2016 Project Abstract
For the Period Ending June 30, 2019

PROJECT TITLE: Developing Biosponge Technology for Removal of Nitrates from Minnesota Waters
PROJECT MANAGER: Lawrence Wackett
AFFILIATION: University of Minnesota
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2016, Chp. 186, Sec. 2, Subd. 04q

APPROPRIATION AMOUNT: $198,000
AMOUNT SPENT: $ 193,805
AMOUNT REMAINING: $ 4,195

Sound bite of Project Outcomes and Results
The project developed methods for detecting and removing nitrate from Minnesota waters. Nitrate comes from nitrogen fertilizer that runs into water. Removing nitrate in Minnesota water treatment plants is expensive. We developed methods to inexpensively detect and lower nitrate levels on land and in water.

Overall Project Outcome and Results

We have developed simple, effective, and inexpensive technology for dealing with nitrate in drinking water, a major problem in Minnesota today. The cost of continuous monitoring and treating nitrate problems with current technology is estimated to be $3500 per household, a huge burden for Minnesotans. To help in alleviating that burden, we have done the following on this project. First, the project director, Dr. Aukema, identified nitrate-removing bio-components. Second, Dr. Aukema investigated and was successful in obtaining different sponge-like materials to maintain the bio-components within the material. Third, water from different sites in the state of Minnesota were obtained and tested with respect to nitrate. The waters were from: Montevideo, Lake Itasca, St. Paul, Zumbro Falls, and Minneapolis. Much work went into designing the matrix that holds the bio-component. It was found that cellulose was best for retaining the bio-component. The bio-component will then reduce the nitrate which is then both measured and removed. The last year of the project was devoted to outreach, disseminating the information: (1) verbally at conference, agencies, universities, (2) in written form in a journal publication, and (3) by the social media platform YouTube. Overall, we conducted outreach over the course of the project, discussing nitrate treatment entirely in, or as part of, 14 outreach items, in Minnesota, other states, and internationally. Note that any and all travel was covered by resources outside of the LCCMR budget. This is important to Minnesotans as it provides a way to cheaply and easily monitor nitrate in any water source they would want, from their local lake to their well. This also provides information for bioremediation of nitrate and other chemicals that are found in Minnesota waters. While generally chemical levels in Minnesota are low, we are glad to develop technology to help in keeping our waters clean.

Project Results Use and Dissemination
We have conducted dissemination activities throughout the project, with the last year of the project being heavily engaged in that activity specifically. For example, we presided over an event at the Institute on the Environment at the University of Minnesota on March, 2019 to discuss and disseminate information dealing with nitrogen and nitrate contamination in Minnesota waters and how to deal with it effectively. We followed that up with a broader meeting on May 16, 2019. In the last year of the project, we also published a research article pertaining to new technology developed. We also posted a YouTube video.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2016 Work Plan Final Report

Date of Report: August 15, 2019

Final Report

Date of Work Plan Approval: June 7, 2016

Project Completion Date: June 30, 2019

PROJECT TITLE: Developing Biosponge Technology for Removal of Nitrates from Minnesota Waters

Project Manager: Lawrence Wackett

Organization: University of Minnesota

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Location: State of Minnesota

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$198,000**
	Amount Spent:	**$193,805**
	Balance:	**$4,195**

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 04q

Appropriation Language:
$198,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to adapt and test an inexpensive biosponge technology for its effectiveness at removing nitrates from drinking water. This appropriation is subject to Minnesota Statutes, section 116P.10. This appropriation is available until June 30, 2019, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Developing Biosponge Technology for Removal of Nitrates from Minnesota Waters

II. PROJECT STATEMENT: We will develop, demonstrate, and disseminate a simple, effective, and inexpensive technology to remove nitrates from drinking water, a major problem in Minnesota today. Minnesotans from all parts of the state are suffering from nitrates in their water. Seventy-five percent of Minnesotans get their water from wells, and 105 water systems in Minnesota were found with nitrate at or above maximum contaminant levels, according to a State Health Department report released in May 2015. The cost of treating these nitrate problems with current technology is estimated to be $3500 per household, a huge burden for Minnesotans. Nitrate in water was linked to fourteen deaths in Minnesota in the 1940's and the National Cancer Institute is studying a link between nitrates and non-Hodgkin's lymphoma. Something must be done. Recent technology developed at the University by the PI and co-PI has demonstrated the efficacy of using a biocatalyst sponge (a biosponge) to remove and destroy unwanted chemicals in water. The technology is clean and safe. It has to this point not been applied to nitrates, but it has been used effectively with numerous other chemicals that are otherwise difficult or expensive to remove from water. In this project, we will conduct the necessary research to adapt and transfer the technology to be useful for nitrates, then optimize it to make it better and cheaper, and finally test it with nitrate-contaminated waters from around the state of Minnesota. Near the end of the project period, we will hold a conference hosted by the Institute on the Environment with many invited stakeholders from around the state and the focus will be squarely on solutions for the nitrate problem in Minnesota. The conference will serve to disseminate our findings and help arrive at the best practices for implementation in the state. Materials and information will be made available to all state agencies. Currently, there are many responses to this problem under discussion by both scientists and the legislature but some of the steps envisioned will take years to implement, and even longer to significantly impact nitrate levels. This project, if funded by the ENRTF, will develop, educate, and foster all best practices for treating nitrate contamination in Minnesota waters and can help institute a long-term, sustainable solution.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of: January 1, 2017

