**2017 Project Abstract**
For the Period Ending June 30, 2021

**PROJECT TITLE:** Wastewater Nitrogen Removal Technology to Protect Water Quality
**PROJECT MANAGER:** Paige J. Novak
**AFFILIATION:** University of Minnesota
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**FUNDING SOURCE:** Environment and Natural Resources Trust Fund
**LEGAL CITATION: M.L. 2017, Chp. 96, Sec. 2, Subd. 04b** as extended by M.L. 2020, First Special Session, Chp. 4, Sec. 2

**APPROPRIATION AMOUNT:** $ 450,000
**AMOUNT SPENT:** $ 450,000
**AMOUNT REMAINING:** $ 0

**Sound bite of Project Outcomes and Results**
A group of bacteria ("anammox") have received attention for their potential in wastewater treatment, transforming harmful reactive nitrogen into harmless dinitrogen gas. However, anammox perform poorly in typical wastewater environments. In this project we developed new materials to selectively enhance anammox growth/retention, supporting more sustainable removal of harmful nitrogen.

**Overall Project Outcome and Results**
Anammox bacteria have received attention for their ability to completely transform harmful reactive nitrogen compounds in wastewater into harmless dinitrogen gas. In addition, when using anammox bacteria, much less oxygen and no supplemental carbon is needed for nitrogen removal, and there is little production of excess biomass in the form of sludge. This reduces costs and energy use for nitrogen removal. It is estimated that the anammox process saves 60% of the energy used in conventional nitrogen removal. Unfortunately, this process has been difficult to implement in typical wastewater systems. Anammox bacteria are slow growing and the ammonium and carbon concentrations in wastewater result in low anammox activity and competition from faster growing bacteria. This leads to the washout of anammox bacteria. In this collaborative research project, our goals were to develop new polymeric materials that could concentrate ammonium to create localized niches for anammox enrichment and retention. We developed two different materials in this project: (1) a porous polymer carrier and (2) a gas-permeable alumina membrane. Both materials were able to concentrate ammonium, while the membrane could also transfer low quantities of oxygen to the surrounding solution. Both materials were also able to enrich and retain anammox when added to a wastewater environment. Further optimization of these materials is needed to enable scale-up and deployment. Nevertheless, given that in the US, the energy used for wastewater treatment costs approximately $2B a year, the predicted energy savings if this technology was implemented would be significant. The impact within the state of Minnesota would also be large, saving millions of dollars and providing more complete removal of harmful nitrogen species. A patent was awarded and the University of Minnesota is exploring commercialization and licensing options. Three peer-reviewed manuscripts were published from this work and have been submitted to the LCCMR.

**Project Results Use and Dissemination**
Information from this project has been shared with several water technology companies who may be able to assist in optimizing and eventually deploying this technology. As stated above, three peer-reviewed manuscripts were published from this work and have been submitted to the LCCMR. Multiple presentations about the research have been given to both regional and national/international conferences. Additional funding is being sought from a large infrastructure company. We anticipate submitting a proposal to the National Science

Foundation for additional funding. The University of Minnesota Technology Commercialization Office is working with us to further the technology.

# Environment and Natural Resources Trust Fund (ENRTF)
# M.L. 2017 LCCMR Work Plan Final Report

**Date of Submission:** August 11, 2021

**Final Report**

**Date of Work Plan Approval:** June, 7, 2017

**Project Completion Date:** June 30, 2021

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**PROJECT TITLE:** **Wastewater Nitrogen Removal Technology to Protect Water Quality**

**Project Manager:** Paige J. Novak

**Organization:** University of Minnesota

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|---|---|---|
| **Total ENRTF Project Budget:** | **ENRTF Appropriation:** | **$450,000** |
| | **Amount Spent:** | **$450,000** |
| | **Balance:** | **$ 0** |

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**Legal Citation: M.L. 2017, Chp. 96, Sec. 2, Subd. 04b** as extended by M.L. 2020, First Special Session, Chp. 4, Sec. 2

**Appropriation Language:**
$450,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to develop a technology for inexpensive low-energy nitrogen removal in wastewater. This appropriation is subject to Minnesota Statutes, section 116P.10. This appropriation is available until June 30, 2020, by which time the project must be completed and final products delivered.

M.L. 2020 - Sec. 2. ENVIRONMENT AND NATURAL RESOURCES TRUST FUND; EXTENSIONS. [to June 30, 2021]

**I. PROJECT TITLE:**
Innovative Nitrogen Removal Technology to Protect Water Quality

**II. PROJECT STATEMENT:**
As we exceed planetary boundaries on nutrients, regulatory directives to remove total nitrogen from wastewater are increasing. Traditionally, bacteria are used to remove total nitrogen from wastewater in a two-step process. Although reliable, this process is energy- and in some cases, material-intensive and also requires significant land as a result of its large footprint. Fortunately, viable new processes that facilitate the transformation of influent ammonium ($NH_4^+$) to harmless nitrogen gas ($N_2$) while minimizing energy use and/or addition of other chemicals, and in some cases plant footprint, are increasing in number. Anaerobic ammonia oxidation (anammox) is one of the most promising emerging nitrogen removal technologies. Anammox microorganisms anaerobically convert 1-to-1 ratios of $NH_4^+$ and nitrite ($NO_2^-$) to $N_2$ in a single step without excessive oxygen input. Some oxygen is required to generate $NO_2^-$, but the aeration, and therefore energy requirements are much lower than that of traditional treatment.

Despite its significant promise, however, there are several problems with the anammox process: (1) anammox bacteria are notoriously slow growing and a method of retaining them in the treatment reactor is needed; and (2) controlling air addition to optimize the anammox process is difficult. As a result, the anammox process has yet to be implemented in the United States for municipal wastewater treatment. Given that each year 19 trillion gallons of wastewater are generated in North America (approximately 130 billion gallons in Minnesota alone), and approximately 3% of the total US electricity use is for wastewater treatment, if the anammox process could be reliably used for nitrogen removal in municipal wastewater treatment plants, it would have an enormous impact on the cost and energy intensity of wastewater treatment in Minnesota, the US, and the world.

We propose to develop a material that can preferentially attract and retain anammox bacteria where they are needed while easily controlling air addition. This will make nitrogen removal from wastewater faster and cheaper. The specific goals of the project are to:

- Develop a material that concentrates the food source of anammox bacteria, enabling selective colonization of these bacteria on the material,
- Develop a material that easily controls the air addition to the system via the material properties,
- Optimize the design of technology (spacing, amount of material, etc.), and
- Test the system with municipal wastewater.

The envisioned system will operate for long time periods and provide improved nitrogen removal from wastewater with much lower energy usage. Patent protection will be sought by the University of Minnesota for the technology.

**III. OVERALL PROJECT STATUS UPDATES:**

**Project Status as of January 31, 2018**:
Project activities over the last six months have included method development, initial material development, and operation of bench scale wastewater reactors for proof of concept experiments. Methods have been developed to quantify specific bacteria in our system: total bacteria, anammox bacteria (via two different methods), aerobic ammonia oxidizing bacteria, and aerobic nitrite oxidizing bacteria. Polysulfone membranes have been made and modified to attach zeolite nanoparticles. Multiple methods to modify both the zeolite particles and the polysulfone membrane surfaces were developed and tested to determine the methods that worked most effectively. Materials were characterized with scanning electron microscopy and elemental mapping to verify zeolite attachment and determine zeolite coverage on the membrane surface.

A bench-scale wastewater reactor fed synthetic wastewater has been constructed and is being operated. The performance of the reactor, in terms of nitrogen and carbon removal, is monitored multiple times a week. Initial experiments are currently being performed with beads of zeolite, glass, or polyethylene for proof of concept testing. Once proof of concept testing is complete, an intellectual property disclosure will be filed with the University of Minnesota and testing with surface-modified polysulfone membranes will begin.

**Project Status as of July 31, 2018:**
Multiple support materials have been generated in a hollow fiber configuration, including a polymer support (polyvinylidene fluoride) and an inorganic alumina support, both of which should enable gas transfer control to facilitate our goals related to Activity 2. Both types of supports have been modified in newly developed protocols such that they can be coated with zeolite materials, either via the growth of the zeolite directly on the support or via attachment or embedding the zeolite onto the support. Efforts will continue along these lines, creating novel materials with beneficial and tunable properties that facilitate ammonium sorption and hence, anammox activity.

Experiments have been and are being performed to test the ammonium sorption capacity of these various materials and to determine how they perform under dynamic operating conditions with respect to the stimulation of anammox activity. Zeolite appears to act as a “battery” in a biologically active reactor system, adsorbing the ammonium, then “discharging” it to the microorganisms, followed by additional sorption/discharge cycles. This appears to facilitate total nitrogen removal via the anammox process, which does not occur in reactors to which no zeolite is added. These studies are informing our future research on how best to operate reactors for maximum performance with respect to total nitrogen removal.

