project will provide a sustainable solution to capture and recycle the reusable resources from runoffs by protecting rivers, lakes, and vital landscape along with protecting terrestrial and aquatic life. The long term strategy is to transform the storm water runoff treatment facility for revenue generation by recovering and recycling the resources, and also to incorporate social responsibility for completely eliminating the runoff pollution, decimating the adverse effects of storm water on water resources. The methods developed in this study and the results obtained will serve as necessary input on future process development for various algae-based technologies for biofuel, bioproducts, and other industrial products. The funding for this research initiative would support a comprehensive understanding of the new “lichen” type biofilms and allow exploration of new avenues in the bioproduction industry with many potential applications. The planned research will build a synthetic ecosystem to provide an innovative platform technology for any processes that involve either heterotrophic fermentation of fungi or autotrophic cultivation of microalgae.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	\$ Amount
Funding from UMN-BTI Synthetic Ecology Program to support Bo Hu’s research group working on microalgae-fungal co-culture to form cell pellets	11/2012-10/2014	\$90,000
Funding from UMN Grant-in-Aid program to support Bo Hu’s research group working on fungal strain screening for phosphorus accumulation and removal	7/2013-1/2015	\$31,000

VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS: N/A

IX. VISUAL COMPONENT or MAP(S): see attached graphic

X. RESEARCH ADDENDUM: See attached research addendum

XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted no later than January 1, 2016, July 1 2016, January 1, 2017, July 1, 2017 and January 1, 2018. A final report and associated products will be submitted between June 30 and August 15, 2018.

It is budgeted to purchase to purchase additional components for Dr. Hu's High Performance Liquid Chromatography (HPLC) for chemical analysis. Dr. Bo Hu's lab has already spent around \$32K for a modular model of the HPLC, including pump, manual injection, multi-wavelength detector and software. This is a request to add an additional modular reflect index detector, an autosampler and an add-on to convert multi-wavelength detector to Photodiode Array detector.	\$30,000	\$0	\$30,000								
It is budgeted to purchase an automatic cell counter to measure the microalgae and fungi cell numbers in the lab experiments.	\$9,730	\$0	\$9,730								
Travel expenses in Minnesota											
Mileage, lodging, meals for travels to the water site for taking water samples. U of M plan for travel expense will be used to process the travel cost	\$500	\$0	\$500	\$515	\$0	\$515	\$530	\$0	\$530	\$1,545	\$1,545
COLUMN TOTAL	\$106,822	\$0	\$106,822	\$74,105	\$0	\$74,105	\$10,073	\$0	\$10,073	\$281,000	\$281,000

