THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF CHEMICAL ENGINEERING

SCALE-UP OF MORINGA COATED SAND FILTERS TO PRODUCE CLEAN DRINKING WATER

PAULA ESPINOZA SOTO
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Reviewed and approved* by the following:

Stephanie Butler Velegol
Associate Teaching Professor of Chemical Engineering
Chemical Engineering Department
Thesis Supervisor

Themis Matsoukas
Professor of Chemical Engineering
Chemical Engineering Department
Honors Adviser

* Signatures are on file in the Schreyer Honors College.

ABSTRACT

Clean drinking water is a global issue particularly in developing countries, affecting more than 844 million people around the world. The objective of the project is to scale-up and redesign a *Moringa oleifera* seed coated sand filter (f-sand) to remove bacteria from wastewater with locally accessible and affordable materials. Moringa seeds contain a cationic protein that will adhere to negatively-charged sand. This sand can then be used to can remove the pathogens present in the water due to electrostatic forces between negatively charged microbes and the positively-charged protein on the sand. For the purpose of scaling up these filter columns, the glass columns packed with 106 um glass beads used previously were replaced by glass columns packed with binary mixtures of larger sand sizes. These materials included recycled materials and more available and cost-effective materials from the field. The column diameter and effluent flow rate were adjusted according to the Clean Bed Filtration Model to obtain the EPA standard approved pathogen removal. To prove the functionality of the filters for bacterial removal, fluorescent *E. coli* bacteria was first utilized as the influent. The results showed that the higher the percentage of 106 um sand size, the higher the removal of bacteria the filter achieves. However, in a more realistic scenario, it was noted that when treating water spiked with *E. coli* and organic matter (TOC), the TOC competes with the bacteria for protein sites, impeding the proper removal. To address this problem, an activated carbon pre-filter was implemented. The results showed that, when this pre-filter is applied, the TOC is contained within the activated carbon particles, allowing the f-sand filter to function properly and remove bacteria above the EPA standard approved removal.

TABLE OF CONTENTS

LIST OF FIGURES

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Chapter 1

Introduction

1.1 Water-Borne Diseases and Water Treatment

Currently, 785 million people around the globe lack access to clean drinking water, according to the World Health Organization[1]. In addition, more than 2 billion people consume contaminated drinking water, which can result in lethal diseases such as diarrhea, cholera, polio, among others[1]. Some of these diseases caused by pathogens transmitted by water result in 6,939 annual total deaths, documented in 2017[2]. Figure 1 shows a representation of the water safety and scarcity around the world.

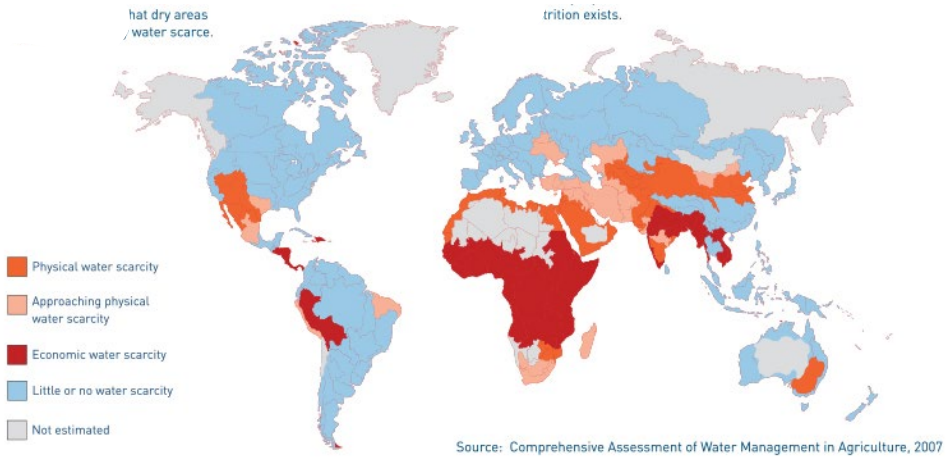

Figure 1. World Map of Water Scarcity[3]

As this represents a growing concern among populations, multiple water treatment methods and technologies have been developed. The employment of point-of-use technologies for household level water purification reduces diarrheal disease by 30−40%[4]; proposed techniques include boiling, chlorination, bio-sand filters, and ceramic filters[5]. However, chemical disinfection techniques may result in the formation of disinfection byproducts, which may have cytotoxic and carcinogenic activities[6]. Boiling water is an energy-exhaustive alternative, which can be economically unattainable, especially in developing countries[7]. Another widely used method is the coagulation process to remove water pollutants, preferable due to its high effectiveness and easiness of application on wide range of water types[8]. Usually, the most widely used coagulants are aluminum, ferric salts and, polyaluminum chloride (PACl). The challenge is that these chemical coagulants affect the water pH and generate significant amounts of non-biodegradable sludge and metal residues in the treated water[8]. Therefore, there is a strong need for sustainable, affordable and energy-efficient water treatment techniques that are derived from locally produced materials to provide safe water in the developing world. Fortunately, the *Moringa oleifera* tree, which seeds contain antimicrobial and coagulant properties, is prevalent in most of these underdeveloped countries and can serve as a potential solution to this matter.

1.2 *Moringa oleifera* Seeds

Moringa oleifera tree is often known as "The Miracle Tree"[11]. Its nutrient-dense leaves, which can be harvested and eaten, contain balanced levels of essential amino acids as well as high levels of protein, calcium, and vitamin A[11]. The seeds can be pressed to extract oil for cooking, cosmetics, and even biodiesel[11]. But most importantly, the seeds are of best interest for water

purification purposes. The *Moringa oleifera* tree (hereafter called Moringa) grows broadly in many equatorial regions of the world, as can be seen in Figure 2, precisely where public health is at risk due to unsafe drinking water conditions[9].

Figure 2. Regions where *Moringa oleifera* Trees grow

Moringa oleifera seeds have been used traditionally as natural flocculants to clarify drinking water[10]. These unique seeds contain cationic and antimicrobial proteins (*Moringa oleifera* cationic protein, MOCP) that form attractive electrostatic forces with the negatively charged pathogens present in water[16]. The antimicrobial properties are produced by the presence of a helix−loop−helix motif that causes fusion of inner and outer cell membranes[16]. The protein structure is shown in Figure 3[17]. The seeds were reported to have strong coagulative and antimicrobial properties on pathogenic strains such as of *Escherichia coli*, *Pseudomonas aeruginosa, Streptococcus faecalis, Salmonella typhi* and *Shigella dysenteriae*[10].