An intellectual property disclosure was made with the University of Minnesota and a provisional patent has been filed.

**Project Status as of January 31, 2019:**
We have continued to create novel membrane materials, including graphene oxide (GO)-coated amine cross-linked-PVDF (c-PVDF) hollow fibers and alumina hollow fibers upon which zeolites have been grown. We believe that, based on literature that shows that GO-coated filters can result in faster *Escherichia coli* growth, such a coated fiber could also improve the activity and retention of anammox bacteria. GO-coated c-PVDF fibers should also allow excellent tuning of the gas transfer properties of the fiber through control of the GO layers. The zeolite-coated membranes should allow ammonia to be concentrated on the fiber surface, providing a competitive advantage to anammox bacteria.

Experiments have continued with zeolite particles and have been started with zeolite-modified alumina hollow fibers. The alumina hollow fiber membranes with zeolite grown on the surface removed substantial quantities of ammonia in batch sorption tests. Batch reactor experiments containing zeolite particles (biologically active and abiotic) and glass particles were continued, with results showing that the total nitrogen removal was much greater in the reactors containing zeolite particles and biomass. A new batch experiment is being performed with the alumina hollow fibers upon which zeolite has been grown.

**Project Status as of July 31, 2019:**
Batch experiments continue to be operated with both zeolite particles and zeolite-coated alumina hollow fibers. An experiment is being performed to optimize the amount of zeolite needed to achieve high rates of ammonia and total nitrogen removal. The information from this experiment was then apply to the batch experiments testing the alumina hollow fibers. The initial experiment with hollow fibers operated previously did not produce results as quickly as anticipated. The hollow fiber experiment currently being operated has been amended to include an optimized amount of alumina hollow fiber and is expected to achieve higher removal rates.

Experiments have been started that incorporate air addition through membranes. This initial experiment achieved high rates of nitrogen removal. Further experiments will be performed to optimize the amount of oxygen delivery. Additionally, it has been determined that the thickness of the zeolite grown on the hollow fibers can control the gas permeation rate. We are pursuing this as a way to precisely control the oxygen delivered to the system.

A non-provisional patent application was filed on the technology June 6, 2019.

**Amendment Request (07/29/2019):**
The addendum is to formally request a re-budgeting of funds for this project.

The supply costs for creating novel zeolite-attached alumina hollow fibers and also for monitoring total nitrogen concentrations in the reactors have been higher than originally anticipated because of the large number of experiments performed. Fewer funds are being used in the "**Personnel**" category than originally anticipated. This has resulted in additional analytical and material costs. Because of this a rebudget of $14,000 is requested from the "**Personnel**" category (Activity 2) to the "**Equipment/Tools/Supplies**" category (Activity 2).

The movement of money between sub-categories will not affect project objectives.

Amendment Approved by LCCMR 8/22/2019

**Project Status as of January 31, 2020:**
Batch experiments with zeolite-coated alumina hollow fiber membranes have been successfully operated to achieve high rates of nitrogen removal. This experiment confirms the quantity of zeolite-coated membrane required for enhanced nitrogen removal for use with future experiments.

Initial batch experiments with aerated membranes demonstrated the conversion of ammonia. Additional experiments are currently being designed to determine the rate of oxygen transfer through membranes with different zeolite-layer thicknesses. Membranes are being tested in an aqueous flow-through system and oxygen concentrations are measured at steady state to determine the oxygen flux per unit membrane area. This information will be used to determine oxygen transfer rates per area of membrane and will help inform the design of flow-through reactors.

Finally, novel carriers have been developed. Polyethylene "sponges" have been successfully synthesized that are able to retain zeolite particles on their surface and can therefore retain desired biomass. These carriers have been tested for ammonia removal.

With respect to project personnel, Co-PIs Michael Tsapatsis and Santiago Romero Vargas have both left the University of Minnesota. Dr. Tsapatsis had been continuing to advise the postdoctoral researcher who was working with him and Marc Hillmyer. This researcher has now left and a new postdoctoral researcher has joined the project (last August) who is being advised only by Dr. Hillmyer. The graduate student on the project is working with Paige Novak and has stayed in touch with Dr. Romero Vargas as needed by email. The project has not been affected negatively by these changes, as Drs. Tsapatsis and Romero Vargas are available for assistance with the project as needed and they were both more heavily involved with the early stages of the research.

**Amendment Request (01/21/2020):**
The addendum is to formally request a re-budgeting of funds for this project.

Again, as reported last period, the supply costs for creating novel zeolite-attached alumina hollow fibers and also for monitoring total nitrogen concentrations in the reactors have been higher than originally anticipated because of the large number of experiments performed. This has resulted in additional analytical and material costs. Because of this a rebudget of $14,000 is requested from the "**Personnel**" category (Activity 2) to the "**Equipment/Tools/Supplies**" category (Activity 2).

The movement of money between sub-categories will not affect project objectives.

Amendment Approved by LCCMR 02/06/2020

**Project extended to June 30, 2021 by LCCMR 6/18/20** as a result of M.L. 2020, First Special Session, Chp. 4, Sec. 2, legislative extension criteria being met.

**Project Status as of July 31, 2020:**
The laboratories of Dr. Novak and Dr. Hillmyer were closed due to the COVID-19 pandemic on March 18, 2020. Dr. Novak's lab was cleared for opening June 15, 2020 and Dr. Hillmyer's was cleared June 22, 2020. Once open, both labs were operated in such a manner as to keep the number of personnel present low, and therefore have not been operating at full capacity. This mode of low capacity operation is expected to continue through at least the end of 2020.

Fed-batch experiments containing zeolite-coated alumina hollow fiber membranes were completed. These experiments clearly demonstrated the ability of the zeolite-coated fibers to stimulate both ammonium and total nitrogen removal in biologically active reactors at rates statistically greater than that observed in reactors lacking bacteria and in reactors containing bacteria but lacking zeolite coatings on the alumina hollow fibers. In addition, molecular analysis showed enrichment of both anammox bacteria and denitrifying bacteria on the zeolite-coated hollow fibers. These bacteria were completely absent from the uncoated fibers and were present at statistically lower quantities in the reactor bulk liquid. These results clearly showed that the zeolite-coated hollow fibers were capable of stimulating biological nitrogen removal.

The manufacture of hybrid polyethylene-zeolite (PEZ) carriers has been optimized. Carriers can be created that contain ~45% zeolite by mass. These carriers have been shown to adsorb ammonium effectively, are mechanically robust, and have been generated in sufficient quantities to now be used for testing in bioreactors.

Finally, oxygen flux testing of the zeolite-coated and uncoated membranes is currently underway in a newly designed abiotic flow-through system and will continue until multiple membranes have been tested. These values will be used to calculate the number of membranes needed for operation in a biological flow-through reactor. This biological flow-through reactor will be expected to achieve complete nitrogen removal from wastewater without supplementing nitrite.

**Amendment Request (08/06/2020):**
The addendum is to formally request a re-budgeting of funds for this project.

Again, as reported last period, the supply costs for creating novel zeolite-attached alumina hollow fibers and also for monitoring total nitrogen concentrations in the reactors have been higher than originally anticipated because of the large number of experiments performed. This has resulted in additional analytical and material costs. Because of this a rebudget of $10,000 is requested, $8,000 from the "**Personnel**" category (Activity 2) to the "**Equipment/Tools/Supplies**" category (Activity 2), and $2,000 total from the "**Travel expenses in Minnesota**" category (Activities 1 and 2) to the "**Equipment/Tools/Supplies**" category (Activity 2).

The movement of money between sub-categories will not affect project objectives.

Amendment Approved by LCCMR 09/14/2020

**Project Status as of January 31, 2021:**
The additional batch experiment with an increased amount of zeolite-coated follow fiber membranes showed promising results with increased quantities of ammonia and total nitrogen removal, as previously mentioned. Nevertheless, there was unexpected, and apparently abiotic, transformation of nitrogen compounds in the

reactors with no biomass. The experiment was operated once more to determine if there was abiotic nitrogen transformation or removal occurring. By increasing the nitrogen loading to the system, the various nitrogen species were more easily monitored and it was clearly determined that abiotic nitrogen transformation occurred (nitrite transformation to nitrate), but there was no overall nitrogen loss from the system without biomass present. A manuscript describing these experiments has been written and is undergoing a final round of edits prior to submission for publication.

Novel zeolite-embedded carriers have been produced in high quantities for use in additional experiments, which was delayed as a result of COVID-related laboratory closures. The adsorption capacity of the carriers in an ammonium solution was determined and they will be tested in synthetic wastewater as well. Experiments are planned to test the carriers in biologically active wastewater reactors to determine their ability to encourage the colonization of anammox bacteria, and thereby, enhance total nitrogen removal.