A Novel Biofilm Technology for Water Nutrient Removal

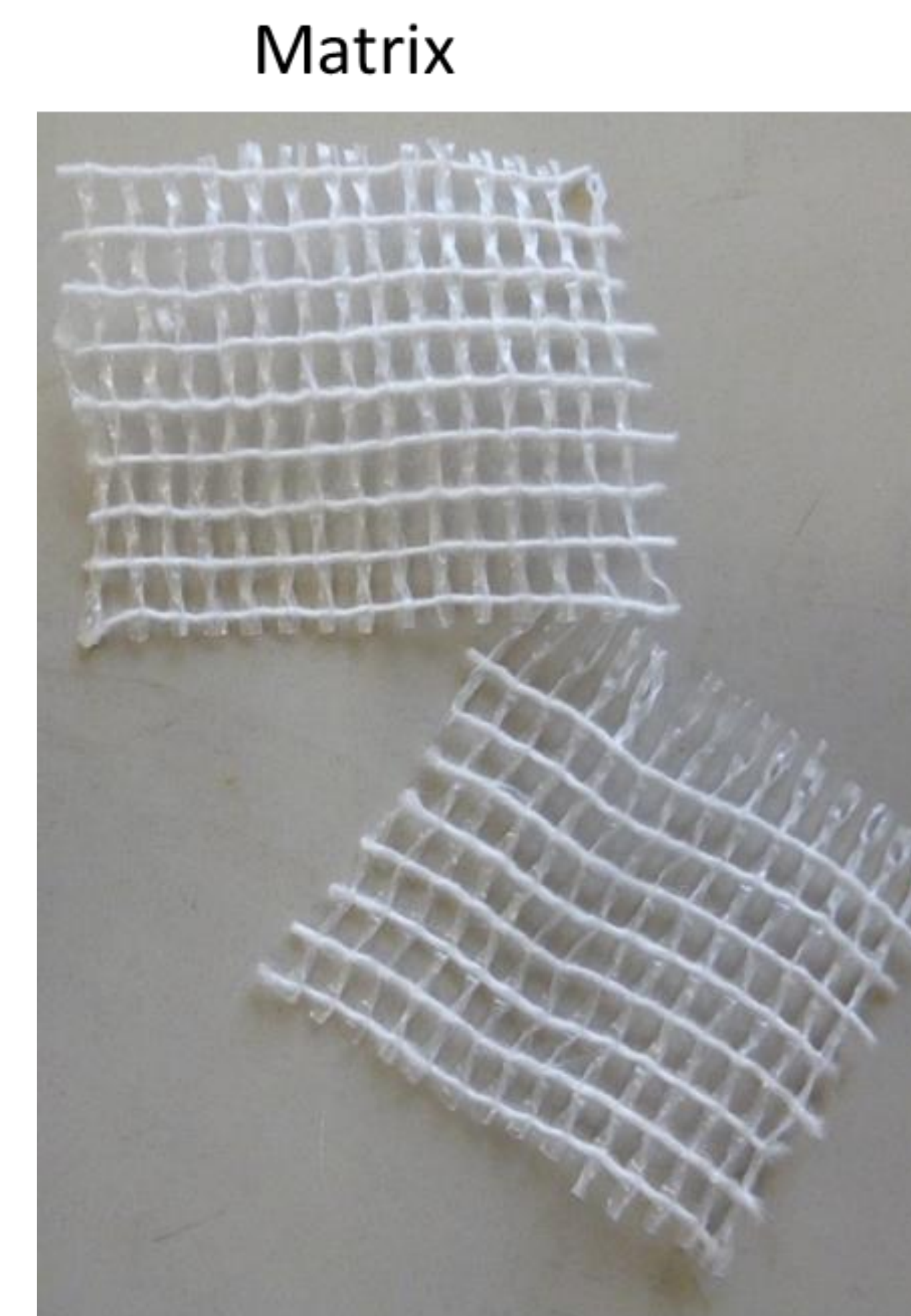
Bo Hu, Aravindan Rajendran and Bruce Wilson
University of Minnesota

Environment and Natural Resources Trust Fund

Project summary - This project proposes to develop a novel simulated lichen biofilm system to remove and recycle nutrients from storm water runoff as well as polluted lakes, ponds, and lagoons.

A natural lichen system:

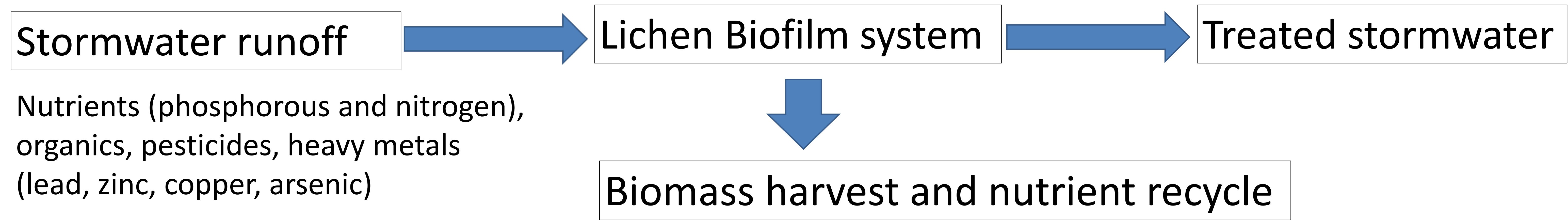
- Phototrophic microalgae
- Fungi
- Supporting surface



A simulated lichen system

- Microalgae with capability to remove nutrients (Selected or wild)
- Selected binding fungi
- Selected polymer easy to be harvested and reused

