SLATT UNDERGRADUATE RESEARCH FELLOWSHIP
FINAL REPORT

SCHOLAR NAME:	Jacob Novitch
FACULTY ADVISOR:	Dr. Robert Nerenberg
PROJECT PERIOD:	Spring 2021-Fall 2021
PROJECT TITLE:	Effect of Hydroxylamine on the Structure and Function of Nitrifying Biofilms
CONNECTION TO ONE OR MORE ENERGY-RELATED RESEARCH AREAS (CHECK ALL THAT APPLY):	**(x) Energy Conversion and Efficiency ( ) Sustainable and Secure Nuclear ( ) Smart Storage and Distribution ( ) Transformation Solar (x) Sustainable Bio/Fossil Fuels ( ) Transformative Wind**

MAJOR GOALS AND ACCOMPLISHMENTS

Summarize your research goals and provide a brief statement of your accomplishments (no more than 1-2 sentences). Indicate whether you were able to accomplish your goals by estimating the percentage completed for each one. Use the next page for your written report.

RESEARCH GOALS	ACTUAL PERFORMANCE AND ACCOMPLISHMENTS	% OF GOAL COMPLETED
Establish a Baseline Microbial Community	The anoxic biofilm reactor reached steady state without the addition on chemicals, after which a DNA extraction was performed to provide insight into the control microbial community	100%
Examine Effects of Perchlorate Addition on Microbial Community	Perchlorate was added to the biofilm through the membrane lumen, after which the community was allowed to reach steady state as in the baseline phase. Another DNA extraction was performed to allow comparison on the two microbial communities. The results of this DNA extraction showed an enrichment of perchlorate reducing bacteria, as was hypothesized. These results were used as proof of concept for future addition of different performance enhancing chemicals through the membrane lumen.	100%
Examine Effects of Predation Inhibitor on Nitrifying Biofilm	For this phase of the project, cycloheximide, an inhibitor for protozoan predators in the biofilm, was added through the membrane lumen in the same manner as chlorate in the preceding phase after a control steady state community was established. As expected, based on the chlorate results, the reactor performance increased, as evidenced by lower effluent COD and ammonia levels upon the addition of cycloheximide, indicating that the protozoan predators were successfully inhibited by the cycloheximide introduced through the membrane lumen.	100%
Publication and Presentation of Results	At the conclusion of this phase of the project, work which is currently ongoing began on the presentation of the findings from phases 1 and 2 at several conferences and the drafting of a journal article for future publication. The initial findings of this work have already been presented at a December 2021 Virtual Biofilm Conference hosted by the University of Notre Dame. An abstract recently submitted for WEFTEC 2022 is attached to this document.	40%
Examine Effects of Hydroxylamine Addition of Biofilm Community and Treatment Performance	Hydroxylamine addition through the membrane lumen will be studied in the same manner as chlorate addition and cycloheximide addition in preceding phases after the completion of the publication and presentation of our findings from previous research. The findings of this phase of the project will likely lead to future presentations and publications.	

RESEARCH OUTPUT

Please provide any output that may have resulted from your research project. You may leave any and all categories blank or check with your faculty advisor if you are unsure how to respond.

CATEGORY	INFORMATION

EXTERNAL PROPOSALS SUBMITTED	(Sponsor, Project Title, PIs, Submission Date, Proposal Amount)
EXTERNAL AWARDS RECEIVED	(Sponsor, Project Title, PIs, Award Date, Award Amount)
JOURNAL ARTICLES IN PROCESS OR PUBLISHED	"A Novel Biofilm Reactor Supplies Performance-Enhancing Chemicals via Microfiltration Membranes." B. Kim, J. Novitch, A. Ontinveros-Valencia, M. Vega, R. Nerenberg. (Publication Information Pending)
BOOKS AND CHAPTERS RELATED TO YOUR RESEARCH	(Book Title, Chapter Title, Authors, Submission Date, Publication Date, Volume #, Page #s)
PUBLIC PRESENTATIONS YOU MADE ABOUT YOUR RESEARCH	IWA Biofilm Reactors 2021 Virtual Conference, "A Novel Biofilm Reactor Supplies Performance-Enhancing Chemicals via Microfiltration Membranes." December 6-8, 2021, Notre Dame, IN (Presentation given by Bumkyu Kim, lead author)
AWARDS OR RECOGNITIONS YOU RECEIVED FOR YOUR RESEARCH PROJECT	(Purpose, Title, Date Received)
INTERNAL COLLABORATIONS FOSTERED	(Name, Organization, Purpose of Affiliation, and Frequency of Interactions)
EXTERNAL COLLABORATIONS FOSTERED	(Name, Organization, Purpose of Affiliation, and Frequency of Interactions)
WEBSITE(S) FEATURING RESEARCH PROJECT	(URL)
OTHER PRODUCTS AND SERVICES (e.g., media reports, databases, software, models, curricula, instruments, education programs, outreach for ND Energy and other groups)	(Please describe each item in detail)

RESEARCH EXPERIENCE

Please let us know what you thought of your research experience: Did this experience meet your expectations? Were lab personnel helpful and responsive to your needs? What else could have been done to improve your experience or achieve additional results?