Finally, experiments to determine the rate of oxygen transfer through the zeolite-coated membranes and to determine whether the membranes can support partial nitritation (ammonium to nitrite formation) are in progress. Improvements to the flow-through system and the mounting process for the membranes used for these experiments have been made. New measurements of gas transfer in the improved system are in progress. The incorporation of these mounted membranes into a wastewater system is currently being designed as well for follow-on experiments.

**Amendment Request (01/27/2021):**
The addendum is to formally request a re-budgeting of funds for this project.

Due to the additional time required to complete this research and several required redesigns of the reactors used, I am requesting that $6,359 be rebudgeted from the "**Personnel**" category (Activity 2) to the "**Equipment/Tools/Supplies**" category (Activity 2). The movement of funds between sub-categories will not affect project objectives.

Amendment Approved by LCCMR 02/05/2021

**Amendment Request 09/02/2021**
To balance the final budget, we request to move $750 from the Personnel category and add it to the Equipment/Tools/Supplies Category. This re-budget is needed because of slight changes between the anticipated supply costs and the actual supply costs that occurred with such a large project.

Amendment approved by LCCMR 09/27/2021

**Overall Project Outcomes and Results:**
Anammox bacteria have received attention for their ability to completely transform harmful reactive nitrogen compounds in wastewater into harmless dinitrogen gas. In addition, when using anammox bacteria, much less oxygen and no supplemental carbon is needed for nitrogen removal, and there is little production of excess biomass in the form of sludge. This reduces costs and energy use for nitrogen removal. It is estimated that the anammox process saves 60% of the energy used in conventional nitrogen removal. Unfortunately, this process has been difficult to implement in typical wastewater systems. Anammox bacteria are slow growing and the ammonium and carbon concentrations in wastewater result in low anammox activity and competition from faster growing bacteria. This leads to the washout of anammox bacteria. In this collaborative research project, our goals were to develop new polymeric materials that could concentrate ammonium to create localized niches for anammox enrichment and retention. We developed two different materials in this project: (1) a porous polymer carrier and (2) a gas-permeable alumina membrane. Both materials were able to concentrate ammonium, while the membrane could also transfer low quantities of oxygen to the surrounding solution. Both materials were also able to enrich and retain anammox when added to a wastewater environment. Further

optimization of these materials is needed to enable scale-up and deployment. Nevertheless, given that in the US, the energy used for wastewater treatment costs approximately $2B a year, the predicted energy savings if this technology was implemented would be significant. The impact within the state of Minnesota would also be large, saving millions of dollars and providing more complete removal of harmful nitrogen species. A patent was awarded and the University of Minnesota is exploring commercialization and licensing options. Three peer-reviewed manuscripts were published from this work and have been submitted to the LCCMR.

**IV. PROJECT ACTIVITIES AND OUTCOMES:**

**ACTIVITY 1:** Develop materials that promote selective colonization of anammox bacteria at their surface

**Description:**
An essential component of material development entails achieving high surface concentrations of ammonia to create an environment naturally selective for the proliferation and retention of anammox bacteria. Materials with different degrees of "stickiness" will also be tested to determine how to best retain anammox bacteria on the surface without simultaneously retaining unwanted bacteria. Our investigations will initially be conducted with commercially available materials, the surface of which will be modified with charged inorganic particles (so called "zeolites"). Additional efforts will focus on ways to incorporate charged particles or other charged chemical groups into novel, but inexpensive materials. The goal will be to develop materials that are inexpensive and scalable while also maintaining a surface that is highly selective for anammox bacteria.

**Summary Budget Information for Activity 1:** **ENRTF Budget: $ 184,900**
**Amount Spent: $ 184,900**
**Balance: $ 0**

| Outcome | Completion Date |
|---|---|
| **1.** *Modify commercially available materials with charged particles for ammonia concentration at the surface* | 12/31/17 |
| **2.** *Incorporate charged particles into novel materials for ammonia concentration at the surface* | 12/31/18 |
| **3.** *Develop novel materials containing charged chemical groups for ammonia concentration at the surface* | 2/28/19 |
| **4.** *Demonstrate anammox bacteria proliferation and retention on the various material surfaces* | 6/30/19 |

**Activity 1 Status as of January 31, 2018**:
A graduate student and postdoctoral researcher have been hired.

Initial experiments to coat membrane surfaces with zeolite particles have been performed. Polysulfone, widely used in the water and wastewater industry as a membrane material, and faujasite, a commercially available zeolite that readily adsorbs ammonium ions, were selected for use and were then fabricated in the laboratory to carefully control material properties. Without modification of the polysulfone membrane, zeolites were unable to attach to the membrane, resulting in poor to no zeolite particle coverage on the membrane surfaces. Zeolite coverage was improved with ultraviolet treatment of the membrane surfaces combined with silane-treatment of both the membrane and zeolite particles.

The zeolite-coated membranes were tested for durability, with mixed results. Sonication of coated membrane samples did not remove the layer of zeolite particles; physical disturbance of the surface did, however. Further material development will focus on improving the durability of the zeolite coating and on exploring imbedding zeolites into the membrane surface.

Methods have been developed to quantify specific bacteria in our system: total bacteria, anammox bacteria (via two different methods), aerobic ammonia oxidizing bacteria, and aerobic nitrite oxidizing bacteria. Quality assurance testing is nearly complete, enabling these methods to be applied with confidence to our membrane materials in the next reporting period.

A bench-scale wastewater reactor has been constructed and operated to test the membrane surfaces for preferential growth of anammox bacteria. The reactor is fed synthetic wastewater and was inoculated with a mixed bacteria community from a wastewater treatment plant and anammox-enriched biomass from a full-scale treatment plant in Virginia. The performance of the reactor, in terms of nitrogen and carbon removal, is being monitored and operation is being modified to support carbon removal but only limited aerobic ammonia transformation to nitrate and nitrite. This should enable anammox bacteria to thrive and should decrease the overall energy used in the reactor for aeration.

Racks have been constructed and printed on a 3-dimentional printer to facilitate membrane coupon deployment in the reactor, which will commence shortly. Initial experiments are currently being performed with beads of zeolite, glass, or polyethylene for proof of concept testing.

**Activity 1 Status as of July 31, 2018:**
We have prepared polymer supports (also used in the experiments described below, made with polyvinylidene fluoride, PVDF) with a hollow fiber geometry. Hollow fibers have a lot of advantages for use in biologically active systems, such as large surface to volume ratios and high production rates (50 meter/min maximum) that are suitable for industrial scale-up. This geometry also enables controlled air addition through the porous hollow fiber walls to support the annamox process. PVDF, as opposed to polysulfone, may have advantages regarding gas transfer across the membrane and zeolite attachment. We have also prepared an alumina hollow fiber material, with the plan that such a material would allow us to directly grow zeolite on the hollow fiber itself.

We have developed and tested a novel crosslinking post-treatment method to improve the affinity between the PVDF support mentioned above and inorganic zeolite, as well as the chemical and temperature stability of the final polymer-zeolite material. Another novel material was developed with the planned application of controlling oxygen permeability through the material (Activity 2). This material consists of graphene oxide (GO), a planar carbon- and oxygen-rich flake material, fabricated as a film directly on the crosslinked PVDF polymer support. The thickness of the GO layer can be controlled easily, which should facilitate control of oxygen delivery. We have also developed a polymer-based material in which zeolite is filtered onto the polymer membrane support, then covered by a second permeable polymer layer (polyamide) to hold it in place. Finally, we have successfully grown zeolite on the alumina hollow fiber materials and confirmed this via scanning electron microscopy.

Zeolite and zeolite-containing polymer have been, and are being, tested to determine the quantity of ammonium that can be adsorbed by the various materials. This data is being used to identify the most promising materials to use in reactor studies and determine the quantity of materials that need to be used to facilitate ammonium sorption and subsequent degradation.

Experiments are also being performed with synthetic wastewater and a mixed wastewater culture to determine how dynamic ammonium sorption by zeolite can alter the nitrogen degradation/loss patterns over time in a reactor. We have observed that over time zeolite appears to act as a “battery,” adsorbing the ammonium, then “discharging” it to the microorganisms, followed by additional sorption/discharge cycles. This facilitates total nitrogen removal via what appears to be the anammox process. No nitrogen removal is observed in the reactors without zeolite present. These experiments are providing critical information regarding how to deploy the zeolite-coated polymers and operate reactors over time for total nitrogen removal.

Finally, samples are being prepared for the "deep sequencing" of the microbial communities present so that the identity of the organisms in suspended versus attached growth anammox cultures can be determined and compared to those organisms that colonize the zeolite itself or zeolite-coated polymers in our experiments. This will provide information regarding how best to seed reactors containing our zeolite-coated polymers for the most rapid and effective colonization. This method is also being used to check our methods for accuracy.

