M.L. 2016, Chp. 186, Sec. 2, Subd. 06b Project Abstract
For the Period Ending September 30, 2018

PROJECT TITLE: Developing Membrane Filtration System to Treat Lake Superior Ballast Water
PROJECT MANAGER: Santiago Romero-Vargas Castrillón
AFFILIATION: UMN, Department of Civil, Environmental, and Geo- Engineering
MAILING ADDRESS: 500 Pillsbury Dr. SE
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E-MAIL: sromerov@umn.edu
WEBSITE: www.cege.umn.edu
FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2016, Chp. 186, Sec. 2, Subd. 06b

APPROPRIATION AMOUNT: $151,000
AMOUNT SPENT: $67,862
AMOUNT REMAINING: $83,138

Overall Project Outcome and Results

This project contributed novel membrane materials for water treatment, as well as new fundamental understanding of graphene oxide surface coatings that show potential in membranes for water purification. The materials explored in this work could find application in the treatment of surface water in Minnesota. An account of our work is provided in the Research Addendum that accompanies this workplan, as well as in our recent publication (*Environ. Sci. Technol. Lett.*, 2018, 5 (1), pp 14–19). In summary, our work showed that graphene oxide coatings, covalently tethered to ultrafiltration membranes, inactivate bacteria and thus prevent membrane biofouling. Our work further showed that the nanoscale morphology of GO surface coatings affects membrane interfacial properties; we demonstrated that randomly oriented GO nanosheets are more desirable for membrane applications, since bacteria are less prone to adhere to disordered GO.

Project Results Use and Dissemination

Additional outcomes of this project were promotion of Minnesota's human capital through training of postdoctoral, graduate and undergraduate students (1 postdoc, 2 graduate and one undergraduate student were supported at various points of the project), a M. S. degree to be completed by one of the graduate students supported by the project (expected completion in early 2019), a conference presentation at the 2017 AEESP Research and Education Conference (presented by the postdoc supported by the project), a recent publication in *Environmental Science & Technology Letters,* a premier environmental engineering peer-reviewed journal, and a further manuscript currently under preparation.

In addition, the PM presented three oral presentations reporting the research funded by this project: a conference presentation at the ACS National Meeting in New Orleans on March 18th, 2018 ("Bacterial Adhesion on Surfaces Functionalized with Graphene Oxide: Insights from Single-Cell Force Spectroscopy"); and two invited seminars at the Department of Physics at Hamline University on April 6th, 2018 ("Computational and Experimental Studies of Aqueous Interfaces") and at the Department of Chemical Engineering at University College London on May 9th, 2018 ("Understanding Microbial Adhesion to Aqueous Interfaces using Single-Cell Force Spectroscopy").

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

Date of Report: September 30, 2018

Final Report

Date of Work Plan Approval: June 7, 2016

Project Completion Date: September 30, 2018

Does this submission include an amendment request? No

PROJECT TITLE: Developing Membrane Filtration System to Treat Lake Superior Ballast Water

Project Manager: Santiago Romero-Vargas Castrillón, Ph. D.

Organization: University of Minnesota, Department of Civil, Environmental, and Geo- Engineering

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City/State/Zip Code: Minneapolis, MN, 55455

Telephone Number: (612)301-1347

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Web Address: www.cege.umn.edu

Location: Cook, Lake, St. Louis.

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$151,000**
	Amount Spent:	**$67,862.12**
	Balance:	**$83,137.88**

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 06b

Appropriation Language:
$151,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to develop a filtration system utilizing bioactive membrane technologies for use in treating Lake Superior ballast water to remove at least 90 percent of suspended pathogens, invasive species, and contaminants. 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 a Membrane Filtration System to Treat Lake Superior Ballast Water

II. PROJECT STATEMENT: The proliferation of invasive species introduced by ballast water discharge is a major threat to marine ecosystems in Minnesota. In the Port of Duluth the discharge of ballast water introduces invasive species of phyto- and zooplankton, bacteria, mollusks and their eggs and larvae; because of their remarkable adaptability, these organisms threaten the biodiversity and disrupt the ecological balance of their new environment. Processes aimed at minimizing the impact of invasive organisms in ballast waters, such as mid-ocean exchange, fail to remove all organisms, while disinfection-based technologies are costly and may produce toxic disinfection byproducts. Membrane filters, in which a polymer film with small pores allows the separation of water from suspended particulates, has shown promise for treatment of wastewaters. In this project, we aim to provide a proof-of-concept demonstration of membrane microfiltration (MF) as a ballast water treatment technology. The proposed effort is structured along two main goals:

- Phase 1: we will develop microfiltration membranes functionalized with graphene oxide, a hydrophilic and bactericidal nanomaterial that will result in a biofouling resistant MF membrane. Our goal is to develop membranes capable of removing >90% of microorganisms and the larvae of invasive species in surface waters sampled in the Port of Duluth-Superior.
- Phase 2: we will develop a pilot-scale unit with a capacity of ~600 gal/day to treat ballast water.

III. OVERALL PROJECT STATUS UPDATES:

Amendment request (9/29/2016)

This amendment affects activities 1 and 2.

As initially budgeted, this project included support for a graduate student research assistant (RA) for 2 years, including stipend, tuition, and fringe benefits. A graduate researcher with the required skill set for the project could not be found, leading the project manager (PM) to hire a qualified postdoctoral research associate, with experience in membrane science, who would be compensated at $36,000 per annum plus fringe benefits (20.1%). In compliance with the Department of Labor's increase of minimum salaries, the University of Minnesota is requiring a salary floor increase for all postdocs to the new minimum of $47,476 per annum (plus fringe benefits at 20.1%). The new minimum salary is effective for all start dates on or after November 14th, 2016; this is the likely start date of the postdoc. As a consequence of the salary increase, the PM requests a budget amendment to support the postdoc at the new minimum salary. Specifically, the PM requests that $18,000 be transferred from "Equipment/tools/supplies/services" to personnel. This will increase the personnel support allocation by $18,000 from $87,395 to $105,395, providing 21 months of support for the postdoc at the new minimum salary. The PM is confident that the postdoc hired for the project will perform at a very high level, and will make more progress in 21 months than a graduate RA would in 24 months. The PM will seek other sources of funds (e.g., NOAA, MN DNR, US EPA) to support the postdoc for the additional 3 months of salary initially budgeted for the project.

To cover the additional personnel costs, the PM requests $18,000 be transferred from the category "Equipment/tools/supplies/services" to "Personnel". The PM expects that the amendment will have little to no impact on the project outcomes, due to the relatively inexpensive reagents that will be used for membrane fabrication. The membranes will be fabricated from commercially available polymers such as polysulfone ($0.67/gram) and polyvinylidene fluoride ($0.94 per gram). The cost of each fabricated membrane ranges from $1 to $2 per membrane, including the price of the solvent. The remaining available funds for chemicals and supplies after the proposed re-budgeting, $10,105, suffices to complete the experiments in Activity 1. The PM will contribute $3,000 from discretionary funds (included in the original "Equipment/tools/supplies/services" allocation) to cover the "services" budget category. A cost-share account will be set up for this purpose.

Amendment approved by LCCMR on 10/5/16

Amendment request (5/18/2017)
An experienced graduate student research assistant (RA) will replace the postdoctoral (PD) associate beginning on May 29th, 2017. The project manager (PM) requests that the PD support be changed to RA, in support of the RA's tuition, salary and fringe benefits through summer 2018 (15 months). The balance remaining for PD salary and fringe benefits is $74,693. RA support for the proposed 15 months is $52,272. The PM requests that the difference ($22,421) be added to the "Equipment/Tools/Supplies" category (split into $11,210 in Activity 1, and $11,211 in Activity 2). These funds will be used for the purchase of reagents and laboratory consumables including polymers for membrane fabrication (polysulfone, polyethersulfone, pvdf), solvents for membrane fabrication (NMP, DMF, acetone, isopropanol), reagents to synthesize graphene oxide and other non-stick coatings, hollow fibers and chemicals to modify hollow fiber membranes, foulants (humic acids, proteins, polysaccharides), supplies for membrane characterization (SEM and AFM sample holders and AFM probes), and analytical equipment user fees.

Amendment approved by LCCMR 6/22/2017

Amendment request (2/15/2018)
In the 1/20/2018 Project Status Update, it was noted that $1965 of the funds for graduate student support from activity 2 were transferred to activity 1, because the salary was slightly overspent for activity 1. The PM requests that this amendment be retroactively approved.

