THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF CHEMICAL ENGINEERING

MORINGA OLEIFERA COATED SAND FILTERS: VIRUS REMOVAL AND SCALE-UP

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ABSTRACT

The clean water crisis affects roughly 1 in 9 people worldwide.[1] An absence of clean water leads to widespread deadly waterborne disease, which kills some 842,000 people each year.[2] Point-of-use technology that can be directly applied to purify surface water, such as membranes, chlorination, ceramic filters, and boiling have demonstrated the ability to significantly decreases the rate of diarrheal disease.[5,8] The *Moringa oleifera* (MO) tree is a tropical tree native to India, whose seeds contain cationic antimicrobial proteins. These cationic proteins will electrostatically adsorb to anionic surfaces such as silica sand, creating functionalized sand, or *f*-sand. When packed into a column, *f*-sand filters demonstrated high removal of *E. coli* and model microbes, making it a potential addition to the list of point-of-use water treatment technology.[15] However, viruses are a large contributor to waterborne disease and are much harder to remove than bacteria due to their size.[17,21,22] In this study, the purification capabilities of the *f*-sand filter are further explored by testing the removal of MS2 bacteriophage, a model virus. Using 106-micron glass beads and a flow rate of 1.6 mL/min, the *f*-sand filter was able to remove >7-$\log_{10}$ (99.99999%) MS2, which is abnormally high for a sand filter. Further experiments revealed the mechanism for virus removal by a binding pocket within a chitin binding protein found in Moringa seed extract.

Additionally, the first ever field scale-up of the Moringa filter was performed in Fort Myers, Florida. Two filters were built using PVC, bamboo, and locally available sand and Moringa seeds. Moringa filters demonstrated superior improvement of the visual clarity of water, as well as >99.9% removal of total coliform. These scale-up experiments also shed light on significant challenges towards implementing the filter in a practical manner, which includes

minimizing the amount of water necessary to construct the filter, extending the filter lifetime, optimizing flow rate, and finding small enough sand.

LIST OF FIGURES

ACKNOWLEDGEMENTS

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Chapter 1

Project Background

Overview of the Clean Water Crisis

The clean water crisis directly or indirectly affects a huge portion of the world's population. 785 million people, or roughly 1 in 9 of the world's population, lack access to safe water while 2 billion people lack access to improved sanitation.[1] Waterborne disease, which can be caused by a variety of microorganisms including bacteria, viruses, and protozoa, accounted for 2.2 million deaths in 2016.[3,4] Diarrheal disease from waterborne pathogens affect the world's most disadvantaged citizens, as 297,000 children under the age of five die each year due to poor sanitation, hygiene, or contaminated water.[5] Aside from issues of global health, according to the United Nations, contaminated water is undermining efforts to end poverty in the world's poorest countries.[6]

Development of Moringa filters

Point-of-use water purification technology, including boiling, chlorination, biosand filters, ceramic filters, and membrane filters can significantly reduce the risk of waterborne disease.[5,8] However, these methods have various practical challenges. Biosand and ceramic filters suffer from low flow rates.[9,10] Membrane filters suffer from filter fouling problems, high energy costs to generate a suitable pressure gradient, and continuous monitoring.[9,11,12] Boiling is also energy intensive and may require the use of scarce or relatively expensive fuels in developing areas.[13] Chlorination is not widely available or accepted in developing countries and can give the water an adverse taste.[8]

Moringa oleifera (MO) is a fast-growing tree that is present across many equatorial regions[14] that are most severely affected by waterborne disease (Figure 1). Moringa seeds

contain cationic proteins, MO2.1 and MoCBP, that have antimicrobial, antifungal, and flocculation activity (Figure 2). Historically, the properties of these proteins have allowed Moringa seeds to be used as a flocculant to purify water.[15] However, disinfection by coagulation is only somewhat effective and the presence of organic matter in the water gives rise to future microbial growth.[16] In order to address these challenges, previous work sought to adsorb these cationic proteins to the surface of negatively charged sand through electrostatic interactions.[17] The resulting positively charged functionalized sand (*f*-sand) is then rinsed to removed organic matter, avoiding the challenge of microbial growth.

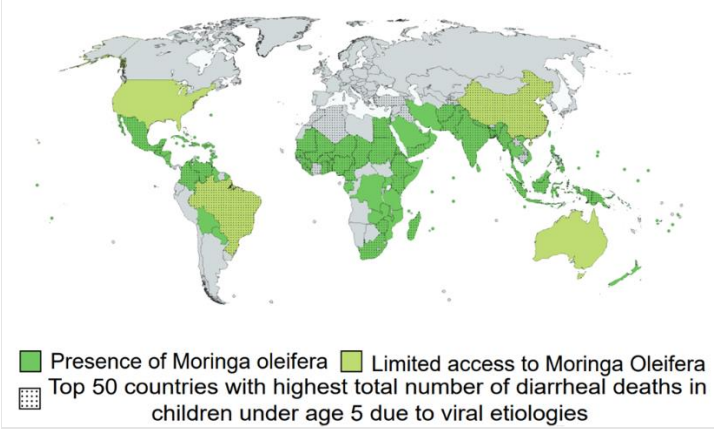

Figure 1. Moringa oleifera grows in equatorial regions that correlate with water stressed areas.

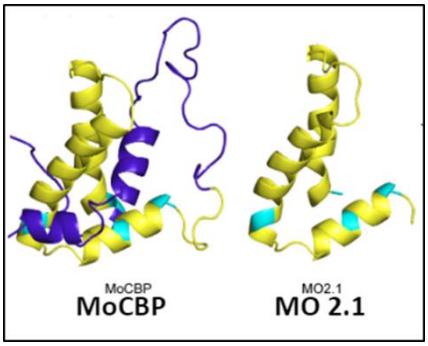

Figure 2. 3D structure of Moringa oleifera chitin binding protein (MoCBP) and MO2.1

The *f*-sand can be packed into a column to create an *f*-sand filter that has significantly enhanced performance compared to bare sand. Previous work demonstrated that these filters could remove 4-log (99.99%) 1-micron sPSL particles and >8-log *E. coli*, both negatively charged model contaminants.[15] Additionally, preliminary scale up analysis showed that a point-of-use *Moringa* filter could provide 10 L of water per day with only 0.21 kg seed per year[15] (mature *Moringa* trees produce 480 kg seed per year).[18] Therefore, *Moringa* filters have the potential to be a locally available, sustainable, and cost-effective point-of-use water treatment technology in developing areas.