**Activity 1 Status as of January 31, 2019:**
Ammonia sorption tests were performed on various zeolite particles and membranes upon which zeolites were embedded, attached, or grown. The alumina hollow fiber membranes with zeolite grown on the surface removed substantial quantities of ammonia compared to other zeolite-augmented membranes. These membranes were therefore selected for further testing in biologically active systems.

Batch reactor experiments containing zeolite particles (biologically active and abiotic) and glass particles were continued. During the first 30 days of the experiment, the total nitrogen removal was greatest in the reactors containing zeolite particles and biomass, as hypothesized. The zeolite particle reactors without biomass achieved high rates of ammonia removal via abiotic sorption, but did not achieve high rates of total nitrogen removal. The reactors containing only glass particles achieved the lowest total nitrogen removal. Samples from this experiment were submitted for the genetic sequencing of the bacteria present and are currently being analyzed via the Minnesota Supercomputing Institute.

A new batch experiment has been started with the alumina hollow fiber membranes. This experiment is being performed similarly to the batch experiment containing particles, with one set of reactors containing alumina hollow fibers with zeolite grown on the outside and the other set of reactors containing alumina hollow fibers without surface modification. This experiment has just started, but results are expected to be similar to those obtained with the particles present.
Finally, we have continued to explore the use of graphene oxide (GO)-coated amine cross-linked-PVDF (c-PVDF) hollow fibers with this system. We believe that, based on literature that shows that GO-coated filters can result in faster *Escherichia coli* growth, such a coated fiber could also improve the activity and retention of anammox bacteria. GO-coated c-PVDF fibers should also allow excellent tuning of the gas transfer properties of the fiber through control of the GO layers attached to the PVDF fiber. To this end, we have optimized the degree of amine cross-linking on the PVDF fiber, allowing for stable GO layer formation. In fact, the GO layer did not detach from the hollow fiber surface after 10 min of extensive sonication. The GO layer thickness upon the membrane support was also controllable. The effect of the GO layer thickness on oxygen transfer through the membrane will be tested in the future. These fibers will also be produced for use in additional biologically active experiments.

**Activity 1 Status as of July 31, 2019:**
With respect to progress on outcomes, we have found that commercially available membrane materials can be modified with zeolites (i.e., charged particles) for ammonia concentration (outcome 1; see Figure 1 below). We have also created novel materials that have been modified with zeolites, most notably the alumina hollow fibers upon which we have grown zeolite, and these are also capable of concentrating ammonia at the surface (outcome 2; again, see Figure 1 below). We are continuing to explore the application of these novel alumina hollow fibers (outcome 4, more on this below), as they can be easily generated, can be "tuned" to have different quantities of zeolite on their surface, and can be used for feeding air directly to the reactors through the fiber (more on this under Activity 2). We are also planning to continue creating novel materials for ammonia concentration for use with a variety of anammox-based applications, such as side-stream anammox treatment, with a focus on polymeric materials. Based on our results we have decided that novel materials containing charged chemical groups, as opposed to charged particles (i.e., zeolites) will not perform well, as they will not be sufficiently specific for ammonia concentration. We are therefore not pursuing outcome 3 further.

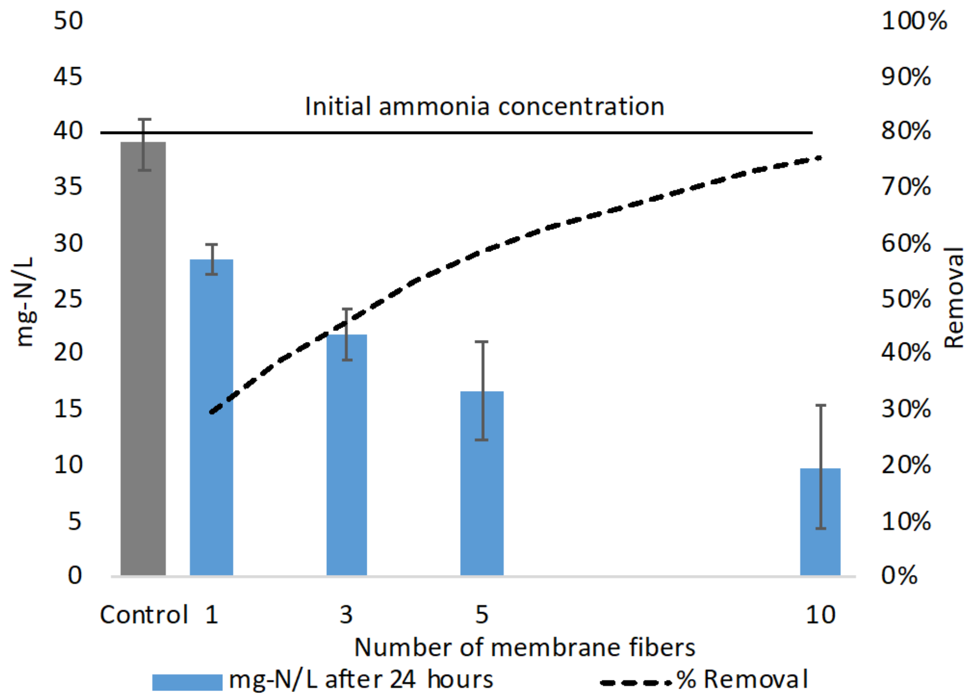


*Figure 1.1. Ammonia removal from synthetic wastewater with zeolite coated alumina hollow fibers*

An initial batch experiment with hollow fibers showed that fibers with zeolite grown on the outside did out-perform the control reactors containing plain alumina hollow fibers, but only after several months of operation. Although the reactors containing these zeolite-modified alumina fibers outperformed the control no-zeolite alumina fibers, the reactors never achieved desired rates of ammonia and/or total nitrogen removal. To achieve better removal rates, more fiber was clearly needed. To determine how much additional fiber is needed, additional batch reactor experiments are being performed with treatments containing a varying mass of zeolite particles to optimize the amount of zeolite needed to achieve high nitrogen removal rates. These reactors are biologically active and use pieces of alumina fibers as the control. We have found that the mass of zeolite can be decreased by a factor of 6 from previous particle experiments and still achieve significant ammonia and total nitrogen removal; the mass of zeolite, however, needed to be increased by a factor of about 5 over previous fiber experiments. The information gathered from this experiment is being applied to current batch experiments with zeolite-modified hollow fiber membranes; these reactors are expected to achieve higher total nitrogen removal rates compared to the previous fiber experiment. These experiments support both outcomes 3 and 4, allowing us to identify the quantity of material that needs to be added to a reactor to achieve a desired outcome with respect to total nitrogen removal.

We have also continued to explore the oxygen transfer capability of graphene oxide (GO)-coated amine cross-linked-PVDF (c-PVDF) hollow fibers. This was initially estimated via the transfer of water across the fiber, which was a function of the GO layer thickness. As the GO layer varied in thickness from about 20 nm to 2µm, the water transfer rate decreased, reaching 4.5 $L/m^2 \cdot h \cdot bar$ with the 90 nm thick GO layer and no transfer at all at with the 2 µm thick GO layer. Based on additional chemical rejection data it was also determined that a uniform GO layer was deposited on the PVDF fiber and that when optimized, the GO layer thickness could be used to control oxygen transfer across the membrane.

Finally, we are currently exploring the effect of pre-seeding zeolite-modified fibers with anammox bacteria to determine if this can decrease the start-up period of reactors. As of now, the pre-seeded zeolite-modified and no-zeolite fibers perform similarly. This is as expected. We expect the performance of the no-zeolite fibers to deteriorate over time while that of the zeolite-modified fibers remains constant. This will be determined by the next update in January 2020.

**Activity 1 Status as of January 31, 2020:**
Batch experiments with an optimized quantity of zeolite-coated hollow fiber were designed based on results from the varying mass of zeolite particle experiment described previously. The biologically active batch reactors with zeolite-coated hollow fiber membranes outperformed the control reactors, which contained uncoated alumina hollow fibers, resulting in lower ammonia effluent concentrations (see Figure 2 below). While the zeolite-coated membrane reactors outperformed the control reactors, higher rates of ammonia removal are desired. An additional experiment with increased quantities of fiber will be performed to demonstrate even higher rates of removal.