Amendment approved by LCCMR on 2/28/2018

Amendment request (6/30/2018)
The project manager (PM) will be resigning from his position on the faculty at the University of Minnesota on September 30th, 2018. On consultation with Becca Nash, LCCMR director, it was recommended that the project completion date be changed to September 30th, 2018, upon which a final report will be submitted with the project findings. Further, it was recommended that student support be terminated at the end of the summer term (August 26th, 2018); this will allow the two students funded by this project (one part-time graduate student and one part-time undergraduate student) to complete their research and submit relevant findings for publication. To reflect the changes to the project duration, the PM requests that the project completion date be changed to September 30th, 2018. Further, given that an alternate project manager could not be found, the PM requests that Activity 2 be removed from the project. Further, the PM requests that the funds for graduate student support in Activity 1 be increased from $40,005 to $64,254 by transferring $24,249 from Activity 2 (this figure includes $17,739 spent in salary and fringe benefits since 1/30/18, plus $6,510 to fund the undergraduate and graduate student through the end of the summer term, i.e., August 26th, 2018). By continuing funding through the end of the summer, the graduate student currently working on the project will finish the characterization of biofouling propensity of the membranes, thus completing Activity 1. It should be noted that funds for graduate student support allocated for Activity 1 were overspent (hence the request for transfer of $17,139 from Activity 2) due to this activity falling behind schedule, which resulted in the experiments to complete activity 1 extending through the spring and summer semester, i.e., well past the initially projected completion date of January 31st, 2018. The experimental challenges that led to slow progress in Activity 1 have since been overcome, and this activity will be completed by the end of the summer.

Amendment Approved by LCCMR 8/2/2018.

Project Status as of January 30, 2017:
Work on activity 1 began in the fall of 2016. Progress has been made in training of the postdoctoral associate and preliminary fabrication and characterization of membrane materials for ballast water filtration, as explained below.

Project Status as of June 30, 2017: Work on Activity 1 continued. UF membranes and graphene oxide nanomaterials have been synthesized and characterized. Further synthesis and testing of the membrane materials and antifouling coatings is currently underway.

Project Status as of January 30, 2018: Significant progress towards completing Activity 1 has been made. A method to modify UF membranes with graphene oxide was developed. Characterization of the biofouling propensity of graphene oxide coatings was carried out, using bacterial adhesion measurements. Results from this project component were reported in a conference presentation (2017 AIChE National Meeting), and one journal publication. Details are provided in the Dissemination section. Expenses in the accompanying spreadsheet are through 12/31/2017. Please note that $1965 of the funds for graduate student support from activity 2 were transferred to activity 1, because the salary was slightly overspent for activity 1.

Project Status as of June 30, 2018:

The properties of membranes functionalized with graphene oxide (GO), a biocidal nanomaterial, are currently being evaluated. Results from this project were reported in a conference presentation and two invited seminars. Details are provided in the Dissemination section.

Project Status as of January 30, 2019:

Overall Project Outcomes and Results:

This project contributed novel membrane materials for water treatment, as well as new fundamental understanding of graphene oxide surface coatings that show potential in membranes for water purification. The materials explored in this work could find application in the treatment of surface water in Minnesota. An account of our work is provided in the Research Addendum that accompanies this workplan, as well as in our recent publication (*Environ. Sci. Technol. Lett.*, 2018, 5 (1), pp 14–19). In summary, our work showed that graphene oxide coatings, covalently tethered to ultrafiltration membranes, inactivate bacteria and thus prevent membrane biofouling. Our work further showed that the nanoscale morphology of GO surface coatings affects membrane interfacial properties; we demonstrated that randomly oriented GO nanosheets are more desirable for membrane applications, since bacteria are less prone to adhere to disordered GO.

Additional outcomes of this project were promotion of Minnesota's human capital through training of postdoctoral, graduate and undergraduate students (1 postdoc, 2 graduate and one undergraduate student were supported at various points of the project), a M. S. degree to be completed by one of the graduate students supported by the project (expected completion in early 2019), a conference presentation at the 2017 AEESP Research and Education Conference (presented by the postdoc supported by the project), a recent publication in *Environmental Science & Technology Letters,* a premier environmental engineering peer-reviewed journal, and a further manuscript currently under preparation.

In addition, the PM presented three oral presentations reporting the research funded by this project: a conference presentation at the ACS National Meeting in New Orleans on March 18th, 2018 ("Bacterial Adhesion on Surfaces Functionalized with Graphene Oxide: Insights from Single-Cell Force Spectroscopy"); and two invited seminars at the Department of Physics at Hamline University on April 6th, 2018 ("Computational and Experimental Studies of Aqueous Interfaces") and at the Department of Chemical Engineering at University College London on May 9th, 2018 ("Understanding Microbial Adhesion to Aqueous Interfaces using Single-Cell Force Spectroscopy").

Note: the student salary budget in activity 1 was slightly overspent by $1,458; to make up for this shortfall, $1458 were moved from the student support budget of activity 2 to activity 1.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Development of low-fouling MF membranes showing complete microorganism removal. The first expected outcome of our investigation is a novel MF membrane with improved resistance toward organic fouling and biofouling (i.e., the clogging of membrane pores by dissolved and suspended contaminants, particles and microbes). A hydrophilic, bactericidal nanomaterial known as graphene oxide will be deposited on the membrane to create a fouling- and biofouling-resistant coating on the membrane surface. The benefits of operation with the GO-functionalized membranes (hereinafter designated GO-MF) include pumping energy savings and less frequent membrane backwashing stages between filtration cycles; an added benefit is longer membrane useful life. Since membrane replacement due to fouling or biofouling can amount to 50% of the operating costs of membrane filtration, considerable savings could result from the materials herein proposed. We will aim to develop membranes with a water permeability in excess of 1000 L m^{-2} h^{-1} bar^{-1}, showing complete removal of microorganisms with sizes > 1 μm.

Membranes will be fabricated via the phase inversion technique. MF membranes with pores < 1 μm will be prepared using poly(vinylidene fluoride) (PVDF). Surface functionalization of the MF membranes will be accomplished using a wet adhesive known as polydopamine. Polydopamine creates an adhesive coating on the PVDF surface for the robust attachment of graphene oxide nanosheets. The GO-MF membranes fabricated will be tested in a bench-scale dead-end filtration cell (~5 cm^2 membrane area) to characterize their fouling and biofouling propensity. Ballast waters and Lake Superior water will be used as feed in the fouling experiments. The objective of these small-scale experiments is to identify the membrane fabrication conditions for optimal biofouling resistance and microbe removal.

Summary Budget Information for Activity 1:

ENRTF Budget: $87,027
Amount Spent: $67,862
Balance: $19,165

Outcome	Completion Date
1. Personnel training, assembly of bench-scale setup	January 31, 2017
2. GO synthesis and membranes fabricated	June 30, 2017
3. Characterization of fouling resistance of GO-MF membranes	January 31, 2018

Activity Status as of January 30, 2017:

The postdoctoral associate has been trained in the fabrication, characterization and testing of polymeric microfiltration (MF) and ultrafiltration (UF) membranes. Preliminary membrane materials have been fabricated using polysulfone and other additives such as polyvinylpyrrolidone. The postdoctoral associate has also been trained in the use of various experimental techniques (electron microscopy, contact angle goniometry, zeta potential, IR spectroscopy, and atomic force microscopy) to characterize membrane properties. Flux measurements to obtain the membrane permeability coefficient are currently being performed in a filtration system in the lab of the PI. Work underway involves the modification of the surface of the membranes to confer upon them anti-fouling and anti-microbial properties.

Activity Status as of June 30, 2017: Graphene oxide-based coatings have been characterized by AFM-based force spectroscopy to evaluate their potential as antifouling coatings. A modification method to attach graphene oxide nanosheets to polymeric membranes for ballast water treatment is currently development. This method uses UV-assisted polymerization of acrylic acid to modify the membrane surface with chemical moieties that can subsequently be used to attach graphene oxide nanosheets. The presence of acrylic acid was verified using FTIR

spectroscopy. The next steps include attachment of the graphene oxide sheets to the membrane using amine coupling chemistry, and characterization of the fouling resistance of the GO-modified membranes.

Activity Status as of January 30, 2018: Using amine coupling chemistry, we have successfully modified UF membranes with GO nanosheets. The presence of GO was confirmed by Raman spectroscopy, showing successful functionalization of the membrane surface. The materials are currently being characterized by electron microscopy and contact angle goniometry.

We have also completed and published a detailed investigation on GO-based coatings being used to improve biofouling resistance. Our investigation found that GO sheet spatial orientation is an important design variable in the fabrication of the GO-coatings. We found that bacterial adhesion is weaker (which is beneficial to fouling resistance), when GO nanosheets are edge-tethered to the surface. Insights from this investigation are currently being translated into the fabrication and modification of UF membranes.

Activity Status as of June 30, 2018: The UF membranes modified with graphene oxide (GO) have been characterized in terms of contact angle goniometry, zeta potential, and water permeability. Preliminary experiments show that the GO coating possesses antibacterial activity towards *P. fluorescens*, a biofilm-forming organism that is common in surface waters. Further, we are characterizing the ability of the GO-functionalized membranes using the techniques developed in our recent LCCMR-funded publication (*Environ. Sci. Technol. Lett.* 2018, 5, 14-19).