Role of Viruses in Water Borne Disease

Out of all waterborne pathogens, human enteric viruses (EVs), such as rotavirus and norovirus, are the leading cause of acute diarrheal disease.[19] Viruses are particularly problematic in drinking water treatment. They pose a higher health risk compared to bacteria and protozoa due to high infectivity (exposure to one rotavirus particle results in a 31% chance of infection[20]). Additionally, viruses are difficult to detect because they exist in low background concentrations[21] and require relatively complex detection techniques such as qPCR.[22] Finally, viruses are more difficult to remove from water compared to bacteria and protozoa. The EPA requires 4-log removal of viruses[23], which cannot be achieved by traditional filtration technology such as rapid sand filtration[24] and slow sand/biosand filtration.[25,26] Membrane filtration, chemical disinfection, and boiling are all effective options to protect against viral contamination of drinking water, but each have significant practical and economic roadblocks against widespread implementation.

MS2 bacteriophage is a nonenveloped RNA virus with an icosahedral capsid that is 22-29 nm in diameter[27] (Figure 3). Due to its similarity in structure and filtration performance to human

enteric viruses, it is a recognized surrogate for human enteric viruses in filtration experiments.[28,29] Additionally, it is nonpathogenic to humans and easier to culture in the lab than human enteric viruses. In this work, we report 7-log removal of MS2 bacteriophage by the *Moringa* coated sand filter. Additionally, we illuminated a specific mechanism for virus removal by *Moringa oleifera* chitin binding protein (MoCBP) through molecular docking simulations.

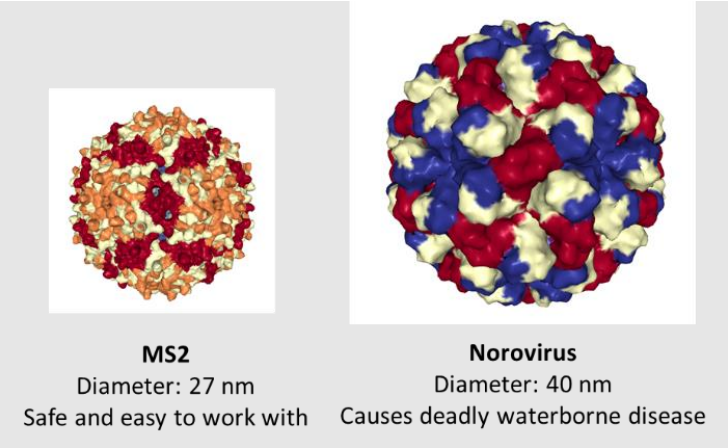

Figure 3. Comparison of MS2 and Norovirus structure

Moringa oleifera Sand Filter Scale-Up

Practical implementation of a *Moringa* filter requires scale-up to a suitable sized column. Various considerations must be made in the scale-up process to construct both an effective, long-lasting, and cost-effective filter. Column parameters such as sand size, column diameter/length, and flow rate all affect log removal.[30] Furthermore, surface water contains a significant amount of turbidity that can compete with pathogens for adsorption sites, decreasing the lifetime of the filter.[31]

In this work, we report the qualitative and quantitative results of intermediate scale up columns that were constructed and tested in a field environment (ECHO Global Farm, North Fort

Myers, Florida). These columns were able to clarify heavily contaminated water obtained from a tilapia pond and remove all detectable coliform bacteria.

Chapter 2

Materials and Methods

MO Seeds

The Moringa seeds used in both lab scale and field work were received from the seed bank at ECHO Global Farm. In the lab, the seeds were stored under ambient conditions in a sealed Ziploc bag. For lab experiments, whole dry seeds were crushed by placing ~15g of seeds into a coffee grinder and grinding for 20 seconds until finely grounded. For field experiments, the seeds were crushed using a mortar and pestle until roughly grounded.

f-sand Preparation

The mass of seed used for both lab and field columns was calculated based on a seed to sand surface area of 5.6 g/m^2 determined from optimization experiments in previous work.[15]

Lab Preparation

To prepare the f-sand in a batch process, 3.1 g of crushed seed was added to 610 mL of DI water. The seed and water were mixed together using a magnetic stirrer and Erlenmeyer flask for 5 minutes. Then, the water extract was vacuum filtered twice through a 4.7 cm diameter 1.5 µm Whatman glass fiber filter followed by a 4.7 cm diameter Millipore 0.22 µm poly(vinylidene difluoride) (PVDF) filter, which removed the seed debris. Next, 25.0 g of Sigma 106 µm glass beads were added to the filtered seed extract. The glass beads were mixed with the seed extract for 5 mins and then allowed to settle with no stirring for an additional 5 mins. The supernatant seed extract was discarded, and excess organic matter was removed by rinsing the glass beads with 600 mL of DI water 3 times. The resultant f-sand was then packed into the appropriate column.

In-situ Preparation

A new continuous process for constructing the *f*-sand columns was developed in order to improve the construction process and reduce the amount of water and seed necessary for scale-up work. Two different MO serums were fed into a packed bare sand column at a flow rate of 1.6 mL/min using a peristaltic pump. The two serum preparations were a single pass 60 mL of 0.005 g seed/mL serum and a single pass of 15 mL of 0.02 g seed/mL serum.

Field Preparation

A similar process was followed to prepare the f-sand in the field. In this case, 30 g of crushed seed was added to 6 L of municipal water. The seed and water were mixed in a plastic tub using a plastic cooking ladle for 5 minutes. Then, the water extract was gravity filtered through a cotton cloth to remove the seed debris. Next, 212 g of sieved sand was added to the seed extract and mixed using a plastic cooking ladle for 5 minutes. The seed extract was then discarded and the sand was rinsed 3 times using municipal water. The resultant f-sand was then packed into the appropriate column.

MS2 Stock Solution Propagation

The virus stock was prepared through bacterial propagation. In our work, 200 mL of *E. coli* (ATCC 1597) culture was inoculated with MS2 phage in a tryptic soy broth (TSB). The culture was then incubated in a shake flask at 200 rpm for 24 hours at 37 °C. Next, purified MS2 phage was harvested by centrifugation of the culture at 500 rpm for 15 min. 500 µL of the resulting MS2 stock solution was diluted with 500 µL of 1:1 glycerol/DI water solution. The resulting 1 mL aliquots were stored at -80 °C. For each experiment, a fresh aliquot of MS2 stock solution was used and diluted to the desired influent concentration for column experiments.