Proposed Process Flowchart

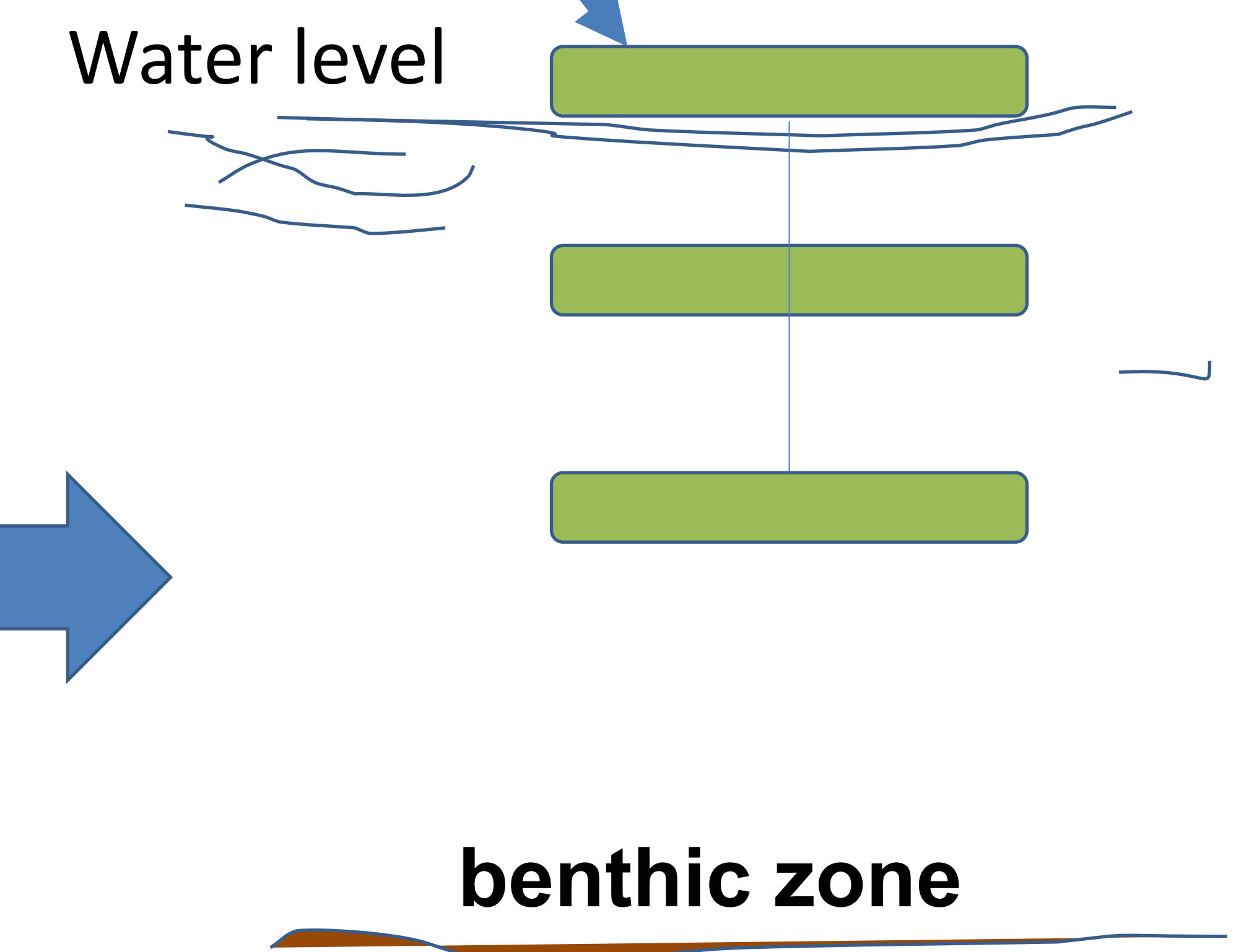


A floating island with simulated lichen systems instead of plants

- Grow much faster
- Can use native microalgae
- No need for soil and easy to harvest



Current floating island with plants



Proposed floating island with simulated lichen system