Final Report Summary: Ultrafiltration membranes functionalized with graphene oxide (GO) where devised, functionalized in the laboratory, and thoroughly characterized by Raman and FTIR spectroscopy, zeta potential, biocidal activity, contact angle, and single-cell force spectroscopy. Detailed experimental protocols are provided in the research addendum that accompanies this workplan. The fabricated membranes show promise for drinking and ballast water treatment given their biocidal activity (i.e., ability to inactivate biofilm-forming bacteria; cf. Figure 6 of the report) and their hydrophilicity (cf. Figure 7 of the report). Ongoing work, supported through non-LCCMR funds, will finalize the characterization of the adhesive properties of the various membranes investigated, using SCFS; preliminary SCFS results are provided in Figures 12 and 13.

Activity 2: Development of a ~600 gal/day pilot-scale microfiltration unit for the treatment of ballast water. In activity 2, a MF pilot-scale unit will be designed and constructed to demonstrate MF as a viable ballast water treatment technology. PVDF hollow fiber membranes will be functionalized with graphene oxide following the protocol developed in Activity 1. The pilot scale unit will consist of an immersed hollow fiber bundle operating in outside-feed mode. Given that typical MF water fluxes are on the order of 1000 L m^{-2} h^{-1}, and considering that the membrane bundle surface area is typically on the order of 0.1 m^2 for pilot-scale units reported in the literature (see Research Addendum), we expect to develop a filtration apparatus capable of processing 100 L/h = ~ 600 gal/day of ballast discharge.

The pilot-scale unit will be demonstrated with waters sampled from Port of Duluth. Permeate quality will be analyzed by total organic carbon and dissolved organic carbon analyses, turbidity, and total suspended solids. Considering that the pore size of the PVDF membranes that will be used in this work is < 1 μm, we expect that the MF pilot-scale unit will achieve >90% removal of suspended pathogens and microscopic larvae.

Summary Budget Information for Activity 2:

ENRTF Budget: $63,973
Amount Spent: $0
Balance: $63,973

Outcome	Completion Date
1. Pilot-scale construction	June 30, 2018
2. Pilot-scale testing	June 30, 2019

Activity status as of January 30, 2017: this activity has not begun

Activity status as of June 30, 2017: this activity has not begun

Activity status as of January 30, 2018: this activity has not begun

Activity Status as of June 30, 2018: no activity to report. Due to the circumstances discussed in the June 30, 2018 amendment request, this activity will not continue and the funds will be returned to the ENRTF after project close-out.

V. DISSEMINATION:

Description: Results will be disseminated via publication in peer-reviewed journals such as The Journal of Membrane Science, Water Research, and Environmental Science & Technology. Results will also be communicated through oral and poster presentations at local, regional and national conferences on water technology.

Status as of January 30, 2017: no activity to report

Status as of June, 30 2017: an oral presentation was delivered by the postdoctoral associate executing the project. The presentation reported on our characterization of the antifouling potential of graphene oxide-based coatings. The talk was delivered on 6/21/17 at the 2017 AEESP Research & Education conference in Ann Arbor, MI.

Status as of January 30, 2018: an invited oral presentation was delivered by the PI on cell adhesion to GO-based coatings at the 1st Pan American Nanotechnology Conference in Guarujá, Brazil, on November 28, 2017. The title of the talk was:
"The interaction of bacterial cells with model graphene oxide surfaces: insights from single-cell force spectroscopy."

In addition, the PI delivered a talk on the same topic at the 2017 AIChE National Meeting in Minneapolis, on October 31, 2017. The title of the talk was:
"Initial Adhesion of Bacterial Cells on Surfaces Functionalized with Graphene Oxide: Insights from AFM-Based Single-Cell Force Spectroscopy."

A publication on our findings regarding GO-functionalized interfaces recently appeared in *Environmental Science & Technology Letters.* The complete reference is:
J. Xue, S. BinAhmed, Z. Wang, N. G. Karp, B. L. Stottrup, S. Romero-Vargas Castrillón, Bacterial adhesion to graphene oxide (GO)-functionalized interfaces is determined by hydrophobicity and GO sheet spatial orientation, Environ. Sci. Technol. Lett. 2018, 5, 14-19
DOI: 10.1021/acs.estlett.7b00509

Status as of June 30 2018:

The PM presented three oral presentations reporting the research funded by this project: a conference presentation at the ACS National Meeting in New Orleans on March 18th, 2018 ("Bacterial Adhesion on Surfaces Functionalized with Graphene Oxide: Insights from Single-Cell Force Spectroscopy"); and two invited seminars at the Department of Physics at Hamline University on April 6th, 2018 ("Computational and Experimental Studies of Aqueous Interfaces") and at the Department of Chemical Engineering at University College London on May 9th, 2018 ("Understanding Microbial Adhesion to Aqueous Interfaces using Single-Cell Force Spectroscopy").

Final Report Summary:

As detailed above, results from this project were disseminated in multiple conference presentations by both the students and the PM, as well as a journal publication in *Environ. Sci. Technol. Lett.* A further manuscript, to be submitted for publication in the upcoming months, is currently under preparation.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	$ Amount	Overview Explanation
Graduate and undergraduate student support. Past support for postdoc (through May 2017), undergraduate researcher support (through December 2017)	$82,974	Current support is for graduate research assistant (25% time during summer term, fringe benefits 10% of cost) and undergraduate student researcher ($18.11/hr for 67 days in the summer (9.57 weeks)).
Professional/Technical/Service Contracts:	$ N/A	
Equipment/Tools/Supplies: reagents, chemicals, consumables, analytical services	$32,526	Reagents and laboratory consumables including, but not limited to, polymers for membrane fabrication (polysulfone, polyethersulfone, pvdf), solvents for membrane fabrication (NMP, DMF, acetone, isopropanol), reagents to synthesize graphene oxide, membrane casting equipment (PET fabric, thin film applicator, glass plates), hollow fibers and chemicals to modify hollow fiber membranes, foulants (humic acids, proteins, polysaccharides), supplies for membrane characterization (SEM and AFM sample holders and AFM probes), analytical equipment user fees. Stirred filtration cell with data logger.
Capital Expenditures over $5,000: MF pilot-scale unit	$ 34,500	Construction of a pilot-scale MF unit.
Travel Expenses in MN:	$ 1,000	Travel in Minnesota for ballast and surface water collection from Lake Superior. Mileage will be reimbursed at $0.55 per mile or current UMN compensation plan.
TOTAL ENRTF BUDGET:	**$ 151,000**	

Explanation of Use of Classified Staff:

Explanation of Capital Expenditures Greater Than $5,000:

MF pilot scale unit: custom-made hollow fiber membrane module, pump and motor assembly, valves, fittings, tubing, flow meters and pressure gauges, data acquisition and logging computer, heater/chiller.

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation:
A full-time graduate student researcher will be employed with this appropriation for 15 months. This results in a total of 1 FTE for the total project.

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

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state	N/A	N/A	N/A
University of Minnesota	$3,000	$0	Analytical services
State	N/A	N/A	N/A
	$	$	
TOTAL OTHER FUNDS:	**$3,000**	**$0**	

VII. PROJECT STRATEGY:

A. Project Partners:

The project manager will be Professor Santiago Romero-Vargas Castrillón (U. of Minnesota), who will supervise a graduate student in the execution of the proposed work. Romero-Vargas has expertise in the development, characterization, and testing of membrane materials for water purification, and membrane-based processes for water production.

B. Project Impact and Long-Term Strategy:

The proposed work will result in membrane materials and processes for the treatment of ballast water discharges in Minnesota. This project therefore directly addresses one of the main vectors for invasive species in the State. We expect this project to lead to further applications in drinking water treatment and, also, to patentable technology.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	$ Amount
United States Geological Survey. Project title: "Improving the (Bio)fouling and Mechanical Resistance of Ultrafiltration Membranes for Drinking Water Production". The project proposed in this work plan partially builds on results and expertise developed during the USGS-sponsored project.	3/1/2015 – 2/28/2016	$30,000
Matching funds from UMN for the abovementioned USGS project.	3/1/2015 – 2/28/2016	$60,000

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):

See attached graphic.

X. RESEARCH ADDENDUM:

See attached research addendum.

XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted no later than January 30, 2017, June 30, 2017, January 30, 2018, and June 30, 2018. A final report will be submitted by September 30, 2018.