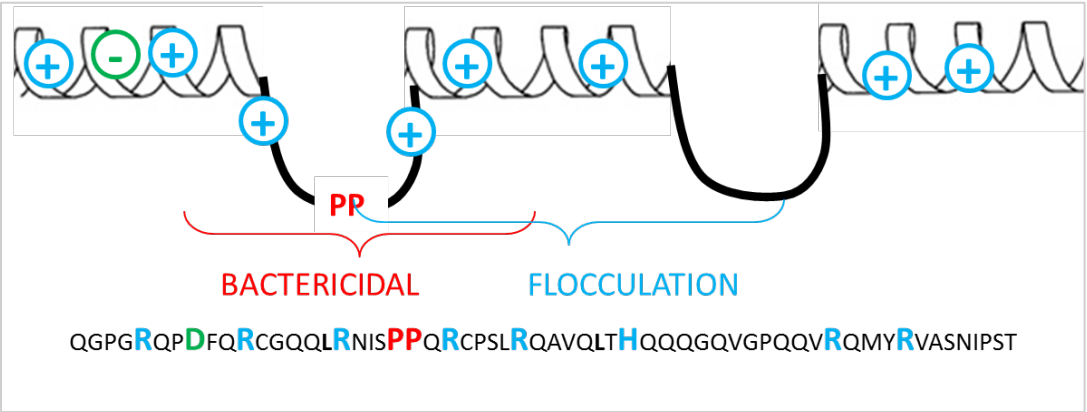

Figure 3. Structure of *Moringa oleifera* cationic protein (MOCP)[17]

Nonetheless, the Moringa seed application as a flocculant may be challenged by the fouling of treated water overtime as a result of the residual organic matter released from seeds[5]. Previous work performed in Dr. Stephanie Butler Velegol's lab studied a solution to this issue: MOCP can be adsorbed onto sand through electrostatic attraction forming what is called functionalized sand (f-sand). The antimicrobial and flocculating capability of the protein adsorbed onto the sand remains comparable to that of the original seeds, while the residual organic matter is eliminated[12]. For the purpose of this study, Moringa seed extract obtained by simply crushing the seed was used, rather than utilizing the isolated MOCP protein. The seed quantity and filter preparation time were minimized for simplicity of field applications. The filter performance was tested using *Escherichia coli* solutions. To determine the essential parameters for filter scale-up design, the classic Clean Bed Filtration Model (CBF) was used.

1.3 Clean Bed Filtration Model

Clean Bed Filtration Theory Classic Clean Bed Filtration Theory has been widely applied describe the colloidal/particle deposition and transport in saturated porous media. The extent of deposition can be estimated from the collector efficiency (η_0), defined as the probability of a particle striking a collector given the column specifics and hydrodynamic conditions. Equation 1 demonstrates the correlation between log removal and η_0:

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

where α is the sticking coefficient, d_C is the collector diameter, *f* is the column porosity, and L is the column length, η_0 is the collector efficiency, N is the effluent particle concentration, and N_0 is the influent particle concentration. The model developed by Tufenkji and Elimelech (TE model) was used to calculate the theoretical η_0 because of the superior agreement of the predicted values with experimental data[13]. The sticking coefficient, α, describes the probability of a particle sticking to a collector upon collision and has a theoretical range from 0 to 1. This model was used to obtain filter running parameters based on the desired log-removal.

Chapter 2

Purpose and Hypothesis of Experimentation

2.1 Purpose

The purpose of this experiment is to develop a simple, accessible, and affordable water filter made from natural resources: *Moringa oleifera* seeds and sand. The ultimate goal is twofold: 1. to achieve the correct and most cost-effective binary mixture of sand particle sizes to obtain the optimum removal of bacteria, and 2. to find a solution for removal of the organic matter that can cause the filters to foul.

2.2 Hypothesis

The first hypothesis states that as the collector (sand) size of the filters is increased, the removal efficiency of bacteria will decrease. However, a binary mixture of different sand sizes can reduce the gap between the bigger particles, increasing the porosity, and therefore improve the pathogen removal.

The second hypothesis is that a f-sand filter run with water containing organic matter (humic acid, HA, for this experiment) spiked with high concentrations of *E. coli* will not remove the desired removal of bacteria as the TOC is negatively charged and it will compete with bacteria for protein sites. This results in the saturation of the column and poor removal of desired pathogen. It is hypothesized that the activated carbon column will remove the TOC (humic acid) from the influent, while the f-sand column will remove the concentrated *E. coli*.

Chapter 3

Materials and Methods for the Preparation of f-sand Filters

3.1 Bacteria Growth and Preparation

E. coli Fluorescent Bacteria

E. coli strain TG1 containing plasmids that express red fluorescent protein (pCA24N-rfp-lasR) were used as model pathogens at an influent concentration of 10^6 - 10^8 colony-forming units (CFU)/mL suspended in 10-fold diluted phosphate-buffered saline (PBS) buffer (0.016 M). Culture medium chemicals were removed from the cell suspension by rinsing pellets three times with PBS buffer. This stock is stored in a -80 ºC freezer.

LB Plates Preparation

Multiple base agar plates are prepared in order to quantify the number of *E. coli* per mL of the influent and effluent. We measured this as CFU (colony forming units). Initially, a solution is prepared using 25 g Tryptic Soy Broth powder and 15 g agar in 1 L of nanopure water. This solution is separated into two 1L flasks that each contain 500 mL of the solution. The flasks are autoclaved in liquid cycle at 121 °C (15 psi) for 30 min. Later, the solution is allowed to cool down to warm temperature (without letting it solidify) and 0.3 mL of antibiotic stock is added to each 500 mL flask. To prepare the plates, roughly 20 mL of solution is poured into multiple petri dish plates of 100 mm diameter until the solution is done

E. coli Stock Plates

The stock of *E. coli* mentioned above is taken from the freezer and transported to a laminar hood. The loop end of a sterile disposable needle is dipped in the stock and used to streak the culture on an LB plate, following the pattern shown in Figure 4. The plate is stored in a 37 ºC incubator overnight and used for the bacterial media preparation procedure (explained next).

Figure 4. Dilution pattern to streak the *E. coli* culture on a plate

Bacterial Media Preparation and Influent Growth Procedure

Two batches of 50 mL each of liquid culture are prepared using the ratio of 25 g Triptic Soy Broth powder for 1 L of nanopure water. Each 50 mL batch is poured in a 125 mL flask and autoclaved in liquid cycle for 30 minutes at 121 °C and 15 psi to sterilize the solution. This media obtains a yellow clear color. The flask is covered with foil and placed in a biohood and contents in the flask are let cool to room temperature. 30 µL of antibiotic stock is then added to the 50 mL of media and mixed to assure bacterium other than *E. coli* are not present in the solution.

Once the liquid media is prepared, a single colony of *E. coli* bacteria from the stock plate is poked using a disposable plastic needle. The needle is dipped into the flask and gently stirred to get the culture into the liquid. This flask is then covered with foil and stirred at 250 rpm in a 37 °C incubator for around 12 hours or more. The liquid is centrifuged twice in a 50 mL sterile falcon

tube at 4000 rpm for 5 minutes each time and rinsed with a phosphate-buffered saline solution (PBS), obtaining 50 mL of $10^8 – 10^7$ *E. coli*/mL concentrated solution which serves as the filter influent.

3.2 Filter Development, Preparation, and Experimentation for water spiked with *E. coli* influent

M. oleifera Seeds Serum Preparation

Various batches of *M. oleifera* seeds from the same origin were used in this work. The seeds were received from Echo Global Farm, Florida. All seeds were stored at room temperature in a sealed bag and crushed before experiment for preparing f-sand columns to ensure that the technique was robust under practical conditions.

Briefly, 0.3 g of unshelled whole *Moringa oleifera* seeds were crushed using a coffee grinder and mixed with 15 mL of deionized (DI) water for 5 min (as determined by previous work[5,16]) to create a protein rich extract. The obtained water extract was filtered through a 1.5 μm glass fiber filter to remove seed debris. This 15 mL solution serves as the serum for the coating of one glass column. A schematic of the batch process to prepare Moringa seed serum is shown in Figure 5.