Dr. Kelly Aukema assumed the position of the project research director at the start of the project on July 1, 2016. Dr. Aukema conducted experiments to develop standardized assays for testing for the removal of nitrates in waters. She also measured the growth of the biological material to determine what was most efficient. This is important to allow for the production of large amounts of biological material inexpensively. The amount of the material and its ability to remove nitrate was tested in a series of experiments. This has been highly successful and we have obtained an optimized biomaterial. In the next steps, the research director put the biomaterial into the sponge material. The biomaterial was shown to work to remove nitrate from water while staying inside the sponge material. The sponge material is shown to allow water to penetrate into the sponge, where it is acted on by the biomaterial and nitrate is removed. This has been tested in the laboratory at concentrations that exceed the allowable concentration of nitrate in water. It has also been tested in the laboratory at concentrations of nitrate in water that are below the threshold of nitrate in water. This is an important result as the sponge material with the biomaterial will also work if waters are showing increasing levels of nitrate but below threshold. Under those conditions, the biosponge will still work. It will work also if the ntrate levels are very high, and bring it down to acceptable levels. No problems have been encountered.

Project Status as of: June 30, 2017

Dr. Kelly Aukema conducted experiments to develop a good method for trapping the biological material within the sponge material. Various sponge recipes were tested. The sponge was found to work with very small holes in the sponge as nitrate diffuses easily into the pores. Dr. Aukema also tested the ability of the sponge to hold up over time and be stable. Storage conditions were tested at room temperature (22^{o}C) and at refrigerator

temperature (4^{o}C), and freezing temperature (-20^{o}C). Freeze drying was tested, with and without additives like glycerol, to test the effects on storage. The requirement for amount of loading of the biomaterial into the sponge was also optimized. There is also a requirement for an electron source to drive the reduction of the nitrate and this was examined. Various slow-release electron sources were tested for this. These included starch, polyvinyl alcohol, and formate. The sponge was then tested in waters at different pH values that are representative of natural lakes, streams and wells in Minnesota. The sponge was found to work at a range of pH values. The presence of dissolved solutes was also tested. The dissolved solutes contained things like salts and metals. The sponge was found to be perfectly functional in waters suitable for drinking or of sufficient quality to be able to be purified for drinking via conventional water treatment methods. We further developed rapid methods to measure the effectiveness of the biosponge. We showed that the method was effective between 1 ppm and 40 ppm nitrate. The allowable level for nitrate in water for drinking is 10 ppm. We expect that virtually waters to be treated in Minnesota will be in the 1-40 ppm range so these methods are ideal to use for biosponge testing.

Project Status as of: January 1, 2018

Dr. Kelly Aukema has worked to set up conditions in which she can reliably test biosponge material. This includes making biosponge material in the laboratory. The biosponge material is then tested with water to which known concentrations of nitrate are added. There was also testing for various delivery platforms. Materials were purchased and product produced. Waters were obtained locally. The project is continuing as expected.

Project Status as of: September 20, 2018

During the first part of this last, materials are tested to determine if there are interfering materials, such as lead, cadmium or other chemicals that could be toxic to the system. The system was not influenced by those chemicals. Delivery platforms were further tested. Materials were purchased. Materials for testing were made with purchased supplied. More waters for testing were obtained locally. In the latter stages of this phase, we were focusing on disseminating information on the project and some additional testing and demonstrations. Activities include publication, speaking engagements, meetings, conferences, and discussions with local and state stakeholders. Also, there is additional testing, and we can test waters for different parts of the state of Minnesota. One publication we are looking at is the journal of *Environmental Science: Water Research & Technology*. This forum provides an international audience. We will be attending and discussing results at the Institute on the Environment's Fall 2018 Annual Meeting to be held in St. Paul, MN at the University's Student Center. Participants include environmental leaders from around the state of Minnesota.

Amendment Request: October 3, 2018

We are requesting here an amendment. There is no amendment to the Work Plan. This is a retroactive request for Budget modification. The overall budget amount is not changing. The amount of funds in each category is not changing. That is, the total amount spent, or to be spent, in each category of Personnel, Service Contracts, Supplies, and Travel are exactly the same.

The request comes about due to changes in a given Activity Period within a given Budget Item due to University mandated wage and benefits changes or the time supplies were purchased at the interface of Activities 2 and 3. Specifically, the change requested in the Personnel (Wages and Benefits) category is due to a mandatory increase in wages and benefits for the project research director, Dr. Kelly Aukema. The University of Minnesota reclassified her position to another level. This was not related to the LCCMR project, but rather new rules at the University. But this had the effect of increasing her wages and benefits. As a result of this, Dr. Aukema drew

more wages and benefits in the Activity 2 period, leading to a negative balance during that period. The Personnel Wages and Benefits for the overall project was not overspent. The final amount spent for Activity periods 1, 2, and 3 maintained at $126,000, the original amount budgeted.