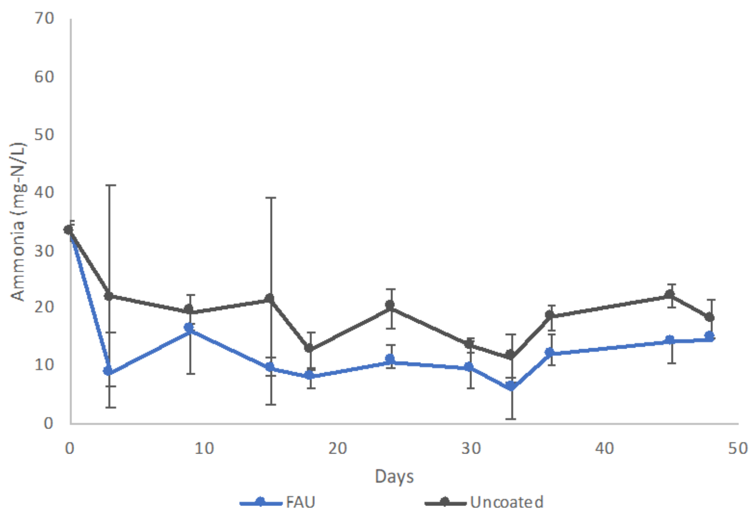


*Figure 1.2. Ammonia effluent concentrations from biologically active batch reactors with zeolite-coated (labeled "FAU" for faujasite-type zeolites) and uncoated alumina hollow fibers. It can be seen that the faujasite-coated fibers removed more ammonia than the uncoated fibers over the course of the experiment.*

Batch reactor experiments with anammox pre-seeded zeolite-coated and uncoated alumina hollow fibers were operated. While quick ammonia removal was demonstrated in both systems, the systems with zeolite-coated membranes were not significantly different from the control systems with uncoated membranes. This is believed to be a result of low quantities of membrane in the systems. Increased quantities of membrane are needed to achieve higher rates of nitrogen removal as shown by previous experiments.

Zeolite-coated carrier development is currently underway. Polyethylene carriers are commonly used to support biofilm growth in biological nutrient removal systems, including sidestream treatment systems in which anammox are active. Zeolite-coated carriers are expected to increase the rate of nitrogen removal and make anammox-supporting carriers feasible for nitrogen removal from mainstream wastewater treatment. We have demonstrated that pellet shaped "sponges" of polyethylene with zeolite on the surface can be synthesized. These initially developed carriers include carriers with no zeolite (control), 10% zeolite, and 20% zeolite. These carriers have been tested for ammonia removal and results are shown in the figure below. Further investigation will be conducted to determine an ideal carrier shape and the maximum mass of zeolite that can be attached to the carrier surface. The carriers will be tested in a biologically active system.

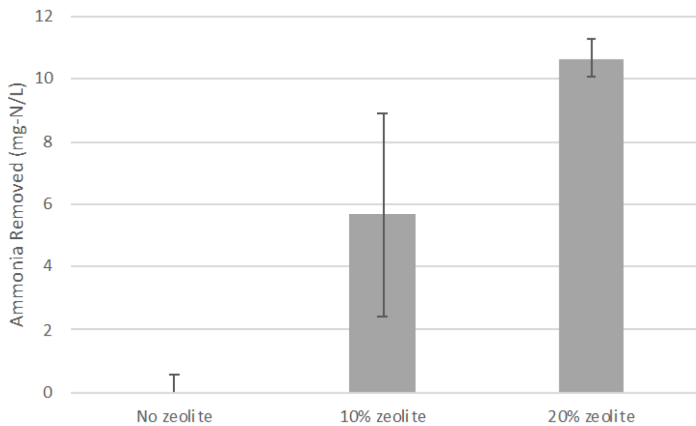


*Figure 1.3. Ammonia removal after 24 hours by sorption to carriers containing different quantities of zeolite on their surface (no zeolite, 10% zeolite by mass, and 20% zeolite by mass).*

**Activity 1 Status as of July 31, 2020:**
As indicated previously, earlier experiments with the zeolite-coated membrane reactors outperformed the control reactors, but higher rates of ammonia removal were desired. An additional experiment with increased quantities of fiber was performed to achieve higher rates of removal. Figure 1.4 below shows the effluent ammonia concentrations in an additional experiment to which zeolite-coated and uncoated fibers were added. Continuous ammonia removal over time indicates that the zeolite-coated fibers were capable of stimulating biological ammonia removal. In addition, molecular analysis showed that anammox bacteria and denitrifying bacteria were enriched on the zeolite-coated fibers but were not present on the uncoated fibers, demonstrating that the zeolite-coated fibers were capable of creating a localized niche that was favorable for anammox and denitrifying bacteria (data not shown).

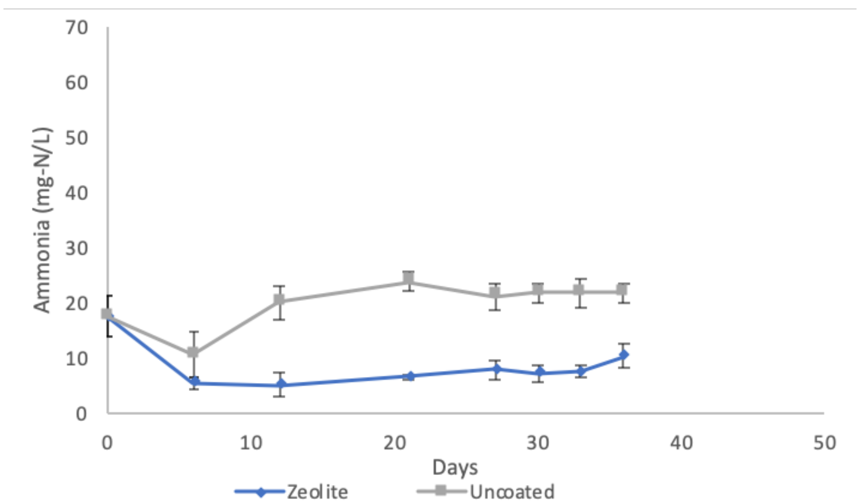


*Figure 1.4. Ammonia effluent concentrations from experiments containing wastewater bacteria and either zeolite-coated or uncoated alumina hollow fibers. It can be seen that the zeolite-coated fibers removed significantly more ammonia than the uncoated fibers over the course of the experiment, which was a result of the stimulation of microbial ammonia removal.*

An optimal route for the manufacture of hybrid polyethylene-zeolite (PEZ) carriers has been identified. Melt blending of polyethylene (PE), polyethylene oxide (PEO) and zeolite (Z), followed by removal of the sacrificial PEO phase by solvent etching in water, was successful for creating these hybrid carriers. Process-property relationships were explored by manufacturing a series of 16 different PE-PEO-Z formulations, varying the PEO and Z loadings relative to the PE matrix. Three critical formulations were identified for scale-up, with one of the formulations containing ~45% zeolite by mass in the finished carrier. The microstructure of this carrier is shown in Figure 5. Carriers for further testing were produced as 11-mm diameter disks with a 1.5 mm thickness.

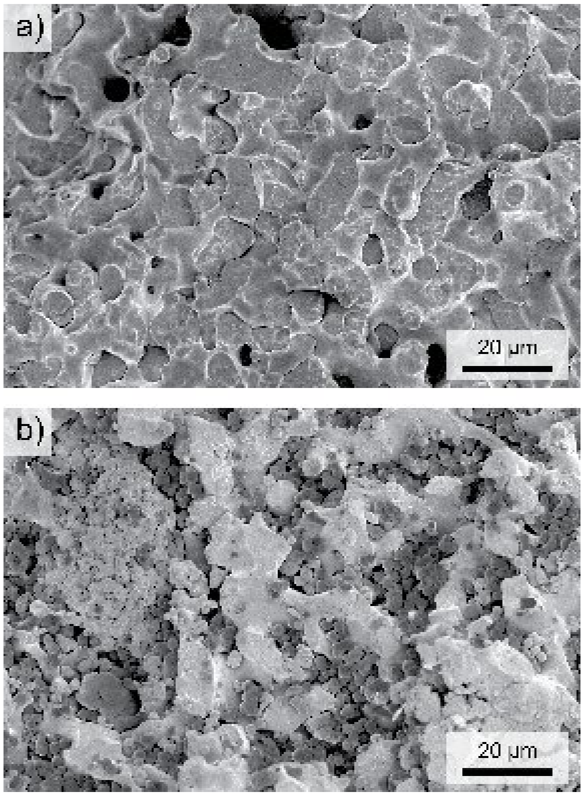


*Figure 1.5. Scanning electron micrographs of the 45% zeolite carrier before (a) and after (b) solvent etching in water to remove the PEO template. The continuous pore structure with physically entrapped particulate zeolite (visible as small darker-toned particles) is clearly visible after etching.*

The ammonium sequestration/adsorption efficacy of the different carrier materials as well as carriers containing no zeolite, was tested by placing them in a solution of water and ammonium (Figure 6). The removal efficacy of the carriers was highly reproducible.