MS2 Plaque Assay

To quantify influent and effluent MS2 concentrations to calculate log removal, a double agar layer procedure (plaque assay) was performed on all virus samples. First, a stock solution of ATCC 1597 *E. coli* was streaked onto a TSB agar plate and allowed to incubate for 12 hours at 37 °C in an incubator. Then, a single colony from this plate was inoculated into 10 mL of TSB liquid media and incubated at 37 °C in a 50 mL Falcon tube at 200 rpm for 6 hours in an incubator.

To make the base agar plates. 6 mL of hot autoclaved (30 min liquid cycle) solid agar solution (15 g bacteriological agar, 30 g TSB powder, and 1 L DI water) was added to a 95mm diameter petri dish.. Next, 5.5 mL of hot autoclaved (30 min liquid cycle) semi-solid agar solution (3.75 g bacteriological agar, 15 g TSB powder, and 500 mL water) was added to a glass tube and heated in a hot water bath until uniformly melted. The tubes were taken out of the water bath and allowed to cool until warm to the touch but still liquid. 100 µL of liquid *E. coli* culture and 100 µL of MS2 sample were added to the tube. The tube was gently mixed by shaking and poured over the base agar plate and allowed to incubate for 12 hours. After 12 hours, the areas with no bacterial growth due to infection by MS2 (plaques) were counted (Figure 3), resulting in a concentration of virus in units of plaque forming units per mL (PFU/mL). The influent and effluent MS2 concentrations were used to calculate the column log removal efficiency (LRE) (Equation 1).

$$LRE = \log\left(\frac{N_0}{N}\right) \quad \textbf{(Eq 1)}$$

Where N_0 is the influent concentration and N is the effluent concentration. The reported log removal values were the average of those calculated from 4, 6, and 8 PV.

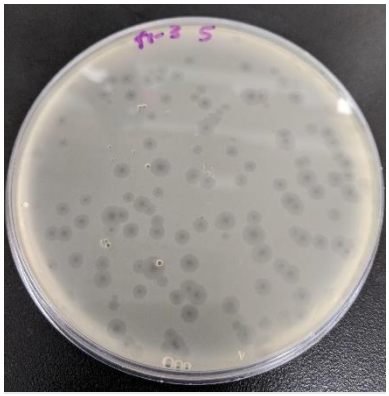

Figure 4. MS2 plaques observed on an agar plate.

E. coli Influent Preparation

The *E. coli* stock solutions were prepared from a stock solution that was stored in the –80 C freezer. A stock solution of *E. coli* strain TG1 containing plasmids that expressed red fluorescent protein (pCA24N-rfp-lasR) was streaked across an LB agar plate and incubated overnight. A single colony was inoculated in 50 mL of LB broth and incubated in a shake flask until the solution turned bright red (~12 hours). The *E. coli* liquid culture was then centrifuged at 2000 RPM for 10 minutes and the pellet was resuspended using 10X diluted PBS. This rinsing cycle was repeated 3 times before dilution to the desired influent concentration.

Lab Scale Column Experiments

The prepared *f*-sand was poured into a 50 mL Falcon tube and filled to the top with DI water. The mixture was shaken into a slurry and quickly poured into a 1.5 cm diameter, 10 cm height Bio-Rad glass chromatography column. Then, the columns were packed overnight under gravity flow with DI water. Before adding influent, the columns were equilibrated with 1 mM NaCl solution for 30 minutes at 1.6 mL/min using a Cole-Parmer peristaltic pump and 1/16 inch silicon tubing. The pump feed line was then switched to the prepared diluted influent bottle. Effluent samples were taken in 1 mL sizes at 4, 6, and 8 pore volumes (PV), which was

gravimetrically measured for each column by measuring the volume of water required to wet the measured mass of sand.

Gel Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)[32] was used to characterize the size of the proteins adsorbed onto *f*-sand. 12 µL of *f*-sand was loaded onto a 12% hand-cast SDS-PAGE gel. A molecular weight ladder was added to one lane to identify the size of the separated proteins. The protein bands were visualized using Coomassie staining and then incised and digested using trypsin. The isolated protein samples from gel electrophoresis were then sent to the Mass Spectrometry Core Facility at Penn State for identification.

Fluorometric Protein Assay

The Thermo Scientific Pierce Quantitative Fluorescent Peptide Assay was used to quantify the amount of protein adsorbed onto the *f*-sand. 100 microliters of *f*-sand was washed with 100 microliters of 600 mM NaCl solution to desorb the protein from the sand. The sand was allowed to settle in a centrifuge tube and the supernatant was collected in a new tube and the amount of protein in the liquid was measured using the fluorometric assay. A calibration curve for the assay was developed using lysozyme samples from chicken egg white.

N-Acetyl D-Glucosamine (GlcNAc) Wash Experiments

To demonstrate the specific virus removal mechanism of MoCBP, *f*-sand columns were washed with a 1M solution of N-Acetyl D-Glucosamine (Sigma) in a 10 mM pH 7 phosphate buffer at 1.6 mL/min following the equilibration step. The rest of the column procedure remained the same, with MS2 filtration and sample collection at 4, 6, and 8 PV.

Molecular Docking Simulations

Molecular docking simulations were performed in collaboration of the Costas Maranas Group at Penn State. Homology modeling[33,34] was used to generate the statistically preferred structures of MO2.1 and MoCBP. Stable binding pockets between each protein and MS2 capsid protein (1AQ3) through a simulated annealing-based approach.[35] CHARMM-energy minimization cycles were performed on the protein complexes until the lowest energy configuration was determined.

Scale Up Column Experiments

Some f-sand experiments were performed in the field at the ECHO Global Farm in Florida. For these experiments, the prepared *f*-sand was poured into both a 1-inch diameter PVC column and bamboo column using a funnel and packed overnight with municipal under gravity flow. Pond water retrieved from the ECHO Global Farm tilapia pond was allowed to settle for 24 hours to remove large solids. The supernatant was poured into a drip bucket and allowed to drip into the columns with the effluent collected in plastic water bottles. Effluent samples were sent to a local water lab for total coliform testing.