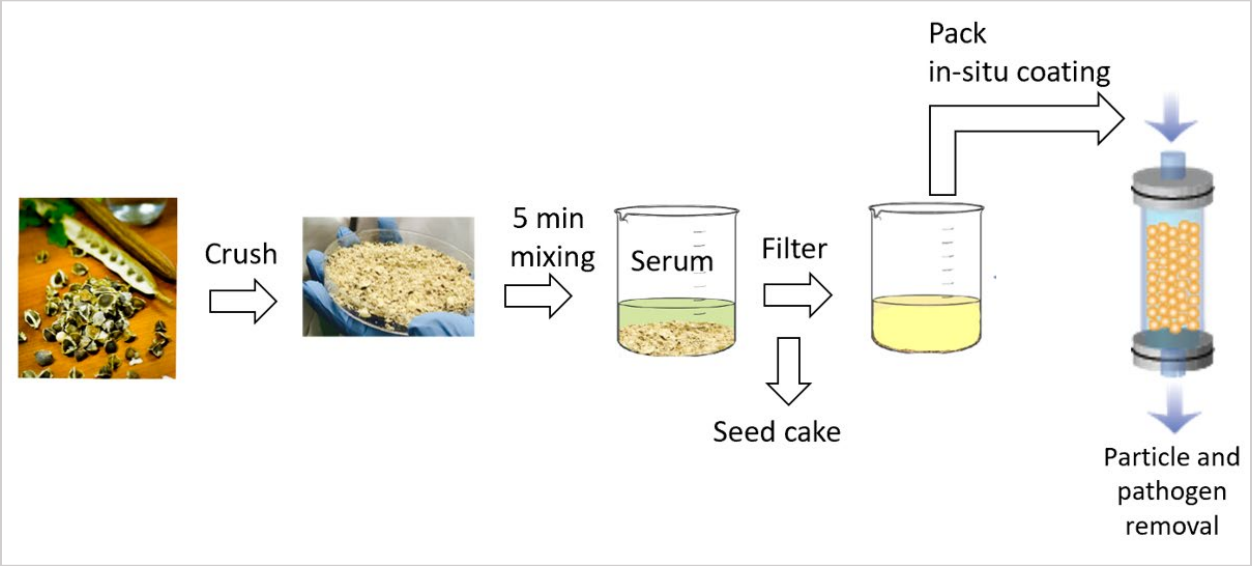

Figure 5. Preparation of Functionalized Sand

Moringa Coated Sand Filter Preparation (f-sand) and Experimentation

For the purpose of testing collector sizes binary mixtures, a total of 25 g of sand consisting of sieved sand (106 μm) and crushed glass (175 μm) particles are boiled until the water appears clear. These are then rinsed and packed into a 10 cm height, 1.5 cm diameter glass column and coated with 15 mL of Moringa seeds serum using in-situ technique with a flow rate of 1.6 mL/min. This results in a positively charged packing material which will be referred to as functionalized sand (f-sand).

After the column has been packed and in-situ coated with the moringa serum, the filter is then equilibrated for 20 minutes at 1.6 mL/min using the PBS buffer solution. The resulting 50 mL solution of 10^8 *E. coli*/mL concentration mentioned above is dissolved 100 times in a 10 times diluted PBS to use as the influent. The columns are then ready to start the removal of bacteria and the inlet is changed to the bacteria influent, run at the same 1.6 mL/min flow rate. Samples of the

influent at the inlet are taken, as well as the outlet at column pore volumes (~7 mL) of 4 (28 mL), 6 (42 mL), and 8 (56 mL). Therefore, each column requires roughly 60 mL of influent.

The influent sample and the outlet samples taken at different pore volumes are plated in the previously prepared LB plates, pouring 100 mL of the sample and spreading it with a cell spreader. These plates are placed in a 37 ºC incubator for ~12 hours to allow the bacteria to grow. Since fluorescent bacteria is used, the colony forming unit (CFU) can be quantified by pink dots grown in the plate. Finally, the CFU on the influent and effluent sample plates are counted and the removal is computed using the following equation, were N and N_0 represent the CFU/mL of the effluent and influent, respectively.

$$\log_{10}\text{removal}_{experimental} = -\log_{10}\frac{N}{N_0} \quad \textbf{(2)}$$

3.3 Hydraulic Conductivity Experimentation

The hydraulic conductivity of each binary mixture of sand sizes was tested to predict bacterial removal efficiency. The experiment was carried out by running DI water through the bare sand packed glass columns (testing different binary mixtures) from a graduated cylinder placed at a higher level (refer to Figure 6 below). The outlet flow rate (mL/min) of the columns was measured at different heights (head lengths). This flowrate was then used to calculate the hydraulic conductivity using the following equation:

$$K = \frac{QL}{Ah_L} \quad \textbf{(3)}$$

where K is the hydraulic conductivity in cm/min, Q is the outlet flow rate in mL/min, L is the length of the column in cm (10 cm), A is the surface area of the column in cm^2 (using a 1.5 cm diameter), and h_L is the head in cm.

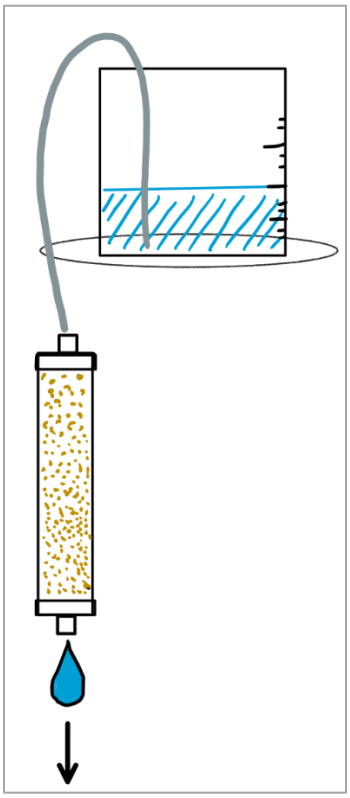

Figure 6. Hydraulic Conductivity Experiment Set-Up

3.4 Preparation and Experimentation for Humic Acid (TOC) spiked with *E. coli* influent

To execute the experiment of *E. coli* removal with TOC present in the influent, all the previously mentioned steps in Section 3.2 above must be carried out in addition to the following:

Influent Preparation

The influent solution replicating organic matter is prepared by adding 58.4 mg of NaCl to 1L of DI water. This solution is autoclaved in liquid cycle at 121 °C (15 psi) for 20 min. Once the liquid solution cools down, 5 mg of humic acid powder are added and stirred overnight, resulting in a 5 mg/L humic acid concentration solution. This solution is then used instead of the 10-fold diluted PBS to dilute the concentrated 50 mL *E. coli* solution by 100 times. This way, an influent that contains both *E. coli* and humic acid is produced.

Activated Carbon Pre-Filter Preparation and Experimentation

To be able to remove the organic matter (TOC) from the water before it is run through the sand column, an activated carbon column must first be prepared as a pre-filter. Roughly 25 g of granular 1.5 mm diameter activated charcoal is weighed (enough to reach the 10 cm height of the column), sieved to achieve a size of 500-2000 µm particles, and boiled until the water appears clean (about 15 minutes). This amount is later rinsed and packed into the glass column without the need of coating or equilibration.

The *E. coli* removal was quantified in this carbon column, taking samples at volumes 25 mL, 37 mL, and 50 mL and plating them as previously explained. Figure 7 depicts the experiment set-up.