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

Project Title: Developing Membrane Filtration System to Treat Lake Superior Ballast Water
Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 06b
Project Manager: Santiago Romero-Vargas Castrillón
Organization: University of Minnesota - Twin Cities
M.L. 2016 ENRTF Appropriation: $151,000
Project Length and Completion Date: September 30, 2018
Date of Report: 9/30/2018 (expenses reported through 9/12/18)

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget (6/30/18)	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM								
Personnel (Wages and Benefits)								
Graduate research assistant, postdoc and undergraduate researcher support. Current support is for graduate research assistant (50% time per year for 15 months, salary 57% of cost, tuition 33% of cost, fringe benefits 10% of cost)	$65,712	$65,712	$0	$17,262	$0	$17,262	$82,974	$17,262
Equipment/Tools/Supplies								
Reagents and laboratory consumables including, but not limited to, Polymers for membrane fabrication (polysulfone, polyethersulfone, pvdf), solvents for membrane fabrication (NMP, DMF, acetone, isopropanol), reagents to synthesize graphene oxide, membrane casting equipment (PET fabric, thin film applicator, glass plates), hollow fibers and chemicals to modify hollow fiber membranes, foulants (humic acids, proteins, polysaccharides, supplies for membrane characterization (SEM and AFM sample holders and AFM probes), analytical equipment user fees.	$21,315	$2,150	$19,165	$11,211	$0	$11,211	$32,526	$30,376
Analytical services ($3,000 **cost-shared by UMN, please see workplan, section B other funds**)	$0	$0	$0	$0	$0	$0	$0	$0

Capital Expenditures Over $5,000								
Construction of a pilot-scale MF unit: custom-made hollow fiber membrane module, pump and motor assembly, valves, fittings, tubing, flow meters and pressure gauges, data acquisition and logging computer, heater/chiller.	$0	$0	$0	$34,500	$0	$34,500	$34,500	$34,500
Travel expenses in Minnesota								
Mileage and lodging. To collect water samples within Minnesota. Mileage will be reimbursed @ $0.55 per mile or current U of M compensation plan.	$0	$0	$0	$1,000	$0	$1,000	$1,000	$1,000
COLUMN TOTAL	**$87,027**	**$67,862**	**$19,165**	**$63,973**	**$0**	**$63,973**	**$151,000**	**$83,138**

Developing Membrane Filtration System to Treat Lake Superior Ballast Water

(M.L. 2016, Chp. 186, Sec. 2, Subd. 06b)

Final Report Research Addendum

30th September, 2018

Contents

Materials and Methods

Membrane Modification

Membrane Preparation

Polyethersulfone (PES) membranes with 30 kDA molecular weight cutoffs (MWCO) were purchased from Synder filtration. PES membranes were soaked in 50% glycerin solution and stored at 4^{o}C. Prior to use, membranes were rinsed with ultrapure (UP) water, soaked in 25% isopropanol 75% ultrapure solution for 24hrs, and thoroughly rinsed again with UP water to remove any residual preservatives.

Acrylic Acid Modification procedure

It has been shown that polysulfone and Polyethersulfone membranes have the unique ability to generate free radicals when exposed to ultraviolet light which can be used to induce graft polymerization of monomers to the surface of the membranes to alter their properties. [1], [2]. Past works have focused on modifying the surface of PS and PES membranes with various monomers to improve their fouling and filtration capabilities, including the grafting of acrylic acid monomers [3], [4]. In this work, we build on the modification procedures for grafting acrylic acid with the intention of further modification with graphene oxide.

A 10% acrylic acid (AA) solution was prepared by diluting 99% AA from Sigma Aldrich with ultrapure water. Dilution was carried out in 500mL covered flasks under 125rpm of stirring for 30 minutes. PES membranes were fixed to a Teflon frame with the active area facing up. The membranes and the AA solution were brought to a glove box, where oxygen had been purged to less than 500ppm. The solution and the membranes were left in the glove box for 15 minutes to remove any dissolved oxygen.

From here, a modified dip coating method was performed. Dip coating is often used for coating membranes with monomer solutions prior to irradiation to increase the UV penetration to the membrane surface. This will in turn increase the free radicals generated on the membrane surface, not in the monomer solution, and increase grafting to the membrane. 10 percent acrylic acid solution was poured over the membrane surface and allowed to sit for 15 minutes. After 15 minutes, all excess acrylic acid monomer solution was poured into a collection vessel. This was done to ensure an even thin layer of AA monomer over the membrane surface. A Spectroline Model EF-160C 120V 60Hz 0.2amp UV lamp was positioned approximately 2cm above the membrane surface. The membranes were then irradiated for 10 seconds. After irradiation, the membranes were rinsed thoroughly and soaked in UP water for 24 hours to remove any unreacted monomer. FTIR Spectroscopy was performed on the unmodified and AA modified PES membranes to test the validity of the AA grafting step.

Preparation of Graphene Oxide solution

A 2mg/mL stock solution of graphene oxide was prepared by dissolving 100mg of GO in 50mL of ultrapure water. Dissolution was carried out in a sealed 250mL flask. The flask was submerged in a bath sonicater and the GO suspension was sonicated for 24 hours to disperse GO particles until colloidally stable.

GO Modification Procedure

PES membranes were functionalized with GO by adapting the procedure developed by Perrault et al. (2013) [5] for polyamide membranes, illustrated in Figure 1. The modification procedure utilized the EDC/NHS coupling reaction in and MES buffer. Instead of the carboxylic acid groups from the polyamide, carboxylic acids groups from the acrylic acid grafted to the membrane surface were activated to amine-reactive esters with a 4 mM 1-ethyl-3-[3-(dimethylamino) propyl]carbodiimide hydro-chloride (EDC, 98%, Sigma) and 10 mM N-hydroxysuccinimide (NHS, 98%, Sigma), buffered by 10 mM MES monohydrate (BioXtra, Sigma) with 0.5 M NaCl, at pH 5 [6]. The membranes were reacted for 60 minutes under ambient conditions while on a shaker table set to 30 rpm. Membranes were then rinsed gently with UP water. The amine reactive esters (NHS) were contacted with 10mM ethylenediamine (ED, BioXtra, Sigma) solution buffered buffered by 19 mM HEPES (99.5%, Sigma) with 0.15 mM NaCl, at pH 7.5, for 30 minutes to replace the esters with ED amino groups on the membrane surface.

The GO sheets were then activated in a similar manner to the membranes. The carboxylic acid groups on the GO nanosheets were substituted for the amine reactive esters (NHS) following a similar procedure. The 2 mg mL^{-1} GO stock solution was diluted to 250 µg mL^{-1}. The following components were then mixed together as follows: 10 parts of 250 mg mL^{-1} GO suspension mixed with 2 parts 100 mM MES buffer, followed with 1.75 parts 20 mM EDC in 10 mM MES buffer, followed by 1.75 parts 50 mM NHS in 10 mM MES buffer. The pH of the solution was then lowered to 5.5 and allowed to react for 15 minutes to ensure the esters were stable while being formed. The pH was then raised to 7.2. The membrane modified with ED groups was then contacted with the activated GO solution for 60 minutes under ambient conditions and 30rpm shaking. This resulted in a covalent linkage between the ED on the membrane surface and the GO nanosheets in suspension. Membranes were then gently rinsed and sonicated for 5 minutes to remove non-covalently bound GO. Following the modification procedure, all membranes were stored in ultrapure water (4^{o}C) prior to use.

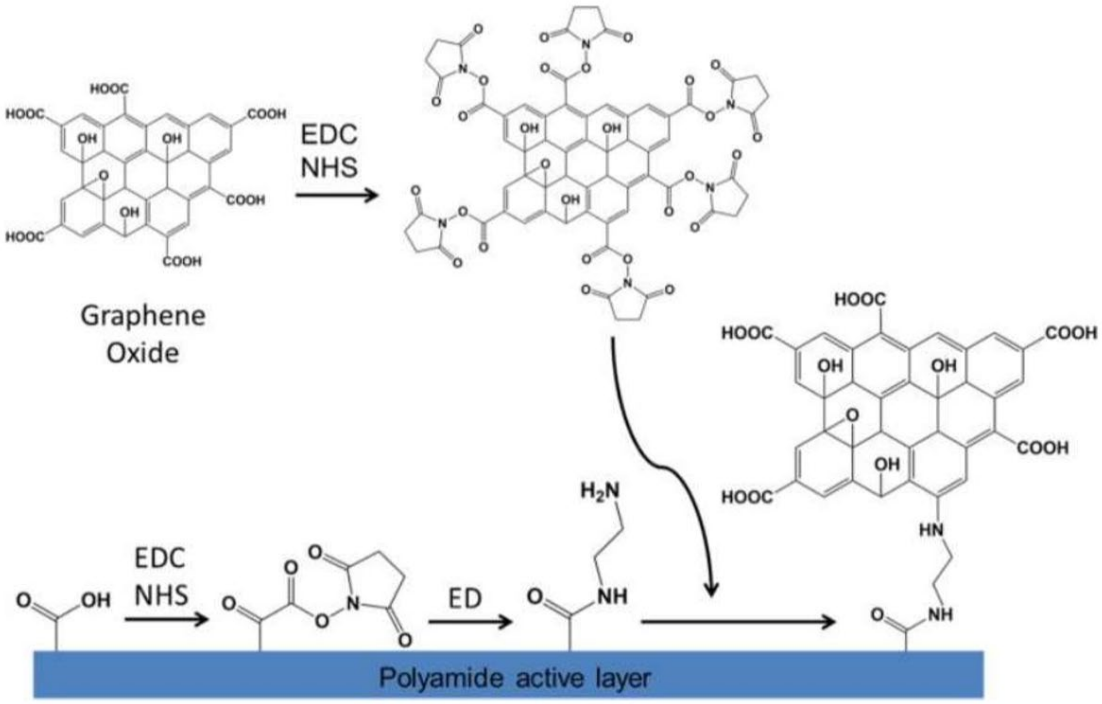


Figure 1 Membrane Modification Procedure with Graphene Oxide [5]

Membrane Characterization Techniques

There were several techniques that were used to characterize the unmodified and modified membranes. Each of the techniques, including the instruments and their respective procedures, are discussed in detail below.