Sedimentation Tests

Sedimentation tests were performed to approximate the sand size in order to model the column post-experimentation. A graduated cylinder of known height was filled to the brim with water. A sample of sand was added to the top of the graduated cylinder and allowed to settle. Using a phone stopwatch, the time it took for the first and last sand particle to reach the bottom of the graduated cylinder was measured and then used to calculate the average velocities. Terminal velocity (Equation 2) derived from Stokes' Law was then used to calculate an approximate sand size.

$$v = \frac{2}{9}\frac{(\rho_p - \rho_f)}{\mu} g R^2 \quad \textbf{(Eq 2)}$$

Where v is the terminal velocity, ρ_p is the density of the sand particle, ρ_f is the density of water, μ is the dynamic viscosity of water, g is the acceleration due to gravity, and R is the radius of the particle.

Chapter 3

Virus Removal

MS2 Column Experiment Results

Lab scale columns demonstrated 7.1 ± 0.4 LRE (99.99999%) for MS2 particles at a flow rate of 1.6 mL/min. Comparatively, bare sand columns (columns prepared with uncoated glass beads) run as a negative control demonstrated just 0.2 ± 0.05 LRE of MS2 (Figure 5). The *f*-sand column performance significantly exceeded the EPA standard for virus log removal/inactivation of 4-log.[5]

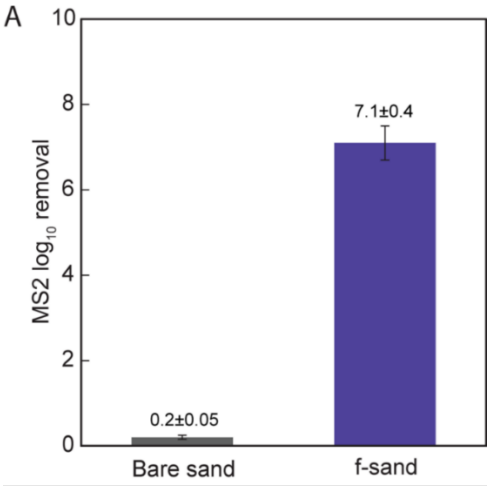

Figure 5. *f*-sand columns removed significantly more MS2 particles than bare sand.

Clean bed filtration theory was also used to model the column performance and inform scale-up considerations. The theoretical log removal can be given by:

$$\ln\left(\frac{N}{N_0}\right) = -\frac{3}{2}\frac{(1-\varepsilon)L\alpha\eta_0}{d_c} \quad \textbf{(Eq 3)}$$

Where ε is the porosity of the column, L is the column length, d_c is the collector diameter, α is the sticking coefficient (the probability that a particle sticks to the collector upon collision) and η_0 is the collector efficiency (probability of a particle striking a collector). The collector efficiency η_0 was calculated based on using the Yao model[36], which models collector efficiency

based on three different mechanisms of mass transport. The first mechanism of mass transport is interception, which is the probability of a contaminant particle (modeled as a sphere) contacting a collector as a result of their relative sizes. The second mechanism of mass transport is sedimentation. If the density of a particle is greater than water, it will follow a different flow trajectory that is influenced by buoyant and gravitational forces and can come into contact with the collector via this flow trajectory. Finally, a contaminant is subject to random motion, which is characterized by the final mechanism, diffusion. The overall collector efficiency, η_0, is defined as the sum of the collector efficiencies from interception (η_I), sedimentation (η_G) and diffusion (η_D):

$$\eta_I = \frac{3}{2}\left(\frac{d_p}{d_c}\right)^2 \quad \textbf{(Eq 4)}$$

$$\eta_G = \frac{(\rho_p - \rho_f) g d_p^2}{18 \mu v} \quad \textbf{(Eq 5)}$$

$$\eta_D = 4.04 * Pe^{-\frac{2}{3}} = 0.9 * \left(\frac{kT}{\mu d_p d_c v}\right)^{\frac{2}{3}} \quad \textbf{(Eq 6)}$$

$$\eta_0 = \eta_I + \eta_G + \eta_D \quad \textbf{(Eq 7)}$$

Where k is the thermal conductivity of water and Pe is the Peclet Number.

For an experimental porosity of 0.37, 106 μm diameter collectors, and 10 cm column length, and a $\log_{10}$ removal of ~7, the sticking coefficient of the *f*-sand column for MS2 was determined to be 0.13, compared to 0.003 for bare sand. The elevated log removal for *f*-sand compared to bare sand demonstrates that the coated sand has higher affinity for MS2 than bare sand.

Additional column experiments using different flow rates and collector sizes were used to test the clean bed filtration model against the actual column performance. The column demonstrated higher than predicted log-removal at elevated flow rates and lower than predicted

log-removal at larger collector sizes (Figure 6). The flow rate trend is encouraging because it suggests that the column could be operated at higher throughput without sacrificing performance. However, the column essentially displayed no removal of MS2 with larger sand particles. Locally available sand used in slow sand filters typically range from 150-300 μm in diameter[37], which presents a significant problem for filter scale-up. The effect of sand size in our columns with respect to virus removal is not very well understood and a subject of further research. One potential solution to the issue of low removal efficiency with increased sand size is to use binary mixtures of larger and smaller collectors, which increases the removal efficiency to an acceptable level while minimizing costs of finding smaller sand. This approach and its optimization in the context of filter scale-up is a subject of other Schreyer Honors College Theses written within the *Moringa* research group.[40,39]

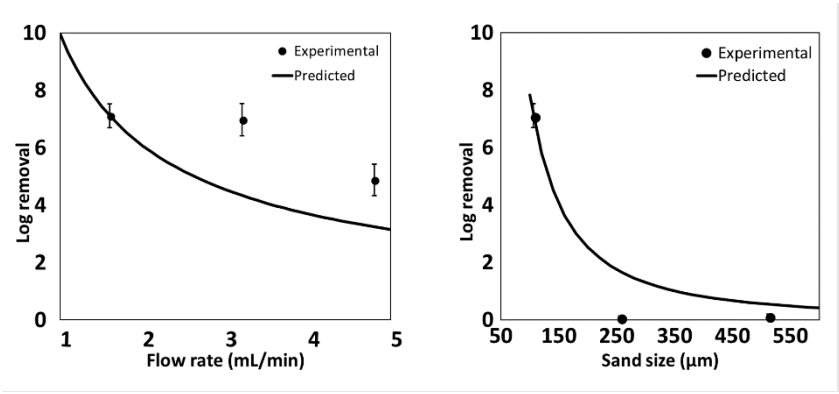

Figure 6. Effect of flow rate and collector size on column performance.

f-sand Protein Characterization

In an effort to illuminate the removal mechanism of viruses, the coated proteins were separated using gel electrophoresis and identified using mass spectrometry. Identification revealed that 2 proteins, MoCBP (*Moringa oleifera* chitin binding protein) and MO2.1 were present on the *f*-sand. MoCBP is a 14 kDa protein that possesses antifungal and chitin-binding activity.[38] It is hypothesized that the Moringa seed produces this protein to protect against fungal

infections. MO2.1 is a 6.5 kDa protein responsible for much of the flocculation activity from Moringa seeds.[39] While MoCBP and MO2.1 are distinct proteins, they share similar sequences (Figure 7).