This research experience has been an extremely valuable supplement to my undergraduate education. The lab personnel have been extremely helpful, and I have been a part of all aspects of a research project through this work. I have applied for and received funding and designed and nonstructured bench scale experiments from which I have collected data. In addition, I am currently in the process of working with my collaborators to publish and present our findings. This work has also been a great discussion point and experience to include in my applications for graduate school.

FINAL WRITTEN REPORT

This project is in the process of studying a novel, biofilm-based treatment technology for wastewater treatment. Wastewater treatment is a major energy sink, accounting for 2 - 4% of electrical energy consumption in the United States. The proposed treatment could greatly reduce these energy demands, or even make wastewater treatment energy positive. The Nerenberg group proposes a new biofilm process combining gas-permeable membranes with water- permeable membranes. The gas-permeable membranes supply oxygen with nearly 100% efficiency. The water-permeable membranes supply a chemical to alter the microbial community of the biofilm growing on the membrane assembly. Specifically, supplying hydroxylamine is proposed, which the group hypothesizes can alter the biofilm community in ways that reduce the oxygen requirements and allow more wastewater organic matter to be directed to the anaerobic digesters. These anaerobic digesters produce methane, an energy carrier that can be used to fuel cars, produce electricity, or provide heat.

As intermediate steps in the project, the membrane system under examination has been or will be studied for effectiveness under a variety of conditions in addition to exposure to hydroxylamine through the hollow fiber membrane. These intermediate phases include studying the effects of chlorate addition through the membrane on the microbial community and treatment effectiveness and also studying the effect of predation on a nitrifying biofilm. For each phase, a baseline microbial community is first established, and a DNA extraction is performed. After the DNA extraction, the chemical of interest is added through the hollow fiber membrane until steady state is again reached. For the first phase outlined above, this chemical is chlorate. For the second phase, this chemical will be a predation inhibitor. At this point, another DNA extraction is performed to analyze differences in the microbial community due to the chemical addition through the membrane. Throughout operation, influent and effluent physical and chemical parameters are documented to track changes in treatment efficiency and reactor stability.

Initially, the project examined delivery of chlorate through a hollow fiber membrane to select for perchlorate reducing bacteria in the biofilm community. This approach has proven effective in other applications and will provide insight and proof of concept into the effectiveness of chemical delivery through a membrane as proposed in this project. As outlined above, a baseline microbial community was established, and chlorate addition was then conducted to induce changes in the microbial community. Resulting changes in the microbial community after chlorate addition will be analyzed through the DNA sample collected to determine the effects of chlorate delivery through the membrane on the microbial community. DNA analysis showed an enrichment in perchlorate reducing bacteria upon the addition of chlorate, proving that chemicals modifying the biofilm community could indeed be supplied through the lumen of hollowfiber membranes. These findings have countless other applications, two of which are tested below. In addition, these findings have many cost-saving implications due to the reduced chemical volumes required when performance enhancing chemicals, which can be expensive, are introduced through the membrane lumen instead of through the bulk liquid.

After the conclusion of the chlorate addition phase of the project, a baseline nitrifying biofilm was established to examine the effects of protozoan predation on the microbial community of the biofilm and on treatment performance, following a similar approach to the chlorate phase of the project. Once the baseline biofilm had reached steady state, cycloheximide, a protozoa inhibitor, was supplied to the biofilm through the membrane lumen. Proving once again that chemical supply from the surface side of the biofilm was effective, the performance of the biofilm reactor increased (evidenced by lower steady state effluent concentrations) when predation was inhibited. Currently, the research team is working on compiling findings from the two phases outlined above for a future publication. In addition, the findings have been presented at a biofilm conference held virtually at the University of Notre Dame in December of 2021 and an abstract has also been submitted for presentation of the findings at WEFTEC 2022.

Following the conclusion of the publication and presentation of the initial phases of the project, the same approach will be taken to evaluate the effects of hydroxylamine addition on the microbial community of the biofilm, growing a baseline biofilm and monitoring changes to the biofilm upon addition of hydroxylamine. The information gathered from this research has the potential to significantly reduce energy demand in wastewater treatment, thus lowering costs and improving environmental conditions. In addition, expensive and sometimes dangerous performance-enhancing chemicals have the potential to be delivered in a much more efficient and economical manner through the membrane lumen when compared to conventional bulk liquid delivery, with minimal chemical losses to reactor effluent. Overall, the current research project has made many important findings, leading to potential presentations and publications, and future research can build upon the initial findings outlined above to further inform energy efficient and environmentally friendly wastewater treatment systems of the future.