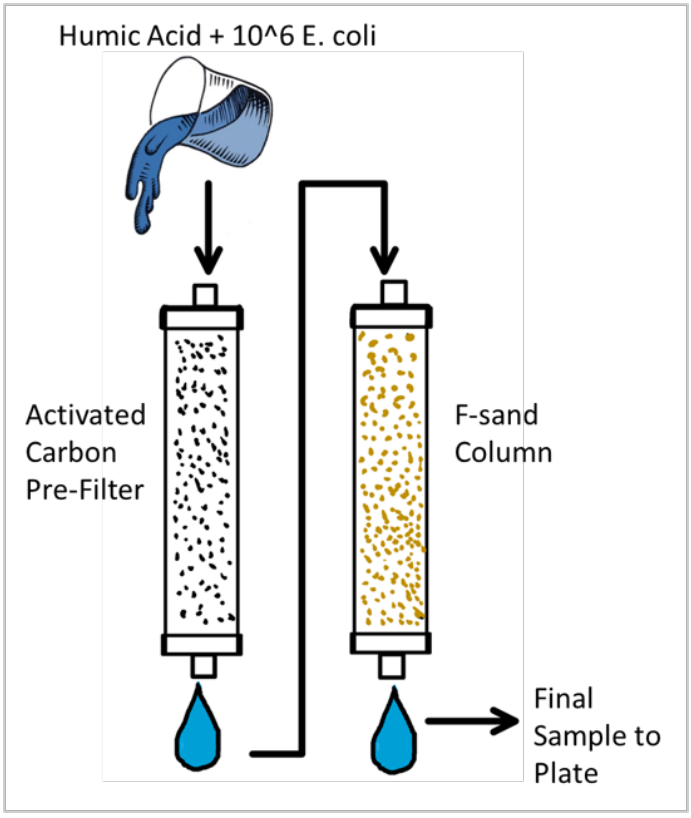

Figure 7. Pre-Filter set-up for TOC and *E. coli* removal

Chapter 4

Experimental Results and Discussion

3.1 Binary Mixtures of Different Sand Sizes

In order to scale-up the laboratory sized filters, multiple parameters are important to consider such as column diameter, flow rate, and collector size. Typical slow sand filters have size sizes above 150 micron[14] while the smallest size of sand in a rapid sand filter is 400 micron. However, both these sands are too large for the scale of this experiment and will not work in the proposed columns. In this study, lab-scale filters were first analyzed by testing the effect of larger sand sizes as packing materials on bacterial removal from water and compared to the prediction from the Clean Bed Filtration Model. For this purpose, three different sand sizes, 106 μm, 175 μm, and 256 μm diameter were tested for *E. coli* removal. Figure 8 shows the experimental data of log removal of bacteria as a function of collector diameter size. Here the upper and lower models are the highest and lowest of the hydrodynamic models for η_o and represent the Ma model[18] and TE model[13], respectively. As can be observed, the efficiency of the filters on bacterial removal decreases as the collector size increases in diameter, just like expected with the model. This occurs due to the increase in permeability and decrease in porosity as the particle size increases.

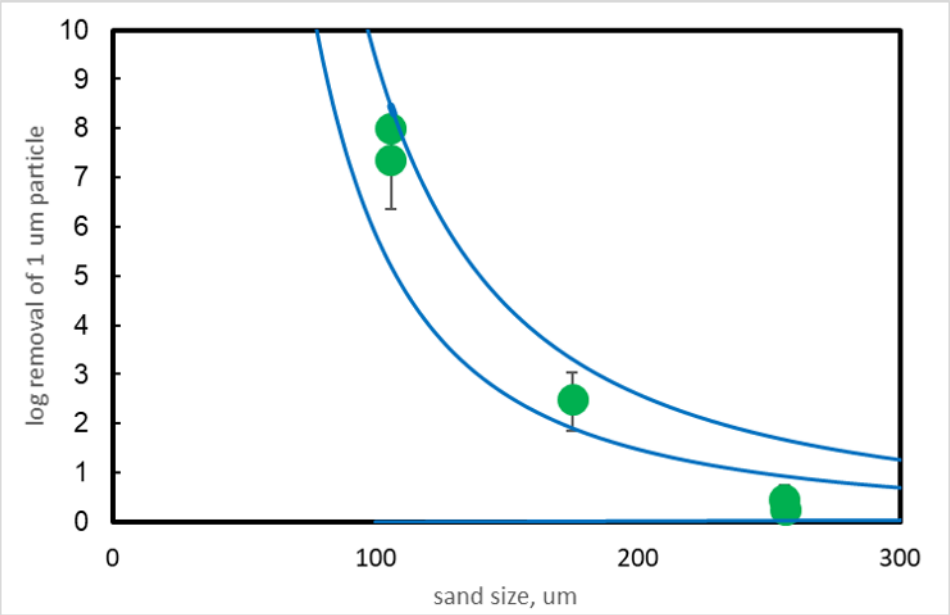

Figure 8. Log Removal of 1 µm particle as a function of sand (collector) size. The upper and lower curves are the highest and lowest of the hydrodynamic models for η_o and represent the Ma model[18] and TE model[13], respectively.

As observed in the previous figure and according to the proposed model, particle sizes greater than 106 µm are not viable as a packing material for the f-sand filters since the results are lower than the desired log removal of 6. Yet, using only 106 µm diameter size glass beads for this purpose is not only unrealistic for bigger filters but it comprises a much higher overall cost. To circumvent the issue with particle size, binary mixtures of sand sizes were designed. A mixture of sand sizes decreases the porosity and gap between the sand grains, allowing for a more efficient pathogen removal. The binary mixtures used were composed of 106 micron sieved sand and 175 micron crushed glass.

To further predict the collector efficiency of these binary mixtures in removing small particles from fluids, the hydraulic conductivity of different ratios of mixtures was obtained. Figure 9 demonstrates that the hydraulic conductivity (or resistance to flow) decreases with an increase

in the mass percentage of 106-micron sand. A low hydraulic conductivity is optimal for the purpose of bacterial removal as more resistance to flow is needed to contain the bacteria within the pores of the packing material. Below a mass fraction of 50% 106 micron sand, the hydraulic conductivity decreases with mass fraction. Above this point the hydraulic conductivity is relatively the same as pure 106 micron (100%). Based on this information, mixtures above 50% 106 µm sand are expected to achieve the desired bacterial removal.