Request will change the following:
Increase Activity 2 Personnel from $49,500 to $63,382 and increase of $13,822
Decrease Activity 3 Personnel from $46,500 to $32,618 a decrease of $13,822

In Professional/Technical/Service Contracts, we spent $7,250 in Activity 1. In Activity 1, $8,000 had been budgeted. The service fees are quoted at the time we request them, not a year before when the LCCMR budget was submitted. In that context, it is not feasible to know exactly what the cost of a service facility will be ahead of the request. Also, in research, the specifications for a service request are not locked in. As we learn more about a system, we refine the specifications of what is requested. This can result in the service costing a little more than expected, or a little less. The $750 balance from Activity 1 (less than 10% of what had been budgeted) was spent in the Activity 2 period. Since $750 more was spent than originally budgeted in Activity 2 period, there was a negative $750 in the Activity 2 balance. We request approval for this rebudgeting retroactively. The overall budget for Professional/Technical/Service Contracts is being maintained as in the original budget for the overall project, at $40,000.

Request will change the following:
Decrease Activity 1 Professional/Technical/Service Contracts from $8,000 to $7,250 a decrease of $750
Increase Activity 2 Professional/Technical/Service Contracts from $32,000 to $32,750 an increase of $750

In the Equipment/Tools/Supplies category, we developed silica precursors in Activity 1 that amounted to $2,000, which is the amount budgeted so the balance for Activity 1 was $0. For Activity 2, the major supply materials were used at the end of the Activity 2 period and into the Activity 3 period. Overall, $28,000 was spent on those supplies overall as budgeted for Activities 2 & 3. But because that spilled over into Activity 3 period, the charges showed up in the Activity 3 period. Supplies were used when they were needed to meet the needs of the project. We did not replenish some of those supplies until the Activity 3 period, and thus charges went through in the Activity 3 budget period. We kept our overall spending exactly within the overall scope of the project. We request retroactive approval for this negative balance figure showing up in the Activity 3 balance.

Request will change the following:
Decrease Activity 2 Equipment/Tools/Supplies from $28,000 to $5,069 a decrease of $22,931
Increase Activity 2 Professional/Technical/Service Contracts from $0 to $22,931 an increase of $22,931

There is remaining $2,163 left in supplies. As we work on our last remaining dissemination activity, we expect to need to purchase some supplies to make materials for demonstrations. We request permission to use this to carry out our remaining dissemination activities as described in the original work plan. This is still part of our plans and we wish to carry that out if we are given permission to do so.
We have developed simple, effective, and inexpensive technology for dealing with nitrate in drinking water, a major problem in Minnesota today. The cost of continuous monitoring and treating nitrate problems with current technology is estimated to be $3500 per household, a huge burden for Minnesotans. To help in alleviating that burden, we have done the following on this project. First, the project director, Dr. Aukema, identified nitrate-removing bio-components. Second, Dr. Aukema investigated and was successful in obtaining different sponge-like materials to maintain the bio-components within the material. Third, water from different sites in the state of Minnesota were obtained and tested with respect to nitrate. The waters were from: Montevideo, Lake Itasca, St. Paul, Zumbro Falls, and Minneapolis. Much work went into designing the matrix that holds the bio-component. It was found that cellulose was best for retaining the bio-component. The bio-component will then reduce the nitrate which is then both measured and removed. The last year of the project

was devoted to outreach, disseminating the information: (1) verbally at conference, agencies, universities, (2) in written form in a journal publication, and (3) by the social media platform YouTube. Overall, we conducted outreach over the course of the project, discussing nitrate treatment entirely in, or as part of, 14 outreach items, in Minnesota, other states, and internationally. Note that any and all travel was covered by resources outside of the LCCMR budget. This project is important to Minnesotans as it provides a way to cheaply and easily monitor nitrate in any water source they would want, from their local lake to their well. This also provides information for bioremediation of nitrate and other chemicals that are found in Minnesota waters. While generally chemical levels in Minnesota are low, we are glad to develop technology to help keep our waters clean.

Overall Project Outcomes and Results:

We have developed simple, effective, and inexpensive technology for dealing with nitrate in drinking water, a major problem in Minnesota today. The cost of continuous monitoring and treating nitrate problems with current technology is estimated to be $3500 per household, a huge burden for Minnesotans. To help in alleviating that burden, we have done the following on this project. First, the project director, Dr. Aukema, identified nitrate-removing bio-components. Second, Dr. Aukema investigated and was successful in obtaining different sponge-like materials to maintain the bio-components within the material. Third, water from different sites in the state of Minnesota were obtained and tested with respect to nitrate. The waters were from: Montevideo, Lake Itasca, St. Paul, Zumbro Falls, and Minneapolis. Much work went into designing the matrix that holds the bio-component. It was found that cellulose was best for retaining the bio-component. The bio-component will then reduce the nitrate which is then both measured and removed. The last year of the project was devoted to outreach, disseminating the information: (1) verbally at conference, agencies, universities, (2) in written form in a journal publication, and (3) by the social media platform YouTube. Overall, we conducted outreach over the course of the project, discussing nitrate treatment entirely in, or as part of, 14 outreach items, in Minnesota, other states, and internationally. Note that any and all travel was covered by resources outside of the LCCMR budget. This is important to Minnesotans as it provides a way to cheaply and easily monitor nitrate in any water source they would want, from their local lake to their well. This also provides information for bioremediation of nitrate and other chemicals that are found in Minnesota waters. While generally chemical levels in Minnesota are low, we are glad to develop technology to help in keeping our waters clean.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Producing biosponge component material