Fourier-Transform Infrared Spectrometer (FTIR)

Attenuated total reflectance (ATR) FTIR was utilized to characterize the functional groups covering the membrane surfaces throughout the modification procedure. Membrane samples were dried overnight in a desiccator to remove any residual water that might appear on the FTIR spectrums. A Nicolet Series II Magna-IR System 750 Fourier-Transform Infrared Spectrometer was then used to irradiate the dried membrane surfaces.

Raman Spectroscopy

Raman spectroscopy irradiates a sample with a laser (either green or red) and measures the scattered light off the sample to yield information about the structural makeup of the surface being irradiated. Different functional groups will yield different characteristic peaks in the Raman spectrum. For this project, membrane samples were tested by first drying them overnight in a desiccator. An Alpha300R Confocal Raman Microscope (Witec) was used to carry out the Raman measurements. For each membrane tested, 20x20 µm area Raman maps at a 0.5 µm resolution were created for randomly chosen sections of the membranes. These maps were used to determine the two dimensional coverage of GO on the membrane surfaces. In addition, an average Raman spectrum was created for each membrane to give information regarding the average coverage of GO.

Contact Angle

Membrane hydrophobicity was characterized using both the water droplet in air and the captive bubble technique. For the captive bubble technique, the contact angle between droplets of n-decane and the membrane were measured in a submerged aqueous environment using a goniometer (Ramé-Hart, Model 200) and DROP Image software (Ramé-Hart). Membranes were inverted and suspended such that their active face was pointing down. The membranes were then submerged in ultrapure water. A J-shaped needle was used to inject n-decane droplets of roughly 10µL onto the membrane surface. The droplet sizes were kept at this volume for each measurement to reduce the skewing effect on the contact angle by increased buoyant forces of larger bubbles. At least 8 angles were measured across three separately functionalized membranes for each membrane type.

For the water droplet in air technique, droplets of ultra pure water were deposited on the membranes and the contact angles between the membrane and the water droplets were measured. Prior to conducting measurements, membranes were dried overnight in a desiccator. Droplets of roughly 5µL were then deposited on the membrane surfaces using a needle. The contact angles were then measured using the same equipment as the captive bubble technique described previously. At least 8 angles were measured across three separately functionalized membranes for each membrane type.

While drying out the membranes for this technique can change the surface properties [7], it also allowed for more consistent, smaller droplet sizes in comparison to the captive bubble technique. This meant that the contact angles were less prone to skewing and spreading from the increased gravitational forces associated with larger droplets.

Zeta potential

Streaming potential of the membrane surface was measured using a SurPASS electrokinetic analyzer (Anton-Paar). Streaming potential was measured over a pH range of 4-10 in 1 mM KCl solution. The pH was initially set at 10 and sequentially reduced to 4 using varying aliquots of 0.05mM HCl. An adjustable gap cell with a set distance of 120µm was used. At least two measurements were carried out for the AA and GO functionalized PES membranes. The pristine PES membrane will need measurements performed.

Biocidal Plate Assay

A biocidal plate assay was performed on the PES, AA, and GO membranes using the colony counting technique. Pseudomonas fluorescens ATCC 13525 bacteria were prepared in an overnight culture in 50mL of autoclaved LB broth under constant 125rpm stirring at 30^{o}C in an incubator. Bacteria were diluted three hours prior to the bioassay in 50mL of fresh LB broth. Stirring was increased to 175rpm for approximately three hours. The colony density in solution was characterized by measuring the OD using a spectrometer. Bacterial dilutions were used when the OD was approximately 0.6nm. 1.5mL of bacterial suspension was washed by centrifuging at 5,000G, dumping the supernatant, rinsing the bacteria with 1mL of fresh sterile PBS saline solution, vertexing, and repeating three times. The bacterial cells were then diluted with 10mL of PBS and applied to 1x1cm membrane coupons for 1 hour. Membranes were then removed, gently rinsed with PBS, and placed in 10mL of fresh PBS in 50mL falcon tubes, where they were bath sonicated for 10 minutes. The resulting solutions were then sequentially diluted in 10:100:1000 ratios. Add 50 µL of each dilution were added to agar plates and speared evenly over the surface with a glass stick. The plates were then incubated overnight at 30^{o}C and the colonies were counted after 24hrs.

Single Cell Force spectroscopy (SCFS)

Single cell force spectroscopy is used to measure the adhesion forces of bacterial cells on surfaces. This is done by adhering single bacterial cells to calibrated AFM tips, followed by contacting the surface and measuring the repulsive and attractive forces associated with pulling the bacteria back off the surface. In this experiment, Pseudomonas fluorescens ATCC 13525 was used for all singlecell force spectroscopy (SCFS) experiments due to its high biodhesion and biofilm formation potential [8]. Cells were grown in an identical manner as those used for the biocidal assays detailed previously. Individual P. fluorescens cells were adhered to the AFM cantilever tips with a polydopamine (PDA) solution used as an adhesive. An MFP-3D-Bio AFM (Asylum Research) integrated to a Zeiss Axio Observer A.1 inverted optical microscope was used for single-cell force measurements carried out under ambient conditions. Force curves were generated with extension and retraction cycles carried out with a cantilever speed of 400

nm/s, force distance of at least 3 μm or longer depending whether long-range interactions were observed, and trigger force of 600 pN. For each membrane type, 100 force curves were to be generated. Currently, all force curves for PES and the AA modified PES membranes have been gathered with about 66 curves for the GO modified membranes. Force curves were acquired at randomly chosen sites on the membrane surfaces. At each randomly chosen location, up to three force curves were performed. This was done to minimize deposition of extracellular polymeric substances.

For each membrane type, at least three individual cells cultured from three different colonies were used to collect the force curves. In addition, at least three separately functionalized membranes of each type were used as well. The cantilever deflection versus piezo Z position curves were converted into force−separation curves. Maximum adhesion forces (FAd) and rupture separation distances (LR) (the separation at which surface forces vanish) for each curve were calculated. After each experiment, the cell viability (alive or dead) using the live/dead assay (Baclight). Only data collected with a live cell that remained at its initial location were reported.

Membrane Characterization

Several of the previously mentioned techniques were used to characterize the membranes throughout the membrane modification procedure to verify the efficacy of the modifications.

Fourier-Transform Infrared Spectroscopy

FTIR Spectroscopy was used primarily to determine the if carboxylic acid groups were present on the surface of the PES membranes after the acrylic acid functionalization step. The primary peak attributed with carboxylic acid groups is a sharp band roughly at 1720cm^{-1} of the IR spectrum and a broad increase in the 2500-3500cm^{-1} region. The sharp band at 1720cm^{-1} comes from the C=O double bond stretching while the broad band roughly around ~3000cm^{-1} comes from the O-H stretching.

As described earlier, PES membranes undergo free radical polymerization upon direct radiation with UV light. In the presence of acrylic acid monomers, these free radicals polymerize the vinyl double bond on the acrylic acid, causing the formation of polyacrylic acid chains covalently bonded to the surface of the membrane. The degree of grafting (i.e. the amount of acrylic acid polymerized to the surface of the membranes) has been shown to be proportional to the time under UV radiation [9]. In addition, it has been shown that the intensity of the UV light increases the rate of polymerization and degree of grafting.

As described in the experimental section, the PES membranes used were irradiated for difference periods of time under the same UV intensity. The UV intensity was set by fixing the distance between the membrane surface and UV light source at ~ 2.5cm. Membranes were irradiated for 10, 20, 40, and 60 seconds with a thin layer of 10 wt% AA solution on top of the membrane. The FTIR results for each membrane are shown in Figure 2. FTIR was performed three times per membrane to account for any spatial deviation with respect to AA grafting. As shown in Figure 2, there is a sharp increase at 1723cm^{-1} and a broad increase in the 2500-3500cm^{-1} for each membrane relative to the control. In addition, the % reflectance for these peaks increased with radiation time. These results are indicative of the increasing presence of carboxylic acid on the surface of the membrane, thus concluding that acrylic acid was successfully grafted onto the membrane surface.