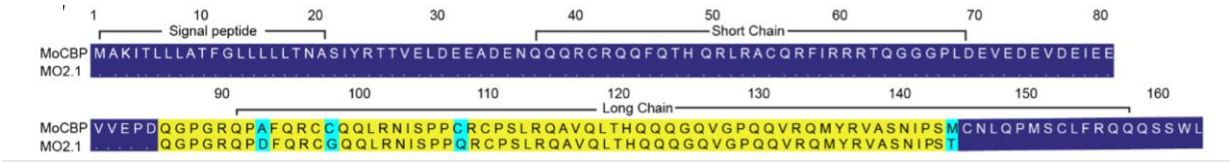

Figure 7. Amino acid sequence comparison of MoCBP and MO2.1. Much of the MO2.1 sequence is the same as the MoCBP sequence with the exception of 4 amino acids.

Molecular Docking Simulations

In silico binding experiments between the MS2 capsid protein and the two Moringa proteins (MoCBP and MO2.1) demonstrated favorable binding between MoCBP and MS2 (Figure 8). The experiment brought 3D models of two different proteins close to each other and measured a change in energy from the binding to nonbinding configurations. MoCBP and MS2 capsid protein demonstrated a negative binding energy, indicating that a binding configuration results in a decrease of Gibbs free energy relative to a nonbinding configuration. In contrast, MO2.1 and MS2 capsid protein had a positive binding energy, indicating that a binding configuration is unstable and therefore unfavorable. The favorable binding between MoCBP and MS2 capsid protein led to the hypothesis that MoCBP was responsible for the mechanism of virus removal.

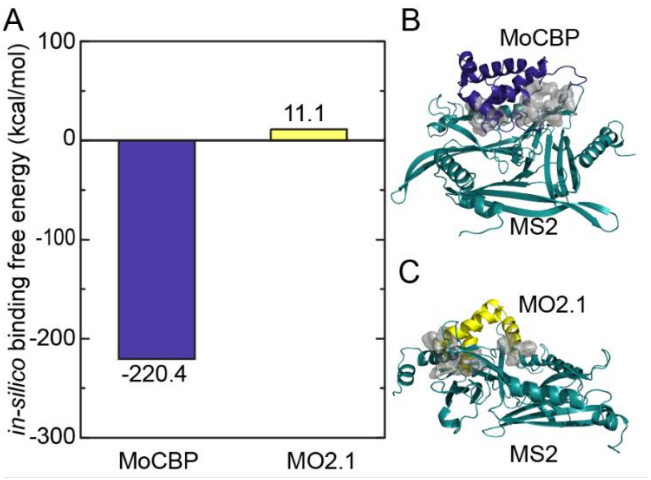

Figure 8. (A) Interaction energies between MoCBP (purple) and MS2 capsid protein (yellow). (B) Homology models of interaction between MoCBP/MS2 and (C) MO2.1/MS2.

Further docking experiments showed a specific binding pocket between MoCBP and MS2 capsid protein that overlapped a binding pocket between MoCBP and N-acetyl glucosamine (GlcNc), a monomer of chitin (Figure 9).

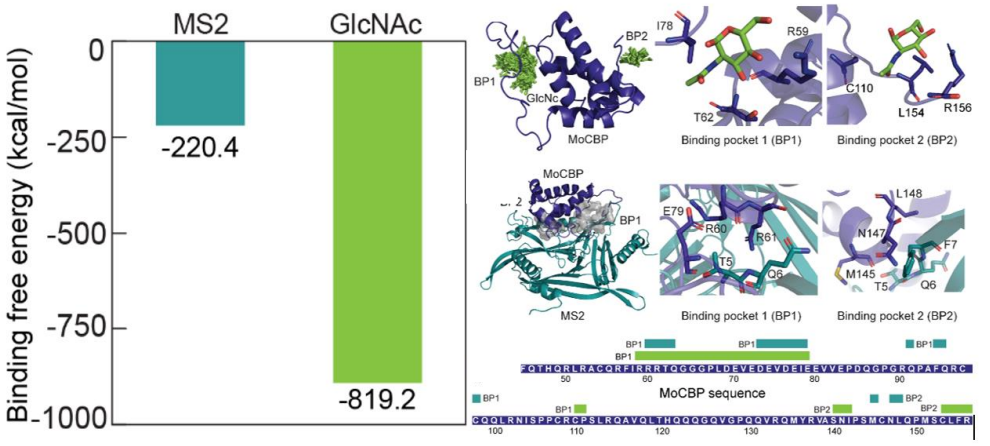

Figure 9. Comparison of binding energy between MoCBP/MS2 and MoCBP/GlcNAc and the corresponding binding pockets.

GlcNc Wash Experiments

Initially, we sought to separate the MO2.1 and MoCBP and coat them onto sand separately to test which one is responsible for virus removal. Unfortunately, attempts to purify the proteins by HPLC in a high enough amount were unsuccessful. These two proteins were probably so difficult to separate due to the high degree of similarity in their primary and secondary amino acid structure.

Another approach is to block the binding pocket of MoCBP with GlcNc (and chitin-binding protein). Our hypothesis was that this binding pocket is also the area where the virus adsorbs. We could confirm our hypothesis if the virus no long adsorbs when the pocket is blocked/. To test this, the *f*-sand columns were washed using a solution of GlcNc to block the binding pocket of MoCBP such that all virus adsorption sites were unavailable. We then tested both *E. coli* and MS2 removal in the GlcNc treated column. While the treated column was able to retain *E. coli* removal, the MS2 removal was completely absent (Figure 10).