Description:

We will use University facilities at the BioTechnology Institute to efficiently and inexpensively produce the bio-component used for the biosponge that will remove nitrate from water. This will require growing cells in fermenters in the Biotechnology Resource Center at the University of Minnesota. The amount and quality of the material will be tested continuously. Assays will be developed to measure the growth and activity of the biological material to be used for the biosponge. The biosponge production will be later in the product, this is only the production of the initial biological material that will be used for the subsequent aspects of the project. These activities will be conducted by and under supervision of the project manager.

Summary Budget Information for Activity 1:	**ENRTF Budget:**	**$ 39,250**
	Amount Spent:	**$ 39,250**
	Balance:	**$ 0**

Outcome	Completion Date
1. *Initial work with Biotechnology Resource Center* - First material production run	September 1, 2016
2. *Second material production run*	October 1, 2016
3. *Testing and measurement of production material by project manager*	November 1, 2016

Activity Status as of: January 1, 2017
We have worked with and made first material production runs and second material production runs. The material produced has been tested by the project research director, Dr. Kelly Aukema. Dr. Aukema first had to develop the test methods and this was done right at the beginning of the project. Then the biomaterial was made in production runs, tested and shown to be effective. The biomaterial alone was shown to be highly effective in removing nitrate. There were no unexpected outcomes for this. Note that Dr. Aukema performed some work for Activity 2 in Activity 1 budget period, amounting to $750 of salary. In light of this, the $750 overspending on her salary in the first report is now moved to Activity 2 budget on the budget spreadsheet.

Activity Status as of: June 30, 2017
The Project Director tested the ability of the biosponge to work under conditions relevant to the use of the sponge in the laboratory under conditions relevant to actual waters to be treated. The biosponge was tested at different temperatures. The effect of storage of the biosponge was tested. Methods were also examined to increase the rate at which nitrate was removed from waters. The biosponge was shown to be effective under different conditions and with different waters in the laboratory. Thus, the project is moving on schedule and will move on the next phase of development and testing.

Activity Status as of: January 1, 2018
A major effort of the Project Director has been to move the project toward scale-up and field testing. In this context, Dr. Aukema has developed field methods for testing water nitrate levels in remote locations without any laboratory equipment. This sets the stage for preparing large amounts of materials and making materials for testing. We have also begun to make arrangements for dissemination of the results of the project by public meetings, reaching out to companies and agencies, and public descriptions of the findings.

Activity Status as of: September 20, 2018
We have continued to produce and test materials. We have collected local waters for testing. The waters have low to intermediate levels of nitrate. We plan to continue to produce testing material. Also to continue to obtain waters and test. A major activity currently is on the dissemination of information regarding this project and its utility. To that end, we speaking at the University and local venues to inform stakeholders of our work. This has been presented at national meetings and we will continue to reach out to interested audiences. We are pursuing interested at the Minnesota Institute on the Environment and will present at their Fall 2018 meeting.

Final Report Summary:
We used University facilities to efficiently and inexpensively produce bio-components for dealing with nitrate in water. The material was tested continuously. Assays were developed to measure the growth and activity of the biological material. The materials were tested in the laboratory under conditions relevant to waters throughout the State of Minnesota. Those conditions include testing at different temperatures, different pH values, and in the presence of other chemicals that may be present, as in actual waters in the environments of Minnesota. The study also investigated the effect of storage on the ability of the biomaterial to remove nitrate from water. A useful material will need to be stored under different conditions and then brought out when needed, so this was an important series of tests. Different materials were tested, such as starch and polyvinyl alcohol. Another key consideration regarding the materials were to anticipate the quality of the waters that would be test later, as it would need to be porous and durable to allow nitrate to penetrate, while being durable enough to store and use over a sufficient period of time. All activities were conducted by or under supervision of the project manager, Dr.

Kelly Aukema. Dr. Aukema managed the production of the bio-components, working with key University facilities. Dr. Aukema further conducted studies on the the immobilizing matrix material as described in greater detail under Activity 2, below.

ACTIVITY 2: Making biosponge material for testing

Description:

The project lab and field director will make sponge material suitable for adsorbing and destroying nitrate. This material consists of silica precursors and particles that constitute the main structural materials that comprise the sponge. This material is porous and so it allows water to permeate the material and contact the biocatalyst that will degrade and remove nitrate. Dr. Aukema will take the bio-component and incorporate that into the sponge material.