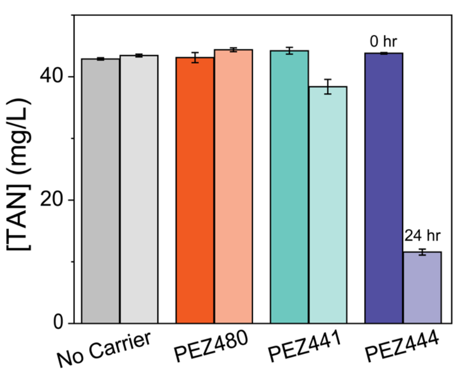


*Figure 1.6. Ammonium removal from 45 mg/L ammonium solutions after 24 hours. The carrier labeled "PEX480" contained no zeolite. The carrier labeled "PEZ441" contained a low quantity of zeolite and the carrier labeled "PEZ444" contained 45% by mass zeolite. The concentrations shown represent the average of three separate experiments, with the error bars representing the standard deviation.*

At this stage, the ammonium sequestration/adsorption efficiency and kinetics for the different carriers have been fully characterized. The carriers are mechanically robust, with consistent performance across multiple samples. A manuscript describing the manufacture and characterization of the PEZ carriers is in preparation. Next steps will involve testing the carriers in the bioreactors.

**Activity 1 Status as of January 31, 2021:**
Enhanced ammonia and total nitrogen removal was observed in reactors containing zeolite-coated alumina hollow fiber membranes plus biomass. Enrichment and retention of anammox bacteria was also observed on the zeolite-coated membranes.

Two sets of control reactors were also analyzed for these reactors: (1) "uncoated" controls, in which uncoated alumina hollow fiber membranes and biomass were present and (2) "abiotic" controls, in which zeolite-coated membranes were present but there was no biomass in the reactors. In the abiotic controls, it was expected that the ammonia would be removed by adsorption to the zeolite, but loss of no other nitrogen species was expected. Nevertheless, the loss of nitrite was observed in the abiotic control reactors. To further determine the fate of all of the nitrogen species in the absence of biomass, this experiment was repeated again with increased influent nitrogen concentrations to facilitate determining the fate of all of the amended nitrogen species. Abiotic zeolite-facilitated nitrite transformation to nitrate was observed in these experiments, but total nitrogen removal did not occur. Total nitrogen removal did occur in the biologically active zeolite-coated membrane reactors (Figure 7).

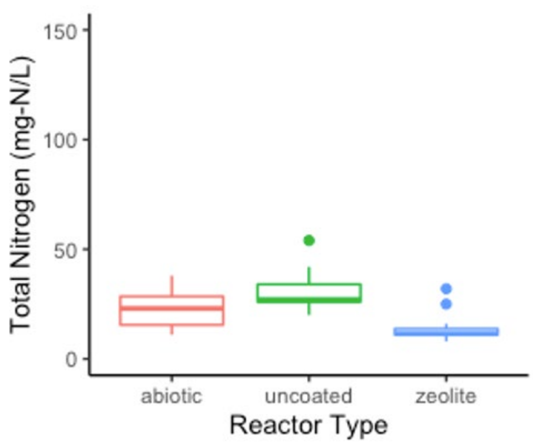


*Figure 7. Effluent Total Nitrogen Concentrations from the batch reactor membrane experiments.*

The zeolite-coated carriers discussed previously were produced in large quantities for further testing. Ammonia removal was characterized thoroughly using a standard ammonium chloride solution and further characterization of ammonium adsorption in the presence of other compounds (e.g., in synthetic wastewater) is in progress. Design of laboratory-scale wastewater experiments in the presence of these carriers is also in progress to determine to what extent they are capable of enhancing the colonization and enrichment of anammox bacteria. The final design will incorporate the additional adsorption information from testing the carriers in wastewater.

**Final Report Summary:**
In the final project period zeolite-containing polyethylene carriers were tested in small lab-scale reactors for enhanced total nitrogen removal as well as their ability to enhance the colonization and enrichment of anammox bacteria over time. Reactors were constructed and operated with a "mainstream" synthetic wastewater containing nitrite to eliminate the need for aeration. During the 50 days of operation, the reactors with the zeolite-coated carriers had higher rates of both ammonia removal and total nitrogen removal compared to the reactors with "uncoated" carriers. In fact, the uncoated carrier reactors had essentially no ammonia removal (average -4%) while the zeolite-coated carrier reactors saw an average of 59% ammonia

removal. Additionally, the total nitrogen removal of the zeolite-coated carrier reactors was 1.5 times that of the uncoated carrier reactors. Both reactors demonstrated removal of nitrite, but this is also a result of denitrification, which readily occurs under anaerobic conditions in mainstream wastewater systems, not only to the anammox process. Additional analysis was performed to determine the quantities of bacteria in the system. While the systems with the zeolite-coated carriers had more anammox bacteria present, the quantities were not statistically different, indicating that the bacteria were perhaps more abundant, but additional replicates would be needed to demonstrate this, as well as more active.

In this project, novel porous polyethylene carriers containing zeolite in the pores as well as novel alumina hollow fiber membranes upon which a layer of zeolite was grown were both developed. The creation of such hollow fibers and carriers has been patented. These materials were shown to sorb ammonium in a complex simulated wastewater solution containing organic carbon and a variety of salts. These materials, when seeded with anammox bacteria and a mixed activated sludge culture were able to enhance the colonization and enrichment of anammox bacteria on their surfaces. When fed synthetic wastewater the presence of these carriers or fibers in a biologically active environment the enhanced loss of total nitrogen from solution was observed. These carriers and fibers are very promising for the enhancement of total nitrogen removal in a low-oxygen environment in existing wastewater treatment plants and should enable intensification of biological nitrogen removal, facilitating more overall nitrogen removal with the provision of less oxygen and no supplemental carbon over time. This work also clearly demonstrated that an important factor in achieving high rates of nitrogen removal and anammox retention is the quantity of zeolite or zeolite-coated material relative to the ammonia concentration in the feed/wastewater influent. Given the experimental nature of the project, these carriers or fibers have not been tested at a large scale, but we are interested in pursuing additional funding to scale up testing of these materials.

Future work beyond the scope of this project will focus on determining whether zeolite does in fact increase anammox bacteria activity, as this would suggest that incorporating zeolite into a system could immediately increase anammox activity. This immediate increase in total N removal is consistent with our experimental results. Additionally, incorporating aeration into the system instead of supplementing nitrite would bring this technology a step closer to application outside the laboratory. The limited time in the laboratory due to the pandemic prevented the exploration of aeration in this system as well as longer-term operation of the system.

**ACTIVITY 2:** Optimization of materials for easily controlled air addition/delivery to support the anammox process

**Description:**
The anammox process requires that very small quantities of air be delivered to the organisms present. To accomplish this, materials will be incorporated into a hollow tube configuration, using the material properties themselves to control the air addition to the surrounding wastewater/microbial culture. We will explore the development of materials (*i.e*., material thickness, permeability to gas) and configurations (*i.e*., "jellyfish" bundle packing, spacing, number of hollow tubes present) that facilitate simple controlled air addition to the wastewater. Eventually, the material used will combine the attributes of those materials developed in Activity 1 to attract and retain the anammox bacteria with those developed in Activity 2 to easily modulate oxygen addition to the system. This will facilitate the anammox process without the need for complex external (i.e., operator) process control.

**Summary Budget Information for Activity 2:**

| | |
|---|---|
| **ENRTF Budget:** | **$ 265,100** |
| **Amount Spent:** | **$ 265,100** |
| **Balance:** | **$ 0** |

| Outcome | Completion Date |
|---|---|
| **1.** *Determine how material characteristics (thickness, diameter, material permeability to gas, material choice) control the diffusion of air across the tube wall to the wastewater* | 6/30/19 |
| **2.** *Optimize the bundle configuration for predictable and controlled air delivery* | 1/31/20 |
| **3.** *Demonstrate robust anammox activity in the absence of complex process control* | 6/30/20 |

**Activity 2 Status as of January 31, 2018**:
No work has begun on Activity 2.

**Activity 2 Status as of July 31, 2018:**
Minimal work has begun on Activity 2 with supplies purchased to enable hollow fibers to be produced (see above also). Work on this activity is expected in the next year.

**Activity 2 Status as of January 31, 2019:**
Work has now commenced using hollow fibers (see above, Activity 1). Although oxygen addition via the hollow fibers has not yet begun, the system has been tested for its ability to deliver oxygen and is capable of doing so. In addition, experiments with fibers to which GO layers are adhered have been performed with the goal of using this to control oxygen delivery through the fiber.