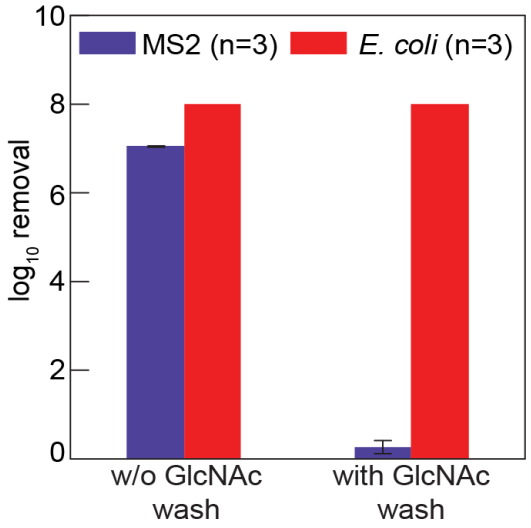

Figure 10. *E. coli* and MS2 removal in untreated vs GlcNAc treated columns.

The results of the GlcNAc wash experiments confirmed the hypothesis that the specific binding site in MoCBP is responsible for virus removal. Interestingly, the *E. coli* removal was

completely unaffected by a GlcNAc wash, indicating that bacteria removal is independent of the MoCBP binding site. Additionally, it seems that bulk electrostatic interactions between positively charged *f*-sand do not contribute significantly to virus removal while *E. coli* was primarily removed from this charge reversal mechanism.

We also performed further molecular docking simulations with MoCBP and the capsid proteins of human rotavirus and norovirus, two common waterborne viruses (Figure 11). Both configurations had highly negative binding free energies, indicating favorable interactions between MoCBP and the two viruses. This is convincing preliminary evidence that the high removal efficiency of MS2 by MoCBP can be extended to human enteric viruses.

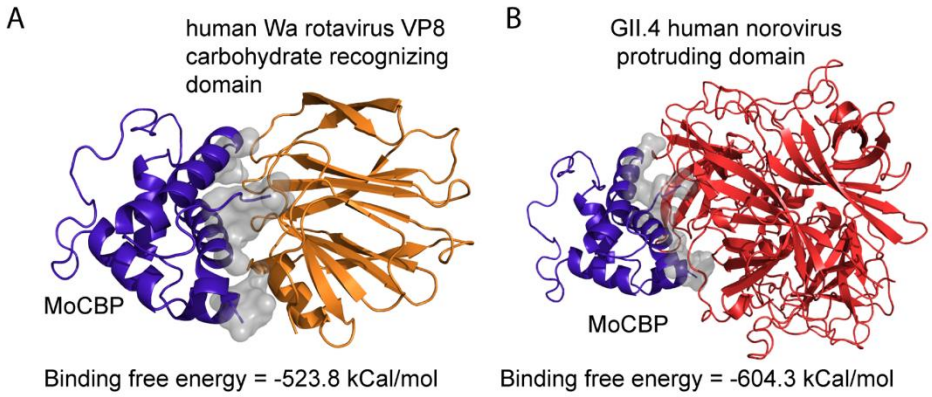

Figure 11. Molecular docking simulation results between (A) MoCBP and rotavirus and (B) MoCBP and norovirus

Chapter 4

Filter Scale-Up

A critical part of the development of the *Moringa* filter is scaling up the column from use in a highly controlled lab environment to a highly variable point of use environment. Material selection and column operating parameters are important considerations when designing a scaled-up filter that is effective, long-lasting, practical, and economical. The first preliminary scale-up prototype was designed, constructed, and tested in North Fort Myers, Florida at ECHO Global Farm. ECHO invited us to work with their Appropriate Technology group, which works to develop and disperse technology that improves sanitation, access to food and energy, and general standard of living in developing countries. We traveled to the farm over spring break in March 2018 to share our knowledge of designing a filter and perform the first field test of a large-scale filter.

Column Construction

The objective of the trip was to use locally available resources to construct a *Moringa* filter prototype and test its performance against locally available influent.

Selection of Materials

PVC was selected as the column material due to its durability and versatility. It is cheap and lightweight, allowing the columns to be constructed locally or transported to the desired location. However, in addition to PVC, thick bamboo segments were also used as the column material. Bamboo is naturally occurring in certain regions and can grow to various thicknesses, making it a potential candidate for constructing a sand filter. Perhaps the most important material that was chosen was the sand. The Appropriate Technology group at ECHO had sand that they

used to mix into clay to make bricks. We sieved this sand to separate the fines from the large grains to use in our columns.

Gravity was chosen as the driving force for flow because pumps and electricity necessary to power the pumps are not widely available in developing countries. This issue highlighted another critical scale-up consideration, as the column must therefore be designed with a hydraulic conductivity that optimizes the flow rate under gravity driven flow, be able to operate under a wide range of flow rates, or both.

Another important consideration we had to consider was how to keep the column as sterile as possible to prevent premature bacterial growth within the column. First, the sand was sterilized by boiling for 10 minutes before being coated. In the lab, sterile water is used to mix and coat the sand in order to prevent contamination or bacterial growth in the column. However, sterile water is, by definition, scarce in areas that this filter will be implemented. At ECHO, two sources of water were available: well water used for irrigation on the farm and municipal water for general use. To keep the column as sterile as possible in the field, municipal water was used for the *f*-sand preparation and column packing process, which highlighted a potential hurdle towards column scale-up. Constructing the column requires clean water, which may be scarce in areas where the column is supposed to be implemented. Processes to minimize the amount of clean water necessary to make the *f*-sand are therefore important to develop. One way to do this is to use an in-situ coating method, in which a high concentration Moringa serum is flowed over a bare sand column to coat the sand instead of using a batch coating procedure. This method can decrease the amount of water necessary for constructing the column by a factor of 10.[42] Once the prepared *f*-sand was packed into the column, the columns were mounted onto plywood and connected to a drip bucket that ECHO uses for a drip irrigation system (Figure 12).

Figure 12. Final column setup with mounted columns attached to drip system

Sedimentation Tests

As previously discussed, sand size is a critically important parameter for successful filtration performance. Since no means such as microscopes were available to measure the sand size directly, a rough estimate was used to determine the range in sand size by measuring the settling velocity. Through this measurement, the range of sand diameter was estimated to be 115-296 microns.