It was decided that only 10s of irradiation time was to be used for subsequent GO modification. This decision was based primarily off the fact that increased irradiation time resulted in damage to the membrane structure. Because PES membranes are reactive to UV light, the polymerization of AA to the surface of PES via free radical polymerization of the PES chains results in irreversible change to the membrane surface structure. Poor collapse is often a side effect of this modification mechanism, resulting in decreased permeability of the membrane. This was not a desired result. As such, the irradiation time was kept to a minimum (10s) to limit the loss in permeability as much as possible. GO was then grafted onto these membranes as described earlier. The results of FTIR of the PES, PES-AA and PES-GO membranes are shown below in Figure 3. This figure shows that both the GO and the acrylic acid membranes display the C=O stretching peak around 1720cm^{-1}. In addition, the GO FTIR spectrum shows increased reflectance for several peaks around 2900cm^{-1}. These peaks are known to be associated with GO and are consistent with other GO FTIR spectrum results.

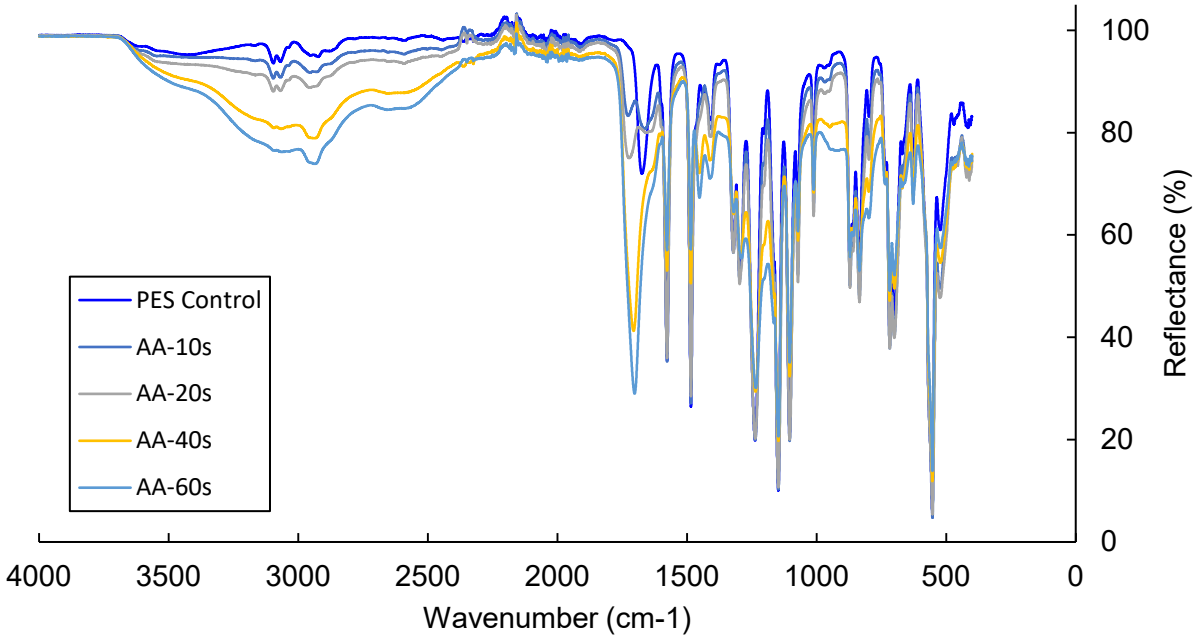


Figure 2 FTIR Results for Varied UV Irradiation Time with 10% AA Solution.

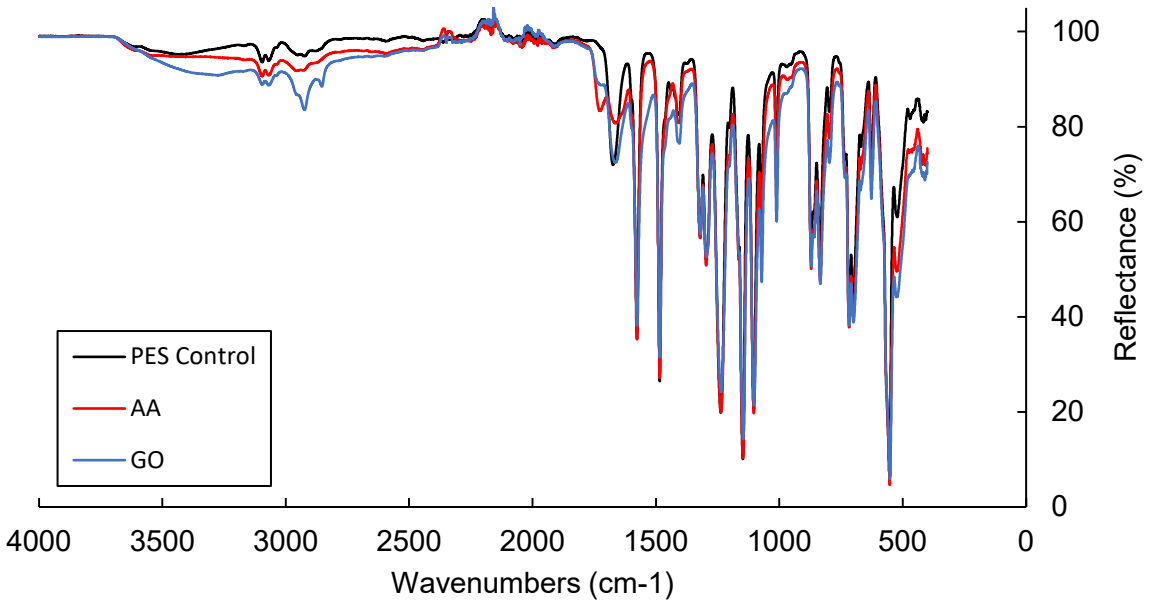
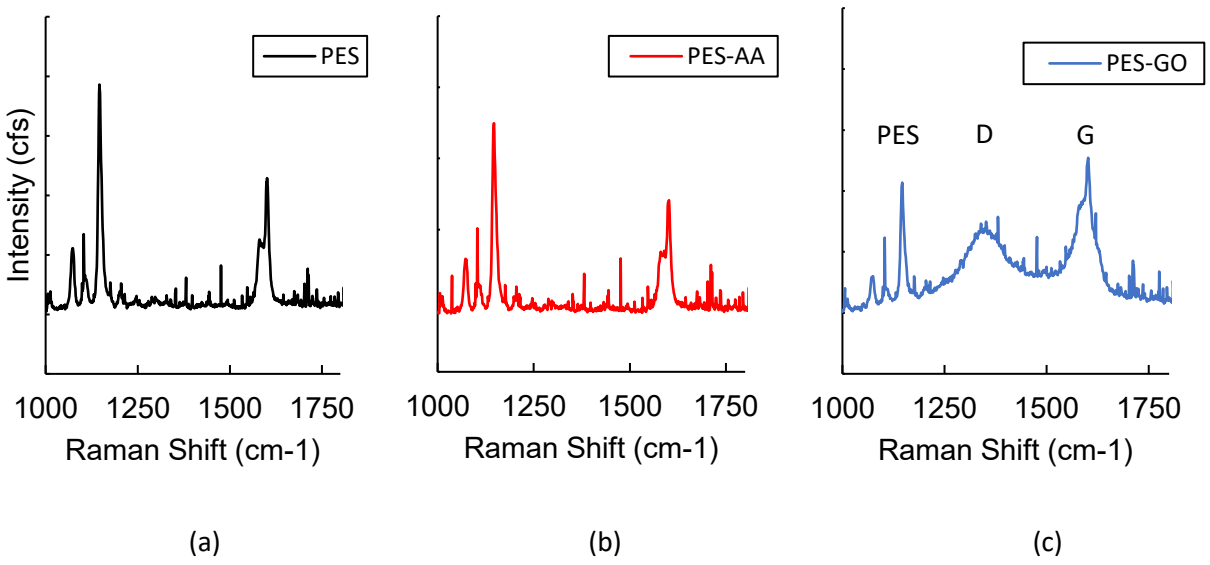


Figure 3 FTIR Results for PES, PES-AA, and PES-GO Membranes

Raman Spectroscopy

The presence of graphene oxide on the modified membrane surfaces was confirmed using Raman spectroscopy. Raman spectroscopy was performed on all three membranes (the pristine PES membrane, the AA functionalized intermediated, and the GO modified membranes) to ensure that the presence of acrylic acid or other functional groups was not mistaken for GO. Three peaks were analyzed to determine if GO was present. The first peak, labeled PES, is inherent to the PES membranes which is the result of symmetric C-O-C stretching of polyether sulfone. The second and third peaks, labeled D and G, respectively, are the characteristic D (~1350 cm^{-1}) and G (~1590 cm^{-1}) bands of GO [10]. In addition, the third (G) peak, is shared by the PES membrane [5]. Figure 4 shows the spectrums of the three membrane types (unmodified PES, AA modified PES, and GO modified PES) averaged over the 20x20um area.