**Activity 2 Status as of July 31, 2019:**
Gas permeation through plain and zeolite-modified ("FAU") alumina hollow fibers has been measured. As the thickness of zeolite grown on the fibers increases, the gas permeation of the fibers decreases. By varying the thickness of the zeolite layer, the delivery rate of oxygen can be controlled; this can be controlled through a simple change in the time taken to grow the zeolite layer (see Table 2.1 below), making this easy to control. These experiments are in support of outcome 1.

*Table 2.1. Time-lag dead-end single-gas ($O_2$ and $N_2$) permeance across zeolite-modified alumina hollow fiber membranes at 25°C.*

| | Bare alumina hollow fiber [mol/m²·pa·s] | FAU layer grown alumina hollow fiber, 24 h growth [mol/m²·pa·s] | FAU layer grown alumina hollow fiber, 48 h growth [mol/m²·pa·s] |
|---|---|---|---|
| **$N_2$** | $9.24\times10^{-6}$ | $4.37\times10^{-9}$ | $9.81\times10^{-10}$ |
| **$O_2$** | $9.12\times10^{-6}$ | $4.18\times10^{-9}$ | $9.14\times10^{-10}$ |

Oxygen addition (in the form of air addition) through plain alumina follow fibers has been tested in biologically active batch experiments with zeolite particles to provide a base case, from which to modify the fiber permeability via zeolite growth. This is in support of outcome 2. The amount of fiber in each reactor was varied and performance, in terms of ammonia and nitrogen removal, was determined. While all the reactors achieved high rates of nitrogen removal, the reactor with the most membrane achieved the highest rate of nitrogen removal. It should be noted that these experiments have not yet been run in triplicate, and this is planned for the near future. Further experiments will be operated to optimize the amount of membrane fiber needed in the reactors (again, in support of outcome 2). Next steps also include testing oxygen addition through the zeolite-modified alumina hollow fibers in similar biologically active reactors.

**Activity 2 Status as of January 31, 2020:**
While previous experiments demonstrated that ammonia removal can be achieved through plain hollow fibers and the thickness of zeolite can control the flux of oxygen, further testing is needed to determine the rate of oxygen flux through fibers submerged in water. A system has been designed to accurately measure the oxygen transfer of membranes in water. This is needed to properly design flow-through experiments and to ensure adequate oxygen control. Once membrane oxygen flux rates have been determined, biological flow-through reactors will be designed and tested for enhanced nitrogen removal. Initial calculations have been performed to

determine the target oxygen delivery rate and hydraulic retention time required for ammonia removal at concentrations found in mainstream wastewater.

**Activity 2 Status as of July 31, 2020:**
Oxygen flux testing of the zeolite-coated and uncoated membranes is currently underway in a newly designed abiotic flow-through system. It appears that the oxygen flux through the zeolite membranes, as expected, is lower than that of the control membranes, as shown in Figure 2.1 below. Oxygen flux rate testing will continue until multiple membranes have been tested. These values will be used to calculate the number of membranes needed for operation in a biological flow-through reactor. This biological flow-through reactor will be expected to achieve complete nitrogen removal from wastewater without supplementing nitrite.

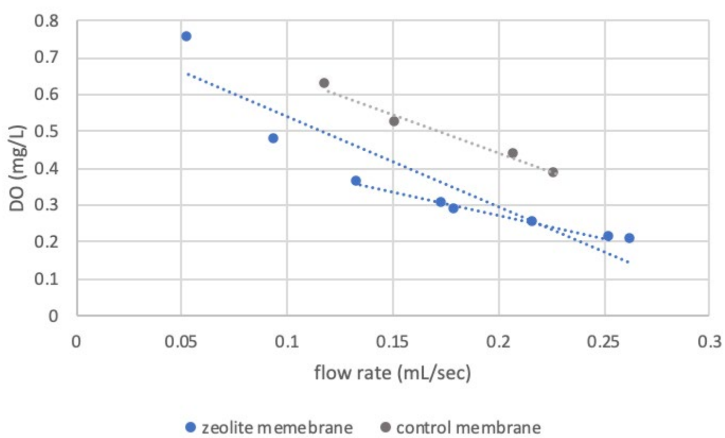


*Figure 2.1. Result from oxygen-flux testing of zeolite and uncoated membranes in a flow-through system.*

**Activity 2 Status as of January 31, 2021:**
Testing of the oxygen permeance through the membranes in water is still in progress. The previously designed system has been improved to include a flow-through oxygen membrane system instead of a dead-end system, which is better-suited to measuring these small quantities of oxygen transfer. Additionally, a system to secure multiple membranes in the flow-through reactor has been designed and tested. This allows for optimization of oxygen delivery in the system by controlling the number of membranes present. Additional experiments to test the membranes in a biologically active system to determine whether they can support partial nitritation is also underway and will incorporate the permeance results.

**Final Report Summary:**
In the final project period, the permeance of zeolite-coated alumina hollow fibers was determined. The ability of these fibers to facilitate ammonium oxidation through oxygen delivery was also tested in a biologically active system. Initially only one fiber was used in each reactor and no ammonia removal was observed. The experiment was repeated with more fiber per reactor (4 and 10 membranes) and ammonia removal was observed. Ammonia removal appears to be dependent on the rate at which the liquid flows through the reactor (the hydraulic retention time) as well as quantity of fiber in the system. Although this process needs to be optimized, these results suggest that simultaneous partial nitritation (partial ammonium transformation to nitrite) and anammox (ammonium plus nitrite transformation to harmless and non-reactive dinitrogen gas) should be possible with these, or similar fibers.

More work is needed in the future to optimize oxygen delivery (pulsing versus continuous oxygen supply) to support both the partial nitritation and anammox processes simultaneously. More work is also needed to develop less brittle membranes that are capable of robust performance in a turbulent wastewater environment. While aeration was successful with the alumina hollow fibers, exploration of additional porous hollow fiber materials is needed because of the brittleness of the alumina and its low permeance. While the low permeance is helpful for precisely controlling oxygen delivery, developing additional materials with a range of oxygen permeance would facilitate a larger variety of deployment configurations. In addition, fibers to which graphine oxide layers were adhered were developed, which should have applications for controlling gas transfer across such fibers and improving filtration efficiency, which should be useful for future applications as well. Through this research, the groundwork has been laid to facilitate total nitrogen removal in existing wastewater treatment plants, again facilitating the intensification of biological nitrogen removal.

**V. DISSEMINATION:**

**Description:**
The target audience for results from this research will be professionals in the area of wastewater treatment and industry. Specific targets will be industries such as Dow Chemical/Filmtec and GE Water, environmental engineers and scientists in academia, industry, state/local government and agencies such as the MDA and MPCA, and environmental consultants. Results will be disseminated through scholarly publications in peer-reviewed journals such as *Environmental Science and Technology* and the *Journal of Membrane Science.* Results from the research project will also be presented at regional conferences such as the *Conference on the Environment*. Results will be used to further scale up this technology and implement it for the treatment of wastewater.

We plan to file an Intellectual Property Disclosure with the Office of Technology Commercialization at the University of Minnesota on the proposed technology. If the University of Minnesota pursues patent protection, this could lead to potential income for the state. Therefore, information contained in the Research Addendum is confidential.

**Status as of January 31, 2018**:
No dissemination efforts have been made, as the project is not advanced enough at this point.

**Status as of July 31, 2018:**
A provisional patent has been filed on this work through the University of Minnesota's Office for Technology Commercialization. No other dissemination efforts have been made at this early stage of the research.

**Status as of January 31, 2019:**
A presentation was made at the regional Conference on the Environment.

**Status as of July 31, 2019:**
A poster was presented at the annual Association of Environment Engineering and Science Professors. A non-provisional patent application was submitted June 6, 2019. Two papers are being prepared, one on the GO-modified membranes and one on the overall idea and performance of the system.

**Status as of January 31, 2020:**
A presentation was made at the National Water Environment Federation Conference ("WEFTEC") in Chicago in September. One paper has been submitted for publication on the GO-modified membranes. A second paper is being written on the zeolite-modified membranes.

**Status as of July 31, 2020:**
A manuscript detailing the manufacture of PE-Zeolite hybrid carriers and their ammonium sequestration abilities is in preparation. Anticipated submission timeline is August-September 2020. Another manuscript describing the ability of the zeolite and zeolite-coated membrane fibers to stimulate total biological nitrogen removal and enrich for anammox bacteria is also being prepared and should be submitted in August-September 2020 as well. Presentations that were planned were canceled as a result of the COVID-19 pandemic.

**Status as of January 31, 2021:**
The manuscript detailing the manufacture of PE-Zeolite hybrid carriers and their ammonium sequestration abilities was submitted and is currently being revised to address reviewer comments. A second manuscript describing the zeolite-coated alumina hollow fiber membranes and their ability to enhance ammonia and total nitrogen removal and retain and enrich anammox bacteria has been prepared. Submission of the manuscript is anticipated in early February 2021 once final co-author comments are incorporated.