Gravity Filtration Results

ECHO maintained a highly turbid halibut pond on the farm, which was chosen as the influent for the column. The influent was allowed to settle for 24 hours to allow large, suspended solids to separate from the rest of the influent to prevent the column from clogging immediately. The remaining influent was still significantly discolored (Figure 13).

Figure 13. Pond water influent after allowing to settle for 24 hours

Effluent from the columns were collected in plastic water bottles such that their visual clarity could be observed. In both the PVC and bamboo columns, the *f*-sand effluent was significantly clearer than the bare sand effluent (Figure 14). The PVC *f*-sand column provided the best visual clarity out of all 4 columns while the bamboo columns overall performed worse from a visual clarity perspective.

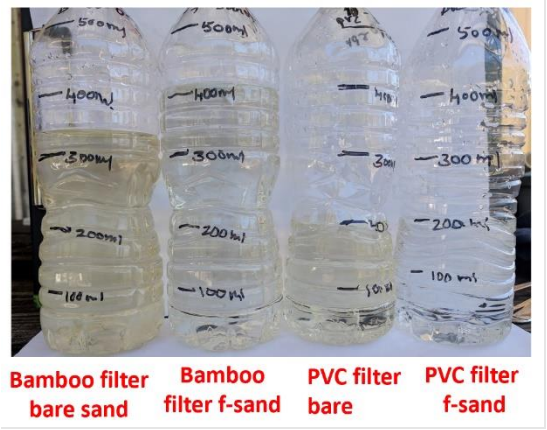

Figure 14. Collected effluent from field columns.

The most noticeable difference in the performance between the bamboo and PVC columns was the flow rate. Despite being similar sizes and using the same sand, under the same liquid head, the PVC filters had a flow rate of 4.7-4.9 mL/min compared to 8-10 mL/min for the

bamboo filters. At a higher flow rate, the contaminants are less likely to be removed, so it made sense why the bamboo filter was less effective. The reason for the higher flow rate in the bamboo filter was likely due to channeling in the bamboo column (Figure 15). Channeling on the walls of the column creates alternative flow paths that allow the water for flow around the sand bed without being purified. Though testing a bamboo filter was a worthwhile side experiment, it is likely that a practical filter would use more reliable materials for constructing the column such as PVC.

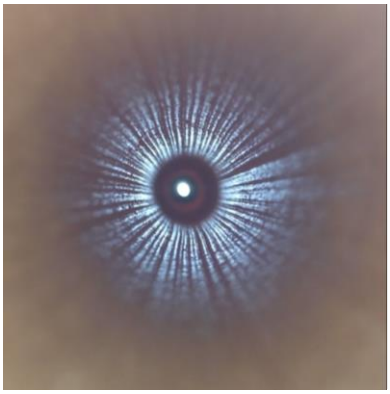

Figure 15. Channeling in bamboo column.

Samples of the effluents from the PVC columns were sent to a local lab for total coliform analysis to quantify bacteria removal (Figure 16). No detectible coliform was present in the *f*-sand effluent samples, corresponding to >2.1 log removal.

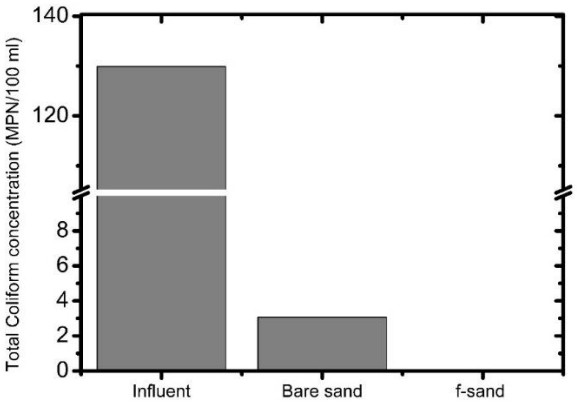

Figure 16. Total coliform concentrations from ECHO field experiments

After departing from the farm after our weeklong Spring Break stay, ECHO's appropriate technology group continued work on the *f*-sand column, building an even larger column and testing it over the course of several weeks (Figure 17). The columns were 10 cm in diameter and 50 cm in length. They used sifted sand between 60 and 120 mesh size, which corresponded to roughly 190 micron diameter sand. Similar to the previous columns, the filters were gravity driven using a drip bucket kit. The head pressure was made adjustable by fabricating a chain lift system that allowed the height of the buckets to be adjusted based on the desired flow rate. ECHO worked with Dr. Ashley Danley-Thomson in the Department of Environmental and Civil Engineering at Florida Gulf Coast University to characterize the performance of these columns when operating as a secondary filtration step to biosand filtration.

The filter was operated for 50 days from September to December 2018 and demonstrated beneficial removal of *E. coli* in the first 20 days. After 20 days, coliform counts in the *f*-sand filter began to rise and a foul odor was observed in the effluent, suggesting filter breakthrough. It is possible that as organic material was filtered out and caught in the *f*-sand filter, bacteria began to grow in the filter, causing the odor after a certain amount of time. These results, while preliminary, suggest that the filter may need to be cleaned periodically to prevent bacterial

growth from blocking adsorption sites and decreasing filter lifetime. Another option is to have a thorough prefiltration of organic matter to extend filter lifetime. Activated carbon and biochar prefilters is the subject of current further research in our lab.

Figure 17. Large scale Moringa filters constructed by ECHO Appropriate Technology

In-situ Coating Results

Up to this point, all *f*-sand was prepared using a batch method. Although effective in coating the sand, this process uses a significant amount of clean water, which may not be available in water stressed areas. Previous work in our lab showed that flowing 600 mM NaCl over the columns will desorb MO proteins from the sand. We combined the desorption procedure with a new in-situ coating procedure to attempt to demonstrate the feasibility of regenerating the columns.

Interestingly, the *f*-sand made from the in-situ process contained significantly more adsorbed protein than the batch process (Figure 18). Additionally, the concentrated serum (Case 3) resulted in the most adsorbed protein. To show regeneration capability, three cycles of desorption using 100 mL of 600 mM NaCl and adsorption using the in-situ coating procedure followed by MS2 removal experiments were conducted. These columns also demonstrated the same high virus removal efficiency, indicating that the in-situ coating procedure is not only more effective in coating the sand, but also just as effective in removing viral contaminants. This set of

experiments demonstrates that the desorption and in-situ coating process may allow for significant conservation of materials when scaling up the column.