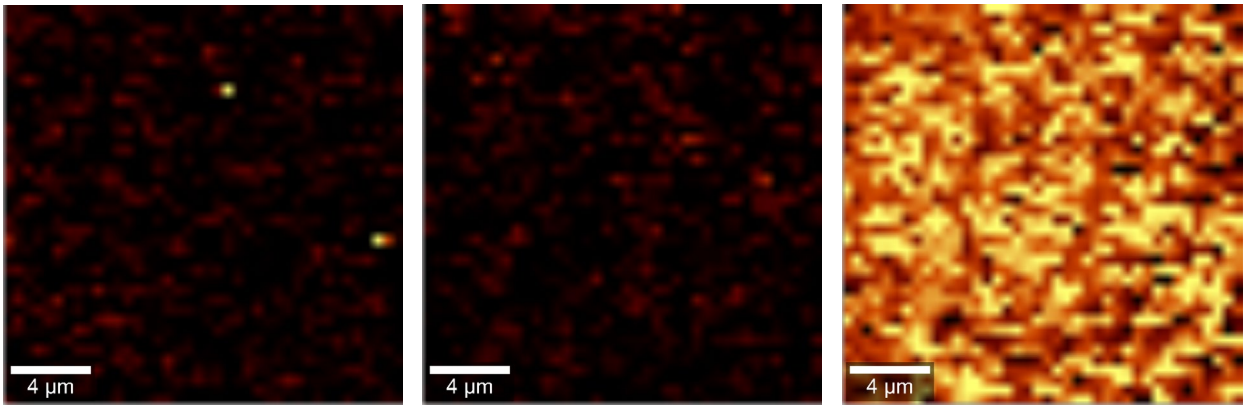


(a) (b) (c)

Figure 4 Raman Results for Pristine PES (a), AA Modified PES (b), and GO Modified PES (b) Membranes.

Figure 5 shows the three spatial Raman spectrums of the modified, AA modified, and GO modified membranes. The unmodified PES membrane and the AA modified membrane shows no evidence of graphene oxide nanoparticles adhered to the surface. The GO modified membranes shows substantial GO coverage across the entire membrane surface.

At each of the scanned points in the spatial Raman maps, the ratio of the GO D peaks to the membrane PES peaks was taken to illustrate the coverage of graphene oxide. The increased intensity of GO to PES is displayed by increased brightness. As expected, the pristine PES and the AA modified membranes showed no apparent GO binding as exhibited by the darker image. The GO modified membrane showed significant coverage throughout the tested area as seen with the brighter intensity of the Raman maps. This confirms the hypothesis that the modification procedure was successful.



(a) (b) (c)

Figure 5 Spatial Raman Maps for Pristine PES (a), AA Modified PES (b), and GO Modified PES (b) Membranes.

Biocidal Assay

Biocidal assays were performed on all three membranes using the techniques described previously. Three separate assays were performed. In each case, the 1:100 dilution was used for counting since the colonies were both distinctive and numerous enough to count after spreading. For each assay, the number of colonies for the AA and GO membranes were normalized to that of PES. The results for the three assays are shown in Figure 6. Both the AA and GO modified membranes showed signs of biocidal activity as seen in the reduction of viable colonies in Figure 6. The GO membranes showed the strongest reduction in colonies, indicating the greatest biocidal effects of the three membranes. The increased biocidal nature of the GO membranes is consistent with previous literature.

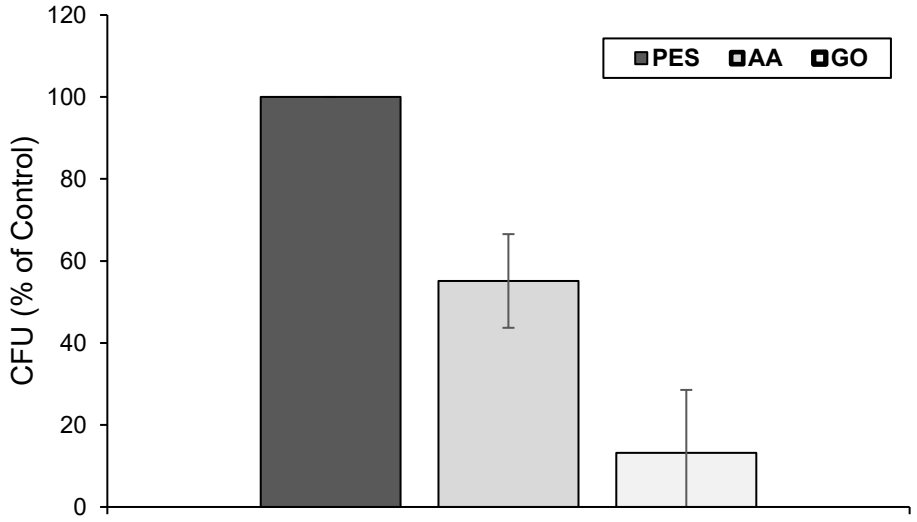


Figure 6 Biocidal Assay Results for Pristine and AA and GO Modified PES Membranes

Contact Angle

Measurements for contact angles were performed using n-decane droplets in water via the captive bubble technique. At least angles were measured for each of the three membrane types. Due to the hydrophilic nature of the membranes (particularly the GO and AA membranes), large droplets of n-decane were required. Efforts were made to keep the droplet size consistent across all three membrane types to minimize. The results for submerged contact angles are shown in Figure 7. The average contact angle for the AA membranes, 20.6°, was slightly higher than that of the GO membranes, 19.6°. Both the GO and AA average contact angles were significantly lower than for the unmodified PES membranes which had an average contact angle of 43.1°.

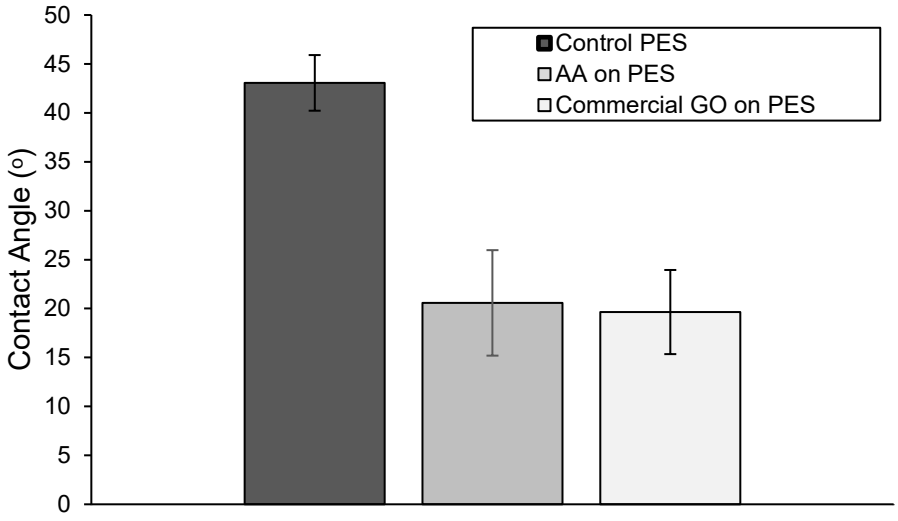


Figure 7 Captive Bubble Contact Angle Results

Contact angles were also measured using the water droplet in air method. For this, a 1uL droplet was deposited on the surface of the dried membranes. The contact angle was immediately measured to minimize the effects of spreading. At least separate angles were used for each membrane. The results are shown in Figure 8. These results show that the unmodified and GO modified PES membranes had similar hydrophilicity when measured in air, while the acrylic acid modified membrane showed significantly higher hydrophilicity.

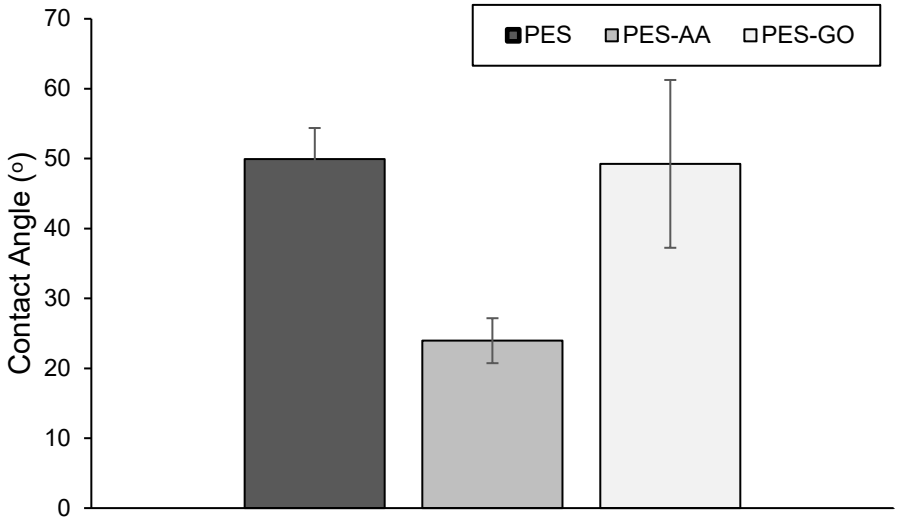


Figure 8 Water Droplet in Air Contact Angle Results

Zeta potential

Zeta potential measurements were taken for three pristine PES membranes, three AA membranes, and three GO membranes. The resulting curves for each membrane are in Figure 9. The acrylic acid modified membranes had a consistent streaming potential of roughly -30mV which was maintained from a pH of 10 to a pH of around 6.5. At higher acidic conditions, the potential dropped to nearly -40mV at a pH around 5 and then gradually increased as the pH was increased to 4. This dip in the potential could have been associated with the presence of carboxylic acid groups on the membrane surface. All three GO membranes followed a consistent trend of increasing in zeta potential initially at a pH of 10, plateauing until a pH of 6.5 and then sharply increasing through a pH of 4. In general, the GO membranes had a lower potential than the acrylic acid membranes until a pH of 6. This more negatively charged surface could be explained by the increase in carboxylic acid density from the GO nanosheets. The generally lower zeta potential (seen in the typical operating conditions of a pH of 6-8) could result in more better rejection due to Donnan exclusion. In addition, this lower charge should cause both the GO and acrylic acid modified membranes to have a lower propensity for bacterial fouling.