**Final Report Summary:**
Information from this project has been shared with several water technology companies who may be able to assist in optimizing and eventually deploying this technology. Three papers have been published from this research (listed below and sent to LCCMR with the final report). An additional 1-2 manuscripts are expected on the performance of the carriers in a biologically active environment and on the ability of the zeolite-coated alumina hollow fibers to support partial nitritation. Copies of these manuscripts will be sent to LCCMR once in press. A patent was granted on the technology (see below).

Eum, K., Kim, D. W., Choi, Y., Duan, X., Hillmyer, M. A., & Tsapatsis, M. (2020). Assembly of Graphene Oxide Nanosheets on Diamine-Treated PVDF Hollow Fiber as Nanofiltration Membranes. *ACS Applied Polymer Materials*. 2:3859-3866.

Huff Chester, A. L., Eum, K., Tsapatsis, M., Hillmyer, M. A., & Novak, P. J. (2021). Enhanced Nitrogen Removal and Anammox Bacteria Retention with Zeolite-Coated Membrane in Simulated Mainstream Wastewater. *Environmental Science & Technology Letters*. 8(6):468-473.

Feinberg, E. C., Huff Chester, A. L., Novak, P. J., & Hillmyer, M. A. (2021). Porous Polyethylene-Supported Zeolite Carriers for Improved Wastewater Deammonification. *ACS ES&T Engineering*. 1(7):1104-1112.

Novak, P. J., Romero-Vargas Castrillon, S., Huff, A., Hillmyer, M., Tsapatsis, M. Systems and Methods for Treating Wastewater, United States Patent No. 16433915.

**VI. PROJECT BUDGET SUMMARY:**

**A. Preliminary ENRTF Budget Overview:**

| Budget Category | $ Amount | Overview Explanation |
|---|---|---|
| Personnel: | $385,000 | Novak (PI) ($31,900), Romero-Vargas Castrillon (co-PI) ($27,800), and Tsapatsis (co-PI) ($22,200) budgeted for 4, 6, and 2% time per year for three years, for Novak, Romero-Vargas Castrillon, and Tsapatsis, respectively, salary 75% of cost, fringe benefits 25% of cost. Co-PI Hillmyer will not take salary on the project. PI and co-PIs will provide project supervision, guidance on the experimental aspects of the project along with guidance on data analysis. A graduate student researcher ($132,000), postdoctoral researcher ($162,100), and undergraduate researcher ($9,000) are also budgeted for three years (72% salary, 14% tuition (for graduate student only), 14% fringe |

| | | |
|---|---|---|
| | | benefits). The students and postdoctoral researcher will perform laboratory experiments. |
| Equipment/Tools/Supplies: | $63,000 | Laboratory supplies include, but not limited to: chemicals for membrane construction, chemicals for bacterial culture, gas tanks for the air supply to the membrane flow, analysis needs such as standards, gas tanks, needles, and septa, supplies for bacterial enumeration and identification, and consumables such as gloves and solvents ($17,000/yr, for a total of $51,000). Additional funds are budgeted for equipment repair and maintenance ($6,000) and laboratory services for microbial quantification and identification ($6,000). |
| Travel Expenses in MN: | $2,000 | Mileage charges to Metropolitan Council wastewater facilities for wastewater collection to feed reactors. Mileage will be reimbursed $0.55 per mile or current U of M compensation plan. |
| **TOTAL ENRTF BUDGET:** | **$450,000** | |

**Explanation of Use of Classified Staff:** N/A

**Explanation of Capital Expenditures Greater Than $5,000:** N/A

**Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation:**
Novak, Romero-Vargas Castrillon, and Tsapatsis will represent 0.04, 0.06, and 0.02 FTE per year, respectively (for 0.12, 0.18, and 0.06 FTE each over the entire 3-year project period). Half of a graduate student researcher (for 1.5 FTE over the entire 3-year project period), one postdoctoral researcher (for 3 FTE over the entire 3-year project period), and a 14% of an undergraduate researcher (for 0.42 FTE over the entire 3-year project period) will be employed with this appropriation per year. This results in a total of 5.28 FTE for the total project.

**Total Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation:**
None.

**B. Other Funds:** N/A

**VII. PROJECT STRATEGY:**

**A. Project Partners:**
**Partners receiving ENRTF funding**

- *Paige Novak, Professor, University of Minnesota, $31,900, Role: Principal Investigator. Novak will oversee the project and direct the testing of microbial colonization and determination of anammox activity.*
- *Santiago Romero-Vargas Castrillon, Assistant Professor, University of Minnesota, $27,800, Role: Co-principal Investigator. Romero-Vargas Castrillon is an expert in material development and will focus on Activity 1.*
- *Michael Tsapatsis, Professor, University of Minnesota, $22,000, Role: Co-principal Investigator. Tsapatsis is also an expert in material development and will work on both Activity 1 and 2.*

**Partners NOT receiving ENRTF funding**

- *Marc Hillmyer, Professor, University of Minnesota, Role: Co-principal Investigator. Hillmyer is an expert in material chemistry and will focus on Activity 2.*
- *The Metropolitan Wastewater Treatment Plant in St. Paul, MN will provide wastewater for the project.*

**B. Project Impact and Long-term Strategy:**
The proposed work fits into a larger research agenda centered at UMN focused on the development of new treatment technologies for water and wastewater. The proposed research complements current and prior research in this area. There are likely to be additional nitrogen removal requirements for wastewater treatment plants in the future. Current technology for the removal of nitrogen is energy and resource intensive. The proposed effort is focused on enabling inexpensive, low-energy nitrogen removal in the absence of complex process control.

**C. Funding History:** N/A

**VIII. REPORTING REQUIREMENTS:**

- **The project is for 4 years, will begin on 07/01/17, and end on 06/30/21.**
- **Periodic project status update reports will be submitted January 31 and July 31 of each year.**
- **A final report and associated products will be submitted between June 30 and August 15, 2021**.

**IX. VISUAL COMPONENT or MAP(S):** N/A

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

**Environment and Natural Resources Trust Fund**
**M.L. 2017 Final Project Budget**

**Project Title:** Wastewater Nitrogen Removal Technology to Protect Water Quality
**Legal Citation: M.L. 2017, Chp. 96, Sec. 2, Subd. 04b**
**Project Manager:** Paige Novak
**Organization:** University of Minnesota
**M.L. 2017 ENRTF Appropriation:** $450,000
**Project Length and Completion Date:** 4 Years, June 30, 2021
**Date of Report:** August 11, 2021

| ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET | Activity 1 Budget 2/5/21 | Amount Spent | Activity 1 Balance | Amended Activity 2 Budget 9/2/21 | Amount Spent 06/30/21 | Activity 2 06/30/21 Balance | TOTAL BUDGET | TOTAL BALANCE |
|---|---|---|---|---|---|---|---|---|
| **BUDGET ITEM** | Develop materials that promote selective colonization of anammox bacteria at their surface | | | | | | | |
| **Personnel (Wages and Benefits) Overall** | $153,400 | $153,400 | $0 | $188,490 | $188,490 | $0 | $341,890 | $0 |
| Paige Novak (PI, 4% time per year for three years, salary 75% of cost, fringe benefits 25% of cost) Est. $31,900 | | | | | | | | |
| Santiago Romero-Vargas Castrillon (co-PI, 6% time per year for three years, salary 75% of cost, fringe benefits 25% of | | | | | | | | |
| Michael Tsapatsis (co-PI, 2% time per year for three years, salary 75% of cost, fringe benefits 25% of cost) Est. $22,200 | | | | | | | | |
| Graduate Research Assistant (50% time per year for three years, 57% salary, 33% tuition, 10% fringe benefits) Est. $132,000 | | | | | | | | |
| Undergraduate Research Assistant (approximately 300 hours per year for three years)Est. $9,000 | | | | | | | | |
| Postdoctoral Researcher (100% time per year for three years, 82% salary, 18% fringe benefits)Est. $162,100 | | | | | | | | |
| **Equipment/Tools/Supplies** | $31,500 | $31,500 | $0 | $76,610 | $76,610 | $0 | $108,110 | $0 |
| Laboratory supplies include, but not limited to: chemicals for membrane | | | | | | | | |
| **Travel expenses in Minnesota** | $0 | $0 | $0 | $0 | $0 | $0 | $0 | $0 |
| Mileage charges to Metropolitan Council wastewater facilities for wastewater collection to feed reactors. Mileage will be reimbursed $0.55 per mile or current U of M compensation plan | | | | | | | | |
| **COLUMN TOTAL** | **$184,900** | **$184,900** | **$0** | **$265,100** | **$265,100** | **$0** | **$450,000** | **$0** |