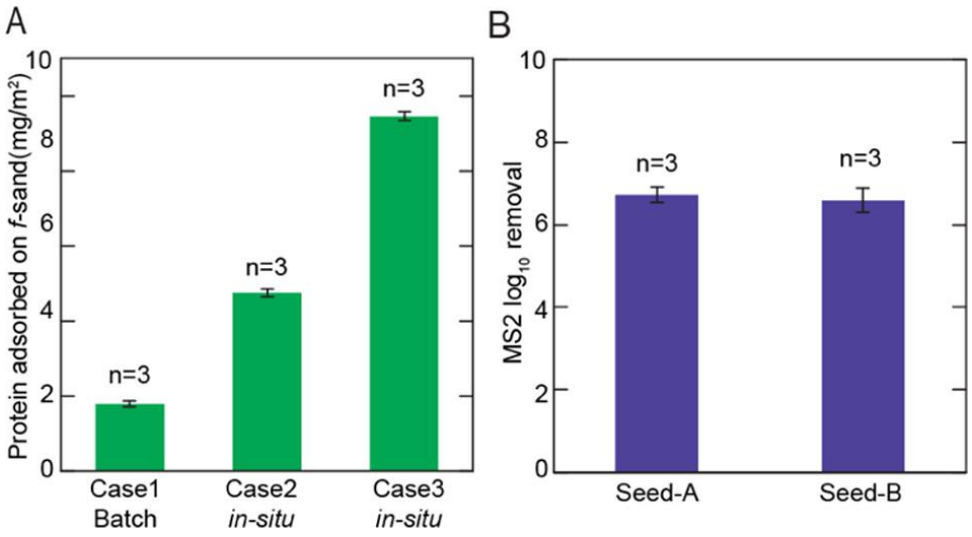

Figure 18. Results of in-situ coating experiments. (A) Case 1: batch coating procedure, Case 2: in-situ coating with 60 mL of 0.005 g seed/mL serum, Case 3: in-situ coating with 15 mL of 0.02 g seed/mL serum (B) MS2 removal of columns made using the in-situ coating process with two different seeds.

Chapter 5

Conclusion

Under optimized lab conditions, a Moringa coated sand filter demonstrates 7-log removal of MS2 bacteriophage, a model virus contaminant. SDS-PAGE and mass spectrometry analysis of the proteins coated onto the sand resulted in the discovery of 2 distinct proteins: MO2.1 and MoCBP. Molecular docking and GlcNAc wash experiments illuminated a specific removal mechanism of viruses by a binding pocket in MoCBP. The high performance on both viruses and bacteria suggest that the filter is a potential point-of-use solution to the clean water crisis in developing areas where Moringa is available.

Additionally, scale-up experiments were performed in the field at ECHO Global Farm. PVC and bamboo filters were constructed with local materials to simulate a practical application of the filter. The filters were tested using pond water, and the PVC *f*-sand filter demonstrated strong performance in removing coliform bacteria and improving the visual clarity of the water compared to bare sand. The ability of the filter to be built and operated in a wide variety of conditions is critical for it to be applied in a widespread manner. This field test was the first step of a long chain of scale-up development of the Moringa filter.

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ACADEMIC VITA

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EDUCATION

The Pennsylvania State University | Schreyer Honors College **University Park, PA**
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WORK EXPERIENCE

Serán Bioscience **Bend, OR**
Research and Development Intern June 2020 – August 2020

- Designed shell and tube heat exchanger that will eventually go into the design of a large-scale recycle spray dryer
- Developed process for API synthesis and formulation into an amorphous dispersion and implemented into GMP manufacturing
- Operated lab and manufacturing equipment, including HPLC, DSC, PXRD, DLS, PLM, jet mills, and spray dryers

Penn State Department of Chemical Engineering **University Park, PA**
Material Balances Instructional Aide (IA) August 2019 – December 2019

- Worked alongside Professor Andrew Zydney to organize materials for introductory material balances course
- Held biweekly office hours to assist students in homework, exam preparation, project calculations, and understanding material
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Early Phase Product Development Intern May 2019 – August 2019

- Developed pressure-flow curves for pressure swirl atomizers used in spray dried dispersions (SDDs) manufacturing
- Characterized glycoconjugate purification membranes using pycnometry, ^{1}H NMR cryoporometry, and flow rate measurements
- Evaluated refractive index effects on Malvern Mastersizer 3000 powder particle size measurements for inhalation grade particles

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Part-time Manager and Racket Stringer May 2019 – August 2019

- Repaired and customized rackets for both men's and women's NCAA Division I teams
- Maintained equipment inventory and detailed personal player records on equipment preferences

RESEARCH EXPERIENCE

Andrew Zydney Research Group **University Park, PA**
Undergraduate Researcher January 2020 – Present

- Investigated unorthodox flux decline profiles in virus filtration membranes for purification of monoclonal antibody solutions
- Developed protocol for zeta potential measurement of monoclonal antibody samples to inform tendency to aggregate

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Undergraduate Researcher January 2018 – Present

- Performed novel research on *Moringa oleifera* modified filters as a means for sustainable, point of use water purification
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Selected Publications and Presentations:

- Samineni et al. (2019). 7 Log Virus Removal in a Simple Functionalized Sand Filter. *ES&T, 53*(21), 12706-12714.
- Pei et al. (2019, November). Enhanced Virus Removal in a Practical Sand Filter. AIChE National Paper Competition
- 2019 AIChE Mid-Atlantic Regional Conference Paper Competition, 1st place presentation
- United Nations Students Seeking Solutions SDG #6 conference oral presentation (one of 6 finalists)
- "Protein fouling profiles and mechanisms in virus filtration membranes, a review" (internal review paper)

LEADERSHIP

Schreyer Honors Orientation (SHO TIME) **University Park, PA**
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- Led a group of 7 incoming freshman Schreyer students through orientation programs, seminars, and entertainment events
- Conducted leadership workshops and organizational meetings during the school year to prepare and train new mentors

Scholar Ambassadors **University Park, PA**
Co-chair and Active Member June 2018 – Present

- Led recruitment of prospective applicants and accepted students through tours, high school visits, overnight visits, and panels
- Worked alongside honors college staff to foster diversity, inclusion, and philanthropy across the Schreyer community
- Led the conversion of Spring 2020 in person recruitment events to virtual webinars amidst the COVID-19 pandemic

SKILLS, HONORS, & INTERESTS

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