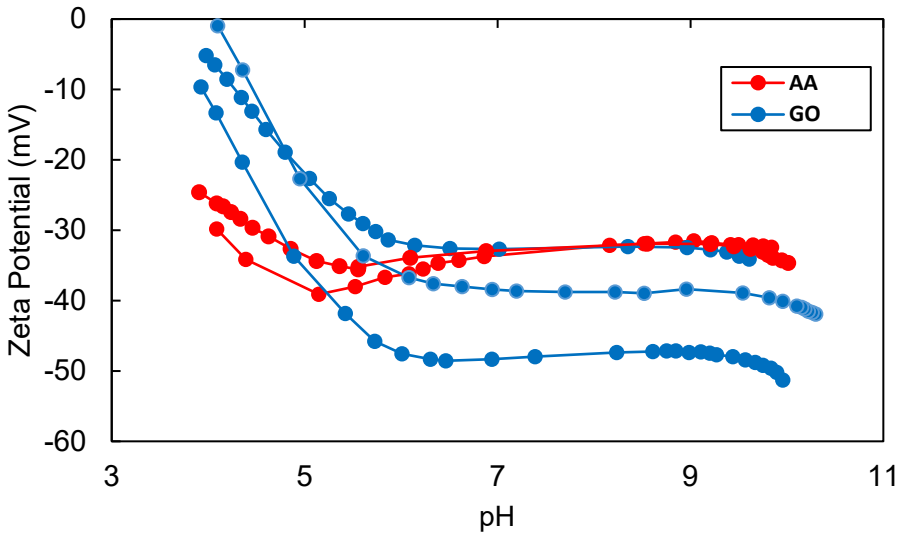


Figure 9 Zeta Potential Results for PES-AA and PES-GO Membranes

Single Cell Force Spectroscopy

A total of roughly 100 force curves were collected from at least three separately functionalized membranes for each of the three membrane types. A single Pseudomonas genus, P. fluorescens bacterium was immobilized on a calibrated cantilever tip. Extension−retraction cycles were performed at a cantilever speed of 400 nm/s, force distance of 2 μm or longer, and trigger force of 600 pN. For each force curve generated, the rupture separation distance and the adhesive forces of the bacteria were calculated. Figures 1-4 represent the data for adhesion force and rupture separation for PES membranes and PES-AA membranes. GO membrane data collection will need to be finished and worked up prior to analysis.

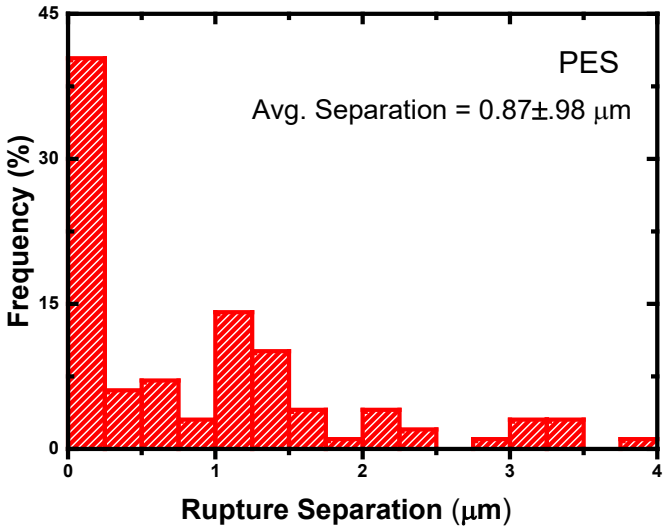


Figure 10: Rupture Separation Data Over PES Membrane

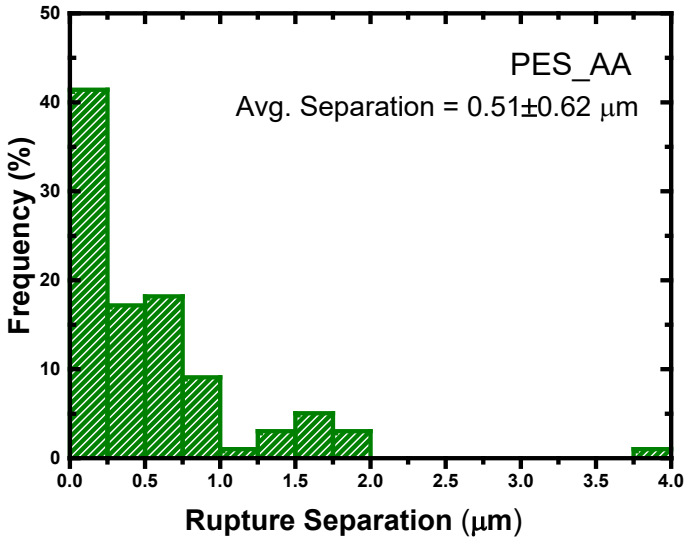


Figure 11: Rupture Separation Data Over PES-AA Membrane

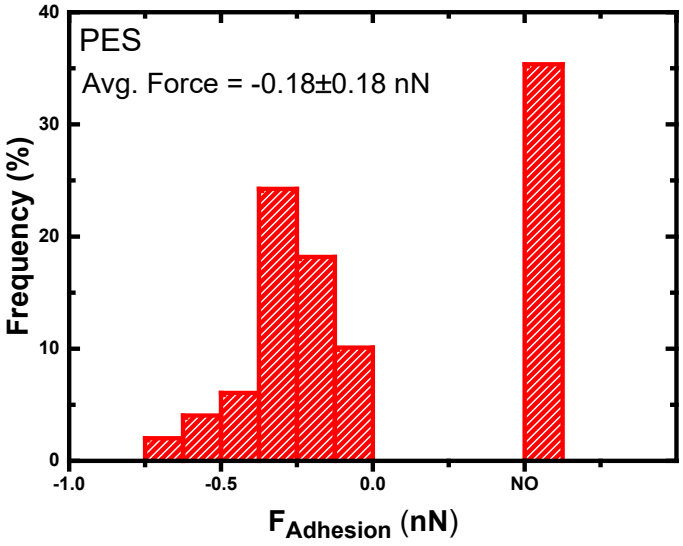


Figure 12: Adhesion Force Data Over PES Membrane

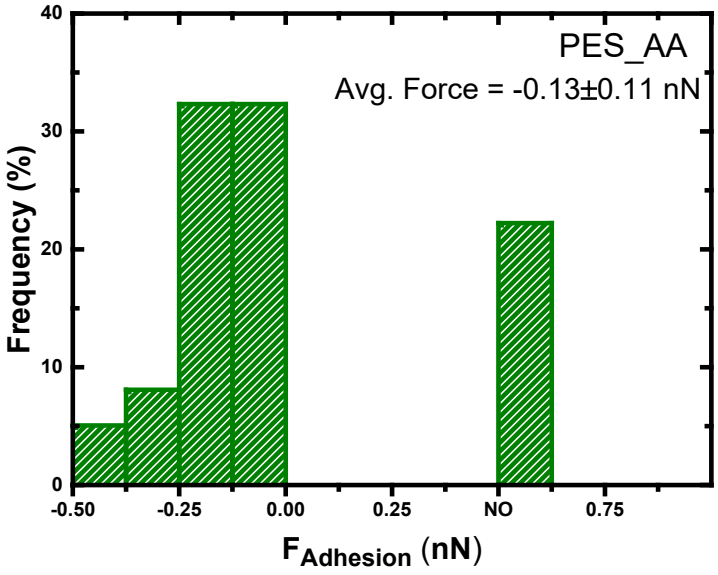


Figure 13: Adhesion Force Data Over PES-AA Membrane

The PES membrane SCFS experiments consist of 99 force curves using three different bacteria cells with an intact cell membrane. The PES-AA membrane SCFS experiments consist of 99 force curves using three different bacteria cells with an intact cell membrane, as well. Future work will consist of obtaining 99 force curves over the GO functionalized membranes for comparison.

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