Outcome	Completion Date
1. Produce biosponge materials and show it works on nitrates	January 1, 2017
2. Optimize the biosponge to work under conditions relevant to use at Minnesota sites	January 1, 2018

Summary Budget Information for Activity 2:

ENRTF Budget: $101,201
Amount Spent: $101,201
Balance: $ 0

Activity Status as of: January 1, 2017

After the biomaterial phase of the project was completed, we moved forward on the project in the last month to work on producing the biosponge materials and showing that it works on nitrates. This involved making the sponge material in such a way that the biomaterial is trapped inside the sponge material. This was accomplished. Next, the research director worked to show that the biomaterial within the sponge material was effective in removing nitrate. This was accomplished and it was shown to remove nitrate present at concentrations below the legal limit in Minnesota waters. It was also shown to be effective in the laboratory at removing concentrations that are one half or one quarter of the allowed limit. The project goals have so far been achieved and the project is currently on track.

Activity Status as of: June 30, 2017

The Project Director tested the ability of the biosponge to work under conditions relevant to the use of the sponge in the laboratory under conditions relevant to actual waters to be treated. The biosponge was tested at different temperatures. The effect of storage of the biosponge was tested. Methods were also examined to increase the rate at which nitrate was removed from waters. The biosponge was shown to be effective under different conditions and with different waters in the laboratory. Thus, the project is moving on schedule and will move on the next phase of development and testing.

Activity Status as of: January 1, 2018

In this report period, we moved toward setting up production, field-testing, and information dissemination. To test in the field, we developed a paper dip-stick type test for nitrate in which a color develops with an intensity proportional to the concentration of nitrate. No enhancement or instrumentation is required and so it can be used at any location, however remote. The method involves a combined use of biological and chemical processes. The methods for this are being disseminated publicly so others can use it. The other key steps are moving toward scaling up production. To accomplish that, we are growing up cells on larger scale. To disseminate information, we are making arrangements for disseminating findings at a MnDRIVE Symposium, an international workshop, in the Institute on the Environment, companies, and agencies.

Activity Status as of: June 30, 2018
In this report period, we established production, field-testing, and information dissemination. We obtained samples from different water sources within the state of Minnesota. The sources were determined to have measurable levels of nitrate. We also spiked nitrate into fractions of the water samples to elevate nitrate levels. Nitrate was tested and activity demonstrated. To disseminate information on our technology, we presented lectures to Minnesota state audiences. The first presentation was at the April 11, 2018 conference for Minnesota's Discovery, Research, and InnoVation Economy, a partnership between the State of Minnesota and the University of Minnesota that aligns areas of research strength with the state's key and emerging industries. The title of the talk was "Ureide Biodegradation for Industry and the Environment." The second dissemination event was the May 21, 2018 International Symposium on Biosensors in St. Paul, MN co-sponsored by the Institute on the Environment and the Grand Challenges program at the University of Minnesota. The topic presented was, "Bio-Detection of Toxicants in Food and Water." An additional event was on May 31, 2018 at the Industrial Partnership for Research in Interfacial and Materials Engineering in Minneapolis. There, we presented under that topic, "Biocatalysis for Health and Agriculture." We also submitted the manuscript entitled, "Inexpensive microbial dipstick diagnostic for nitrate in water," for publication in the journal *Microbial Biotechnology*, distributed by John Wiley & Sons publishers. The manuscript is now undergoing scientific peer-review and we hope to hear of its suitability for publication within 8 weeks. All of these dissemination activities, as well as the earlier research, has been presided over by Dr. Kelly Aukema.

Activity Status as of: September 20, 2018
Dr. Aukema is writing a manuscript on nitrate in water. The PI's research group has submitted an abstract for presentation of the results to be presented at the Institute on the Environment's 2018 Annual Meeting to be held in St. Paul, MN. Participants include environmental leaders from around the state of Minnesota. Dr. Wackett presented research findings at the Annual Meeting of the Society for Industrial Microbiology and Biotechnology on August 13, 2018.

Final Report Summary:
In this research, we trapped biomaterials within a sponge matrix and optimized the consumption of nitrate. The biomaterial was tested with various inexpensive materials that could serve as an electron donor to remove the nitrate. Example materials tested were table sugar, starch, and formate. The latter was shown to be the ideal material that led to optimum nitrate removal rates. There was also optimization required to test the immobilizing matrix material for best performance. Among the materials tested were alginate, silica, and cellulose. For most purposes, a cellulosic immobilizing matrix proved best. A key development was our discovering the ability to both reduce the nitrate and to detect it within the same spongy matrix holding the bio-materials. The materials then served a dual purpose. The nitrate could be removed by reduction from the materials embedded within the matrix. The attachment of the biomaterials was investigated. We used fluorescent methods, using inherent fluorescence in the biomaterials and the cellulosic materials to visualize the juxtaposition of each material. This showed that the biomaterial would be retained when water was applied. Overall in this project, we produced materials, tested them, went to various places in Minnesota, disseminated the information at numerous state, national and international venues. Testing the efficacy of the materials is discussed below, under Activity 3, below.

ACTIVITY 3: Testing efficacy of product with Minnesota waters
We will work with our contacts in the Minnesota Department of Health, Department of Agriculture, and the MPCA, to identify, obtain, and test actual nitrate contaminated waters. Note that we have experience in conducting environmental cleanups approved by state agencies and the U.S. EPA and so we understand the complications that can arise in moving from the laboratory to the field. We also have contacts in the field near Northfield, MN and plan to explore the use of nitrate mitigation in a field setting using the first derivative production of the nitrate biosponge. We will explore the best places for field mitigation, which might be at tile

drain sites, or edge of field. We will use assays developed for nitrate to conduct the experiments. The field project will be presided over by the project manager, Dr. Kelly Aukema.