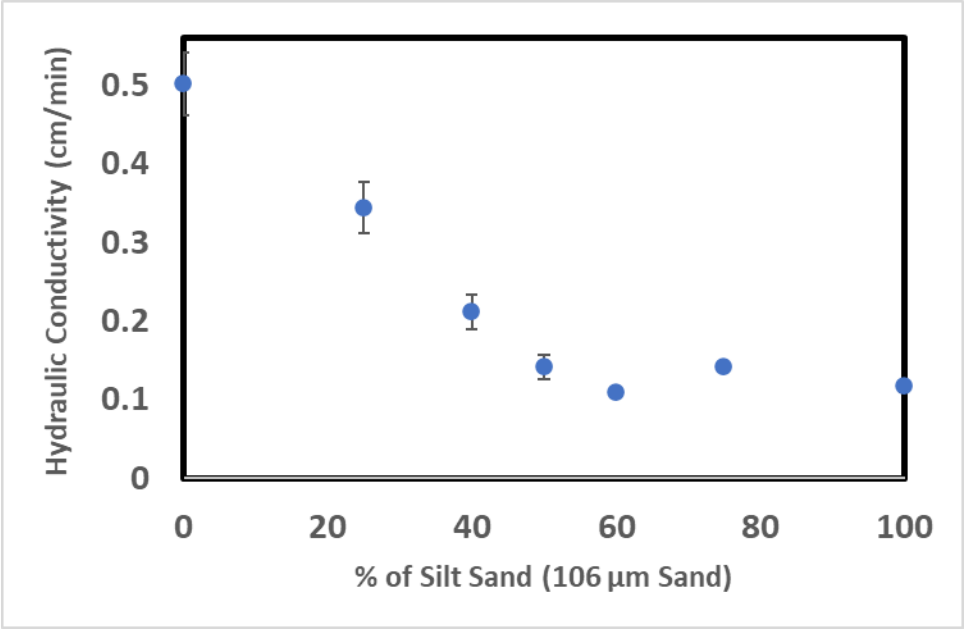

Figure 9. Hydraulic conductivity of sand particles as a function of percent of silt sand (106 um) in a binary mixture

After obtaining the hydraulic conductivity of several binary mixtures to predict removal efficiency, it was then necessary to test the effects of this mixture of collector sizes on bacterial removal. Different percentages of 106 µm sieved sand mixed with 175 µm glass powder were used as packing materials and 10^6 *E. coli*/mL influent was run through the columns. As can be observed in Figure 10, the removal of bacteria increases as the percentage of 106 µm particles increases, as

predicted by the model. This is due to the decrease in permeability. It can be observed that after a percentage of 40% of 106 µm sand, the desired EPA standard log removal of 6 is achieved. This is closely related to the 50% 106 µm results obtained from the hydraulic conductivity tests. Based on this information, to obtain an affordable yet effective filter, the optimal binary mixture was determined to be 40% of 106 µm sieved sand with 60% 175 µm glass powder. This judgment considers the lowest possible percentage of 106 µm sand with a minimum log-removal of 6.

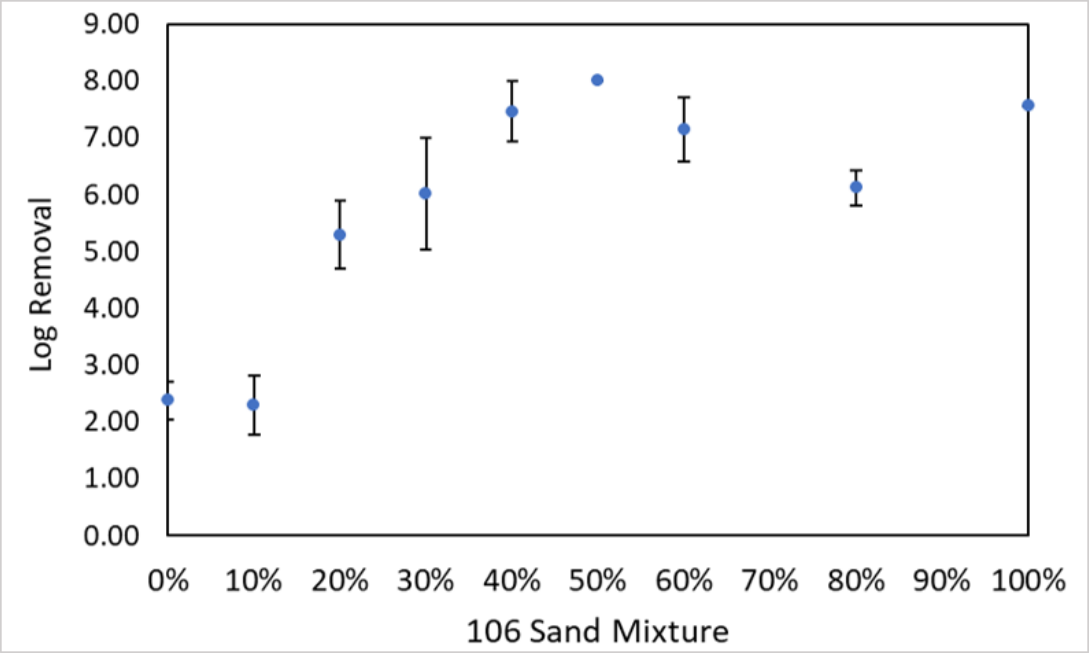

Figure 10. Log Removal of *E. coli* as a function of 106 µm sand size percentage

3.2 Removal of Bacteria using Humic Acid Solution Spiked with *E. coli* as the Influent

The presence of organic matter in ordinary dirty water is another realistic issue that must be addressed when attempting to remove pathogens using Moringa coated sand filters. The Total Organic Carbon (TOC), or organic matter, is also negatively-charged and causes the filters to clog

because it competes for protein sites with bacteria. This occurs mainly due to electrostatic forces between the cationic protein of *Moringa oleifera* and the negative charge on both TOC and bacteria. As a solution to this problem, activated carbon was introduced as a pre-filter to remove the organic matter. This removal process is due to hydrophobic interactions between AC and TOC.

To assess the efficiency of these pre-filters on removing organic matter from water, three different columns were tested: 1. Activated carbon alone, 2. Activated carbon pre-filter followed by f-sand filter, 3. One f-sand filter alone without a pre-filter. Figure 11 below illustrates the experimental procedure set-up of the experiment.

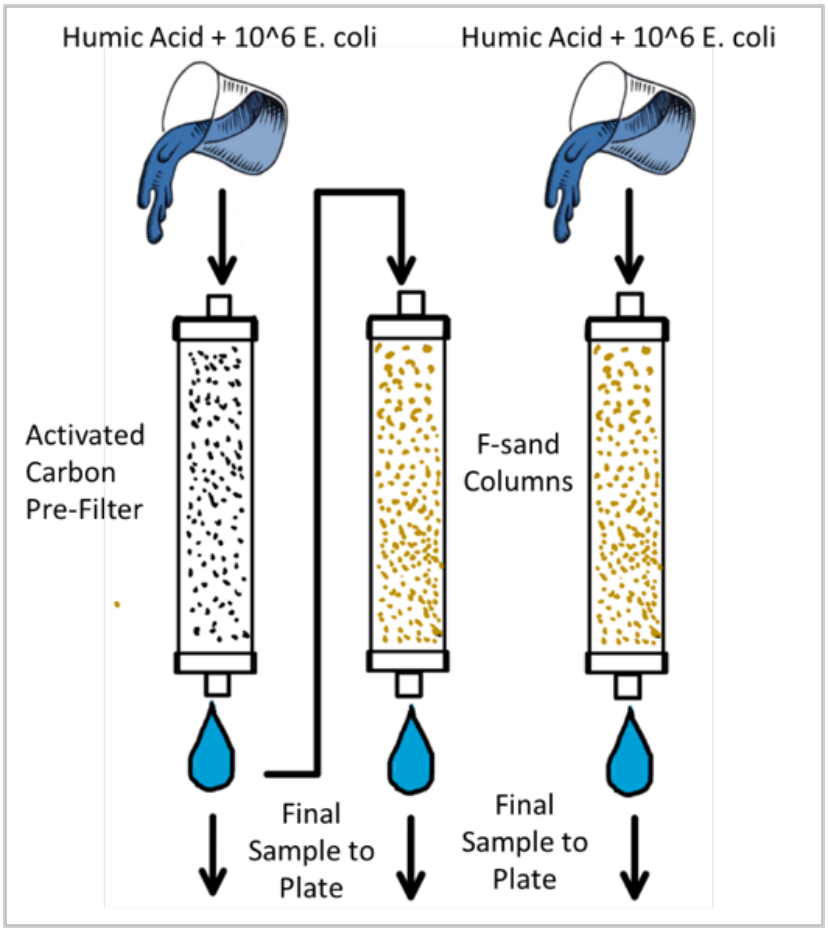

Figure 11. Experimental procedure to remove TOC from water and test efficiency on bacterial removal