Outcome	Completion Date
1. Show biosponge greatly lowers nitrates in impacted waters from sites around the state	June 30, 2018

Summary Budget Information for Activity 3:

ENRTF Budget: $ 57,549
Amount Spent: $ 53,386
Balance: $ 4,195

Note: A budget amendment was requested and granted.

Activity Status as of: January 1, 2017
This part of the project is to be carried out at a future stage of the project.

Activity Status as of: June 30, 2017
This part of the project is to be carried out at a future stage of the project.

Activity Status as of: January 1, 2018
This part of the project is to be carried out at a future stage of the project.

Activity Status as of: September 20, 2018

The major activity has been making and testing materials, and presenting and discussing results. Dr. Aukema is describing the methods for nitrate in water to the publication, *Environmental Science: Water Research & Technology*. The PI's research group has submitted an abstract for presentation of the results to be presented at the Institute on the Environment's 2018 Annual Meeting to be held in St. Paul, MN. Participants include environmental leaders from around the state of Minnesota. Dr. Wackett presented research findings at the Annual Meeting of the Society for Industrial Microbiology and Biotechnology on August 13, 2018.

Final Report Summary:
We collected Minnesota waters for testing. The waters had low to intermediate levels of nitrate. Nitrate in water continues to be an issue in Minnesota. There are many facets of the issue. First, there is the question of what waters are impacted, how impacted they are, and determining if the problem is increasing or decreasing. Given recent health-related research done by others, it is clear that we all want to minimize the amount of nitrate in water, especially sources of drinking water. Having rapid testing methods that anyone can use on the spot is a major advance for detecting and treating nitrate-containing waters. In this research, we trapped bacteria within a sponge matrix, had the bacteria consume the nitrate, and that also made for a rapid testing method for nitrate. The nitrate was turned into a material that formed a purple color within the entrapped matrix. Most importantly, the level of color was directly proportional to the level of nitrate in the water. The person using this system would then visually determine the level of the color compared to a known scale and could instantly know the nitrate level in the water. We considered that color blind people might have some difficulty. We have also explored cell phone apps in which a user photographs the biosponge material and gets a direct numerical readout from their phone. We have made different biosponge materials. Some of them do not have the built-in measurement feature. But those other designs might be better in standalone applications in fields or in remote areas where people would not be routinely monitoring the nitrate levels. Overall in this

project, we produced materials, tested them, went to various places in Minnesota to obtain and test waters, disseminated the information at numerous state/national/international venues, and published a peer-reviewed journal article that highlights the problem, presents the methods, and discusses uses.

V. DISSEMINATION:
Description: Conference at end of project, participation in other conferences, invited lectures, and peer-reviewed scientific

It is expected that the technology developed under this project will be broadly useful. In this context, it is anticipated that research publications will result that will disseminate the findings to the scientific community so that many can use and implement the results here. In addition, if there is patentable material, invention disclosures will be filed with the Office of Technology Commercialization at the University of Minnesota. They will decide if the results should be filed for patents. Patents publish but they also retain rights for the University and the State of Minnesota. At the conclusion of the project, we will organize and host a conference at the Institute on the Environment. The conference participants will include political leaders, people from relevant state agencies, concerned citizens, and the private sector. We will disseminate our findings from the project. We will also discuss the major state sites that are most impacted by nitrates and the best means for deploying the biosponge, and any other technology that can help solve the problem. It is understood that nitrate contamination of waters has been a long- term problem in Minnesota and other agricultural states and so we seek to help devise and implement long-lasting, sustainable solutions.

Outcome	Completion Date
1. Disseminate information and move the best technology into practice in Minnesota	June 30, 2018

Status as of: January 1, 2017
This aspect of the project will be carried out at a later stage of the project.

Status as of: June 30, 2017
This part of the project is to be carried out at a future stage of the project.

Status as of: January 1, 2018
This part of the project is to be carried out at a future stage of the project.

Activity Status as of: September 20, 2018

Dr. Aukema is writing a manuscript describing methods for nitrate in water to the publication, *Environmental Science: Water Research & Technology*. The PI's research group has submitted an abstract for presentation of the results to be presented at the Institute on the Environment's 2018 Annual Meeting to be held in St. Paul, MN. Participants include environmental leaders from around the state of Minnesota. Dr. Wackett presented research findings at the Annual Meeting of the Society for Industrial Microbiology and Biotechnology on August 13, 2018. We also discuss the results with interested colleagues at Water sessions hosted at the University of Minnesota. We are hosting a company specializing in reducing nitrate runoff in corn fields. We have submitted a grant to the United States Department of Agriculture on novel methods for reducing nitrate contamination. All of these activities serve to both disseminate and extend the current work.