The influent containing 5 mg/L of humic acid and 10^6 *E. coli*, and the final effluent samples obtained from each column were plated to assess the removal of bacteria *E. coli.* Figure 12 illustrates the results from these experiments. It can be observed that the f-sand column alone without the pre-filter process is not capable of removing bacteria from water with TOC due to the clogging of the column. As a matter of fact, the bacterial removal is as low or lower than that of the AC pre-filter, a filter not even designed to remove such small particles. However, when a pre-filter is used before running the f-sand filter with water and TOC, the f-sand column performs as expected, achieving a log removal higher than 6. This occurs because the AC pre-filter can remove a significant amount of organic matter from the water before it enters the actual filter.

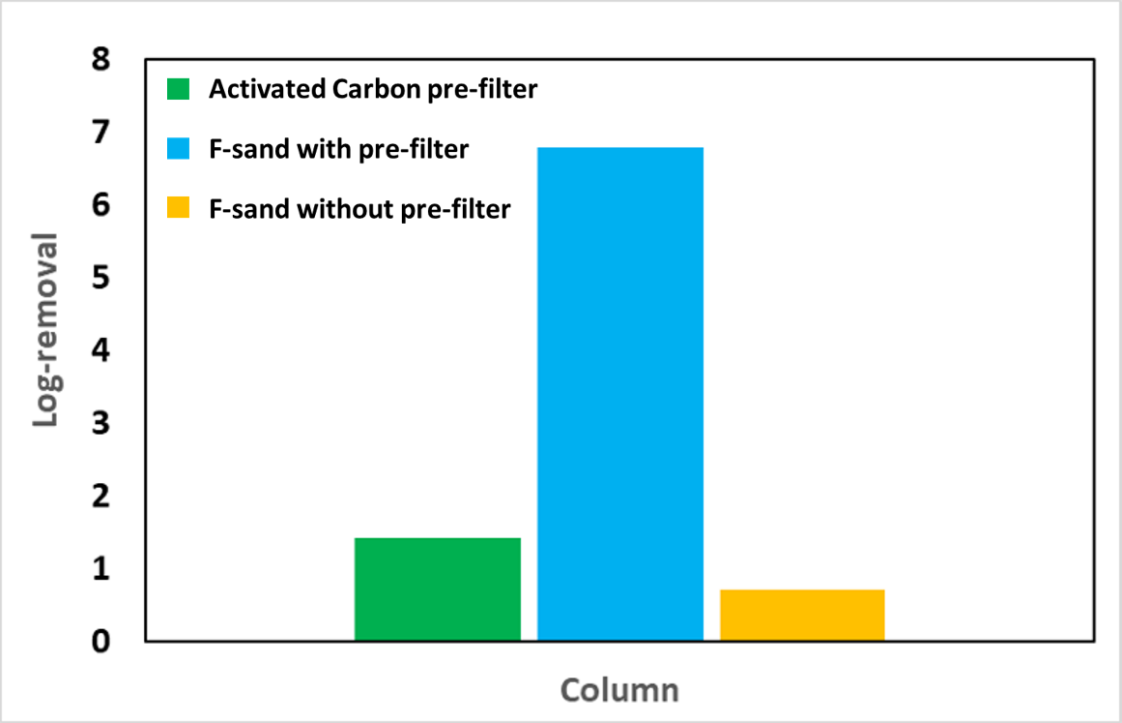

Figure 12. Log removal of *E. coli* of f-sand with pre-filter vs. f-sand without pre-filter

To further assess the feasibility and durability of the pre-filter/filter system, a breakthrough experiment was carried out using the same setup of f-sand with pre-filter and f-sand without pre-filter as in Figure 11. The two sets of columns were assessed by taking samples from both f-sand filters every 5 pore volumes, starting at pore volume of 10 and concluding at 60. Figure 13 shows the results of this experiment. It can be observed that the f-sand column with pre-filter starts breaking through at about 50 pore volumes, while the f-sand without pre-filter starts at about 25 pore volumes. This confirms that the presence of the organic matter blocks the sites available for the bacteria and reduces the removal.

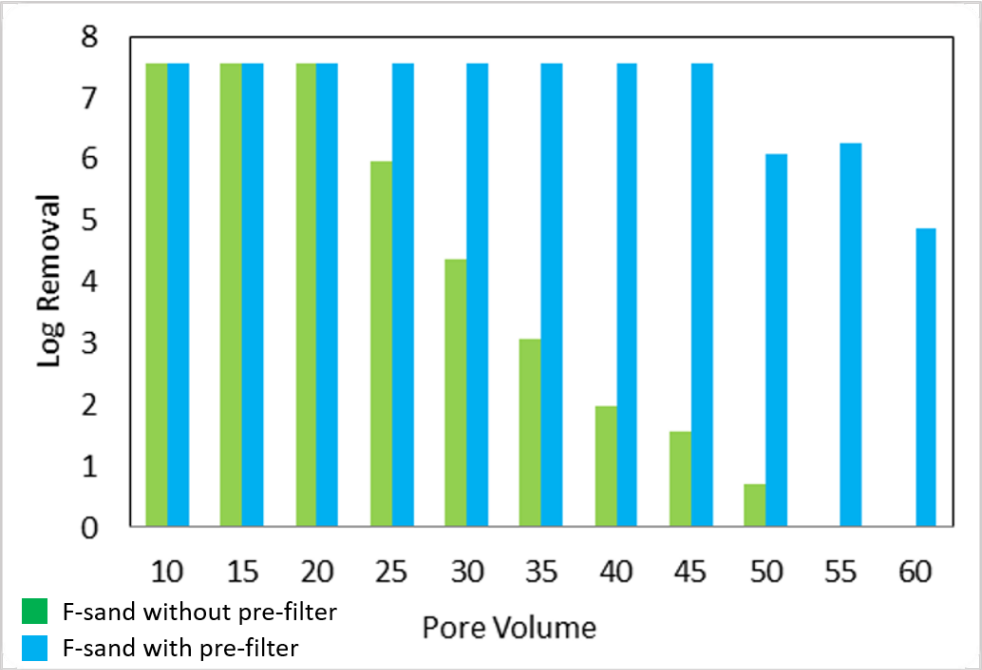

Figure 13. Breakthrough experiment of f-sand with pre-filter (blue) vs. f-sand without pre-filter (green), illustrating the Log Removal of bacteria as a function of column pore volume.

Chapter 5

Implementation Challenges and Future Work

The application of f-sand to point-of-use water purification in developing countries is still limited for the following reasons: (1) limited removal (<1 log removal) of pathogens at dilute concentrations (commonly occurring in surface water) by larger particles in f-sand suspensions due to mass transport limitations[5], (2) a lack of optimization of seed dosage and preparation procedures, and (3) the low practicability of sand of small size as a packing material for scale-up.

As sand with diameters less than 100 micron is relatively expensive, hard to manage, and not widely accessible, a different packing material is being studied by the lab team. This packing material consist of store-bought cotton balls, which are widely available, easy to handle, and inexpensive. It is advantageous over sand because it contains a much higher surface area that allows for a better electrostatic interaction with the moringa cationic protein. In addition, initial experiments show that the flow rate can be increased almost ten-fold and the column has a greater breakthrough capacity. Work is being done at the lab to test the TOC effects on bacterial removal for this material as well.

In addition to alternative packing materials for the moringa filter columns, biochar is being studied as a replacement material for the activated carbon used as a TOC removing pre-filter. This material is more cost effective and sustainable.

Regarding the construction of the columns, work is being done to implement a column scale-up utilizing PVC pipes instead of glass columns and changing the column diameter from 1.5 cm to 5 cm. This allows for the flow rate to increase from 1.6 mL/min for the lab-scale columns to about 17 mL/min.

Chapter 6

Conclusion

In summary, the primary purpose of this paper was to demonstrate the possible development of a scale-up Moringa coated slow sand filters using a binary mixture of sand sizes as the packing material to remove pathogens from water and result in clean drinking water. For the purpose of this investigation, binary mixtures of 106 μm and 175 μm sand diameter sizes were tested to obtain the hydraulic conductivity, residence time, and bacterial removal efficiency of these filters. Results showed that the optimal binary combination, meaning the lowest amount of 106 μm sand percentage with the highest log-removal, was 40% 106 μm with 60% 175 μm sand resulting in greater than 6 log-removal (EPA standard for drinking water).

Additionally, the impact of naturally occurring organic matter from water running through the filters was assessed. The results demonstrated that TOC present in water causes the f-sand columns to clog since it competes for protein sites with bacteria. However, as the *E. coli* water influent containing organic matter is run through an activated carbon pre-filter beforehand, the f-sand filters perform with the desired efficiency, reaching a log-removal greater than 6.

Future work is however needed for the full practical implementation of this technology. This includes building a real and practical scale-up model of the laboratory filters, studying the performance and lifetime of the filters, and performing long term experiments. In addition, testing different packing materials for the optimal efficiency of the columns. Another application of f-sand technology is to use Moringa coated masks for the COVID-19 pandemic. These masks would potentially be able to filter through the virus.

Appendix A

Hydraulic Conductivity of Binary Sand Mixtures Raw Data

Hydraulic Conductivity Experiments

Glass powder 175	**Height h_L (cm)**	**K1 cm/min**	**K2 cm/min**	**K3 cm/min**	**Flowrate (mL/min)**
	42	0.5676357886	0.5654800391	0.5742781917	93.86888889
	41	0.5735581845	0.5486730799	0.565291411	90.56744268
	40	0.5618381698	0.5409570412	0.5493927585	86.50835979
	39	0.5639544317	0.5190319288	0.5383714892	82.77174603
	38	0.5514541939	0.5276125973	0.520559609	79.56843034
	37	0.5426371057	0.5097852313	0.506816706	75.51844797
	Area (A)	A= pi/4 d^2 [=] cm^2	19.63495408	0.001963495408	m^2
	Length (L)	[=] cm	5	0.05	m
	diameter (column)	[=] cm	5	0.05	m
	diameter (collector)	[=] um	175	0.000000175	m
Silt	**Height hL (cm)**	**K1 cm/min**	**K2 cm/min**		**Flowrate (mL/min)**
	42	0.113879488	0.123696906		19.59216667
	41	0.1169717234	0.1255993877		19.52777778
	40	0.1221001356	0.1125444728		18.42894444
	39	0.1242660031	0.1079453212		17.78188889
	38	0.1213680228	0.1028353139		16.72844444
	Area (A)	A= pi/4 d^2 [=] cm^2	19.63495408	0.001963495408	m^2
	Length (L)	[=] cm	5	0.05	m

	diameter (column)	[=] cm	5	0.05	m
	diameter (collector)	[=] um	106	0.000000106	m
Echo	**Height hL (cm)**	**K1 cm/min**	**K2 cm/min**		**Flowrate (mL/min)**
	42	0.6444311307	0.6563707492		107.2729778
	41	0.6432298524	0.6460476799		103.7911111
	40	0.6284608534	0.6260954572		98.53262222
	39	0.6180802438	0.6157946519		94.4856
	38	0.6061311207	0.6036543348		90.26551111
	Area (A)	A= pi/4 d^2 [=] cm^2	19.63495408	0.001963495408	m^2
	Length (L)	[=] cm	5	0.05	m
	diameter (column)	[=] cm	5	0.05	m
	diameter (collector)	[=] um	175	0.000000175	m

Percent silt	**K**
0	0.539254
	0.54488
	0.533746
	0.535757
	0.523881
	0.515505
	0.509337
	0.504905
	0.505698
	0.510759
	0.537206
	0.521239
	0.513909
	0.49308
	0.501232
	0.484296
	0.485683

	0.460945
	0.424506
	0.376639
avg 0	0.501123
25	0.419049
	0.381108
	0.355874
	0.322389
	0.313876
	0.345139
	0.342492
	0.328832
	0.316277
	0.315167
avg 25	0.34402
avg 40	0.211439
error 40	0.021946
50	0.180764
	0.155206
	0.143455
	0.140392
	0.126893
	0.13029
	0.133404
	0.13601
	0.132142
	0.12777
avg 50	0.140633
75	0.150747
	0.147252
	0.143994
	0.139592
	0.134959
	0.144396
	0.140658
	0.136695
	0.131974
	0.134891
avg 60	0.109191
error 60	0.004534
avg 75	0.140516
100	0.113879

	0.116972
	0.1221
	0.124266
	0.121368
	0.123697
	0.125599
	0.112544
	0.107945
	0.102835
avg 100	0.117121

Log Removal Experiments

	Colony Forming Units					
	4/17/2019			**4/3/2019**		
Percentage 106 silt	**PV4**	**PV6**	**PV8**	**PV4**	**PV6**	**PV8**
0%	430000	35000	45000			
10%				1100000	6600000.00	76000.00
20%	19	65	171	0	2500	0
30%				0	0	450000
40%	2	4	24	250000	300000	0
50%						
60%	2	6	35			
80%						
100%						
Influent	14000000			48000000		

Colony Forming Units								
4/24/2019			**10/20/2018**			**11/8/2018**		
PV4	**PV6**	**PV8**	**PV4**	**PV6**	**PV8**	**PV4**	**PV6**	**PV8**
21000	90000	311000						
10	10	0						
0	0	0						
						0	0	0
10	30	30						
			0	0	0			
30000000			3.1E+08			31000000		

Appendix B

TOC Removal Experiments Raw Data

Log Removal Experiments

	1 (charcoal)			Column 2 (post-charcoal)		
	Original	**10^4 diluton**	**Original calculated**	**Original**	**10^2 dilution**	**Original calc**
4 PV	high	2	2.00E+05	0	N/A	0
6 PV	high	3	3.00E+05	0	N/A	0
8 PV	High	2	2.00E+05	0	N/A	0
	Influent	6.00E+06				