Final Report Summary:
We have conducted dissemination activities throughout the project, with the last year of the project being heavily and specifically engaged in that activity. For example, we presided over an event at the Institute on the

Environment at the University of Minnesota on March, 2019 to discuss and disseminate information dealing with nitrogen and nitrate contamination in Minnesota waters and how to deal with it effectively. We followed that up with a broader meeting on May 16, 2019. In the last year of the project, we also published a research article pertaining to the new technology developed. We also posted a YouTube video. Detailed information on these activities and products are listed under "Overall Project Outcomes and Results." In that list, we provide detail of the organization, venue, and the date of the discussion or presentation.

The project research director, Dr. Kelly Aukema, is a career scientist. In addition to organizing, coordinating and carrying out the detailed aspects of this project, Dr. Aukema has also helped demonstrate the technology to students at the University of Minnesota and to outreach groups. Our goal has been to develop, discuss, and disseminate the technology so that it reaches as wide a potential user group as possible. Results, data, and conclusions derived from this project were submitted to a peer-reviewed scientific journal. The process of writing and having reviewed a scientific journal article was lengthy and required much of the last year of the project. There was an initial review of the data and conclusions and some comments by the scientific reviewers and journal editor. We responded to those questions and comments with additional explanations. In the end, the work was found to be of high quality and accepted for publication. The published paper appeared in print in the journal in early 2019. In addition to the journal article, that is read by scientists internationally, we discussed our results at the University of Minnesota and at other Minnesota venues to disseminate the information obtained. We spoke to stakeholders from environmental groups, government agencies, and professional water treatment experts.

Lectures/Discussions/Workshops

Minnesota Community Organizations
West Metro Naturalists Association, Minnetonka Community Center, July 9, 2016

Minnesota direct outreach event
IonE Salon, St. Paul, MN, March 27, 2019

Minnesota Conferences and Workshops
Nanotechnology Center, Minneapolis, MN, February 20, 2017
Society for Environmental Toxicology and Chemistry, Minneapolis, MN, November 14, 2017
Minnesota's Discovery, Research, and InnoVation Economy Conference, April 11, 2018
International Symposium on Biosensors, St. Paul, MN, May 21, 2018
IPRIME, Minneapolis, May 31, 2018

Conferences outside of Minnesota
Society for Industrial Microbiology and Biotechnology, Chicago, August, 2018

International Conferences and Workshops
EU Workshop on Biodegradation, Munich, Germany, November 14, 2016
TransCon, Ascona, Switzerland, April 28 – May 3, 2019

Universities
University of Wisconsin, Department of Biochemistry, November 21, 2016
University of the Andes, Department of Civil and Environmental Engineering,Bogota, Columbia, November, 2018
Marquette University, Milwaukee, WI, February 15, 2019

Government agencies
Central Intelligence Agency (CIA), Washington, DC, October 30, 2018

Research publication
Aukema, K.G. and L.P. Wackett (2019) Inexpensive microbial dipstick diagnostic for nitrate in water. *Environmental Science: Water Research Technology,* Volume 5(2), pages 406-416.

YouTube video
Nitrate technology
https://www.youtube.com/watch?v=UzBynyqZtGk&feature=youtu.be

VI. PROJECT BUDGET SUMMARY:
A. ENRTF Budget Overview:

Budget Category	$ Amount	Overview Explanation
Personnel:	$ 126,000	1 project manager, Dr. Kelly Aukema, at 100% FTE for two years ($126,256). Dr. Aukema is a highly experienced lab and project manager with a Ph.D. from the University of Wisconsin and research management eperience at the University of British Columbia.
Professional/Technical/Service Contracts:	$40,000	University facilities at the BioTechnology Institute will be used and there is a fee for the facility and the materials used to produce the biological material ($30,000). There will also be fees for the use of the Characterization Facility at the University of Minnesota ($10,000).
Equipment/Tools/Supplies:	$30,000	Funds are for producing nitrate-reducing materials for field testing, in addition to routine lab supplies are budgeted. These include silica encapsulation materials, solvents, buffers, chemicals, flasks, pipetters, glassware ($30,000)
Travel Expenses in MN:	$2000	Funds are requested to travel to state sites for acquiring water samples, and testing our materials in actual field environments.
TOTAL ENRTF BUDGET:	**$198,000**	

Explanation of Use of Classified Staff: NA

Explanation of Capital Expenditures Greater Than $5,000: NA

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 2

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

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
State			
Institute on the Environment funds	$30,000	$	Funds are secured and held in the Institute on the Environment at the University of Minnesota to hold

			conferences, workshops and other working groups to explore solutions to water issues in Minnesota and beyond the region. Funds will be used to sponsor on meeting on nitrate in state waters and the best solutions to the problem. This will inform and guide or research and help educate people in the state on best-practice methods.
In-kind services to be applied during project period	$15,000		Faculty salary time paid by the University of Minnesota that the PI will devote to the project over the summer months.
In-kind services to be applied during project period	$20,000		BioTechnology Institute Pilot Plant fee waiver. Since the PI is a member of the BioTechnology Institute, this project will have the entry fee waived for the use of the facilities to prepare nitrate-reducing biocatalysts to be used in this proposal. The project will only be charged for materials used in production and the hourly wages of the staff at the facility that they work on this specific project.
TOTAL OTHER FUNDS:	**$ 65,000**	**$**	

VII. PROJECT STRATEGY:

A. Project Partners:

BioTechnology Institute
Institute on the Environment

B. Project Impact and Long-term Strategy:

Nitrate contamination of water is a Minnesota problem but also it is a national issue. The development of technology for cheaply and efficiently removing nitrate will have major impact on our state and country.