	1 (charc)			column 2 (post charc)			column 3 (control)		
	Original	**10^4 dilution**	**Original calculated**	**original**	**10^2dilution**	**Original calc**	**original**	**10^4dilution**	**Original calc**
4 PV	high	14	1.40E+06	high	3	3000	high	112	11200000
6 PV	high	16	1600000		9	9000		120	12000000
8 PV	High	25	2500000		2	2000		1.13E+02	1.13E+07
	Influent	3000000							

Breakthrough experiments

	7/12Column 1 with Pond Water (TOC)				7/12Column 2 DI water (NO TOC)			
PV	**Original**	**Dilutions**	**Original calculated**	Percent rem	**Original**	**Dilutions**	**Original calculated**	Percent removal
10	0	0	0	100	0	0	0	100
15	0	0	0	100	0	0	0	100
20	0	0	0	100	0	0	0	100
25	4	0	40	99.99989	0	0	0	100
30	150	0	1,500	99.99583	0	0	0	100
35	too high	311	31,100	99.91361	0	0	0	100

40	too high	374	374,000	98.96111	0	0	0	100
45	too high	1000	1,000,000	97.22222	0	0	0	100
50	too high	700	7,000,000	80.55556	3	0	30	99.99992
55								
60								
65								
Influent	36000000							

6/26 Pond water spiked with 10^7 E. coli/mL				**6/26 Pond water spiked with 10^7 E. coli/mL**			
Original	**Dilutions**	**Original calculated**	Percent Removal	**Original**	**Dilutions**	**Original calculated**	Percent removal
0	0	0	100	0	0	0	100
0	0	0	100	0	0	0	100
0	0	0	100	1	0	10	99.9995
0	0	0	100	1	0	10	99.9995
11	0	110	99.9945	1	0	10	99.9995
36	0	360	99.982	>100	17	1,700	99.915
67	0	670	99.9665	>100	84	8,400	99.58
too high	46	46,000	97.7	too high	46	46,000	97.7
too high	1	10,000	99.5	too high	35	350,000	82.5
2000000							

6/23 E. coli				***6/23 E. coli***			
Original	**10^3**	**Original calculated**	Percent removal	**Original**	**10^3**	**Original calculated**	
0	0	0	100	6	0	60	99.99983
0	0	0	100	0	0	0	100
0	0	0	100	6	0	60	99.99983
0	0	0	100	4	0	40	99.99989
0	0	0	100	6	0	60	99.99983

4	0	40	99.99989	7	0	70	99.99981
0	0	0	100	6	0	60	99.99983
0	0	0	100	6	0	60	99.99983
15	0	150	99.99958	3	0	30	99.99992
36000000							

6/18 E. coli (40%)				6/18 E. coli (40%)			
Original	**10^3**	**Original calculated**		**Original**	**10^3**	**Original calculated**	
0	0	0	100	0	0	0	100
0	0	0	100	0	0	0	100
1	0	10	99.9996	0	0	0	100
1	0	10	99.9996	0	0	0	100
1	0	10	99.9996	0	0	0	100
0	0	0	100	0	0	0	100
2	0	20	99.9992	0	0	0	100
0	0	0	100	0	0	0	100
3	0	30	99.9988	0	0	0	100
2500000							

6/17 E. coli (100%)				6/17 E. coli (100%)			
Original	**10^3**	**Original calculated**		**Original**	**10^3**	**Original calculated**	
1	0	10	99.99997	0	0	0.00E+00	100
0	0	0.00E+00	100	0	0	0.00E+00	100
0	0	0.00E+00	100	28	0	28	99.99993
0	0	0.00E+00	100	0	0	0.00E+00	100
0	0	0.00E+00	100	0	0	0.00E+00	100
0	0	0.00E+00	100	0	0	0.00E+00	100

0	0	0.00E+00	100	0	0	0.00E+00	100
0	0	0.00E+00	100	0	0	0.00E+00	100
0	0	0.00E+00	100	0	0	0.00E+00	100
38000000							

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ACADEMIC VITA

PAULA ESPINOZA SOTO

EDUCATION

The Pennsylvania State University **University Park, PA**
Schreyer Honors Scholar, Dean's List in all semesters to date
Major: Bachelor of Science in Chemical Engineering

WORK EXPERIENCE

Coherus Biosciences, Inc **Redwood City, CA**
Process Engineering Part-Time Intern *October 2019 – May 2020*

- Managed and maintained cloud-based databases to ensure the availability of manufacturing data in a useful format for statistical analysis, trend review, and troubleshooting
- Aided the process engineering team with data analysis and compilation of technical reports
- Supported troubleshooting efforts for Drug Substance manufacturing related issues, such as deviations and Corrective and Preventive Actions

Coherus Biosciences, Inc **Redwood City, CA**
Process Engineering Intern *May 2019 – August 2019*

- Completed automation analysis for chromatography unit operations for a biosimilar downstream manufacturing process
- Managed and maintained cloud-based databases to ensure the availability of manufacturing data in a useful format for statistical analysis, trend review, and troubleshooting
- Assisted with the design, execution, and analysis of process optimization and characterization studies
- Aided the process engineering team with data analysis and compilation of technical reports
- Supported troubleshooting efforts for Drug Substance manufacturing related issues, such as deviations and Corrective and Preventive Actions

Dr. Stephanie Velegol and Dr. Manish Kumar Research Laboratory **University Park, PA**
Undergraduate Researcher *May 2018 – May 2020*

- Use cationic protein from *Moringa oleifera* seeds to create a sustainable water sand filter and produce clean drinking water
- Contributed to the design and experimentation of the scale-up of the laboratory experiment with accessible and affordable materials
- Participated in the Research Experience for Undergraduates (REU) program during the summer of 2018
- Attended the AIChE Student Conference 2018 and participated in the Poster Competition

LEADERSHIP EXPERIENCE

Outcast Dance Team **University Park, PA**

President *May 2018 – May 2020*

- Organization is a student-run dance group to create, teach, and learn choreographies for different performances, working in teams
- Organized and planned with team members Outcast's dance performance at THON 2017, 2018, 2019, and 2020 and Homecoming 2018 and 2019
- Increased the number of team active members and number of performances/dance competitions per semester

Venezuelan Student Organization **University Park, PA**

Awareness Representative *January 2017 – May 2017*

- Spread the word throughout the community about the daily issues occurring in Venezuela
- Planned and organized fundraising events in order to support "SOS Worldwide Venezuela" and "Water for Venezuela's" mission to generate global awareness about the crisis in Venezuela and provide water filters to the affected population

Society of Hispanic Professional Engineers (SHPE) **University Park, PA**

January 2017 – May 2020

- Student organization dedicated to empowering the Hispanic community to realize its fullest potential in STEM

VOLUNTEER EXPERIENCE

Special Olympics Venezuela **Caracas, Venezuela**

Volunteer *November 2015*

- Planned and organized multiple activities for disabled athletes, including painting, dancing, swimming, and other recreational sports
- Lead and guided the dance activity for over 40 athletes and students

ACHIEVEMENTS AND SKILLS

AIChE Regional Conference 2019

Larry Duda Scholarship/ Research Award, year 2019

Whitfield Endowment/ Research Award, year 2018

Larry Duda Scholarship/ Research Award, year 2018

The President's Freshman Award at The Pennsylvania State University, year 2017

Bilingual fluency in both Spanish and English

Skills: Microsoft Excel, JMP, Mathematica