The existing technology platform uses non-growing, actively-metabolizing bacteria that are encapsulated in a cheap, safe, extended life-time, silica-based hybrid gel. The silica material is robust and retains the bacteria within, yet it is highly porous to water and chemicals.

There is an opportunity for developing patentable material on this project and this bring an additional impact to the project.

The long-term strategy is to develop an easy to use and inexpensive treatment material for removing nitrate from waters. This material could be used in wells, municipal water systems and for treating runoff from fields.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	$ Amount
National Science Foundation funds from the federal government were used to develop some of the key background for this platform technology to be applied here to nitrates	September 1, 2012-August 31, 2015	$601,000

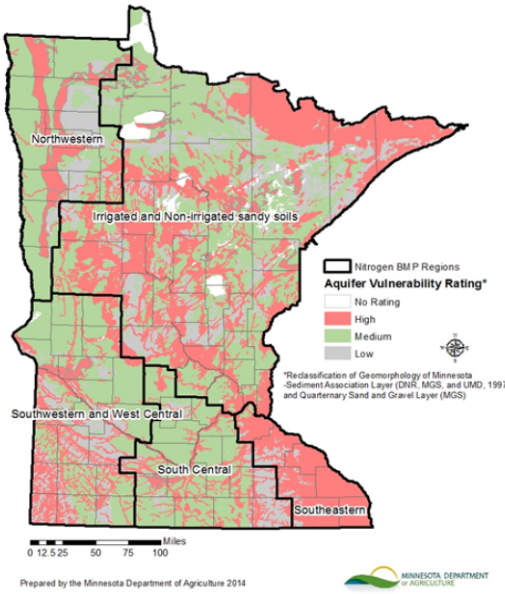
VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS:

A. Parcel List: N/A

B. Acquisition/Restoration Information: N/A

IX. VISUAL COMPONENT or MAP(S):

Aquifer Vulnerability, Largely from Nitrates; Map, *Minnesota Department of Agriculture*



Solutions are controversial and expensive!

1. Remove agricultural land from production
2. Use reverse-osmosis water treatments – expensive, removes at most 80%
3. Use cheap biotechnological solution, >95% removal, to be developed here

Reverse osmosis system

Biosponge for chemicals in water
(For illustration: nitrate biosponge
to be developed in this project)

X. RESEARCH ADDENDUM: N/A – Project contains confidential information and and has been confidentially peer-reviewed following the protocol designated by LCCMR staff.

XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted no later than January 1, 2017, June 30, 2017, and January 1, 2018. A final report and associated products will be submitted between June 30 and August 15, 2019.

Environment and Natural Resources Trust Fund
M.L. 2016 Project Budget

Project Title: Developing Biosponge Technology for Removal of Nitrates from Minnesota Waters
Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 04q
Project Manager: Lawrence Wackett
Organization: University of Minnesota
M.L. 2016 ENRTF Appropriation: $ 198,000
Project Length and Completion Date: 3 years, June 30, 2019
Date of Report: FINAL Report

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised Activity 1 Budget 10/9/18	Amount Spent	Activity 1 Balance	Revised Activity 2 Budget 10/9/18	Amount Spent	Activity 2 Balance	Revised Activity 3 budget	Amount Spent	Activity 3 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	***Producing biosponge component material***			***Making biosponge material for testing***			***Testing efficacy of product in Minnesota***				
Personnel (Wages and Benefits)	$30,000	$30,000	$0	$63,382	$63,382	$0	$32,618	$32,618	$0	$126,000	$0
Kelly Aukema, Ph.D.: $63,128 (salary 82% salary; 18% fringe)- 100% FTE for 2 years											
Professional/Technical/Service Contracts											
Biotechnology Resource Center, University of Minnesota: Fermentation for producing bacteria to be used in assay development and subsequent production of nitrate-removing materials; Characterization facility, University of Minnesota: Electron microscopy, hardness testing, water content testing, porosity testing	$7,250	$7,250	$0	$32,750	$32,750	$0				$40,000	$0
Equipment/Tools/Supplies											
Materials for assay development: Silica precursors, silica particles,solvents, catalysts, enzymes, chemicals for assays, test tubes, cuvettes, pipettes, pipette tips, screw cap vials, flasks, buffers,disposable gloves, materials for demonstrations associated with dissemination activities (approximately $2,200)	$2,000	$2,000	$0	$5,069	$5,069	$0	$22,931	$20,565	$2,366	$30,000	$2,366
Travel expenses in Minnesota											
Travel will be conducted to test biosponge technology in the field in Minnesota.							$2,000	$171	$1,829	$2,000	$1,829
COLUMN TOTAL	**$39,250**	**$39,250**	**$0**	**$101,201**	**$101,201**	**$0**	**$57,549**	**$53,354**	**$4,195**	**$198,000**	**$4,195**