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**SCALE-UP OF MORINGA OLEIFERA COATED SAND FILTERS TO REMOVE
PATHOGENS FROM WASTEWATER**

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ABSTRACT

The World Health Organization (WHO) has reported that waterborne infectious diseases cause more than 2.2 million deaths in 2016.¹ It is estimated by 2025, half of the world's population will be living in water-stressed areas.² In 2015, 884 million people worldwide lacked access to a basic drinking water service, while 2.3 billion people lacked even basic sanitation facilities such as toilets or latrines.³ Point of use household technologies are able to significantly reduce diarrheal diseases using boiling, chlorination, bio-sand filters and ceramic filters.⁴ This study explores the use of functionalized sand filter (f-sand) created with the seeds of a *Moringa oleifera* (MO) tree to offer a potential adsorbent for the removal of *E. coli*. MO grows widely in many equatorial regions of the world where people are threatened by diarrheal diseases caused by drinking water contamination. The seeds of MO contain cationic proteins that have antimicrobial, antifungal, and coagulant properties. The use of clean bed filtration model led to the scaled-up design of a lab-scale f-sand column. *E. coli* strain TG1 containing plasmids that express red fluorescent protein (pCA24N-rfp-lasR) were used as model pathogens at an influent concentration of 10^8 colony-forming units (CFU)/mL suspended in 10-fold diluted phosphate-buffered saline (PBS) buffer (0.016 M). Initial scale-up results showed no enhanced removal using the f-sand columns due to an increase in collector size. The use of binary mixtures of collector sizes ($\leq 106 \mu\text{m}$ and 212-300 μm glass beads) showed the need for a critical percentage of $>55\%$ small collector sizes for f-sand columns to see enhancement from that of a bare sand column. Experiments were performed using different ranges of collector sizes and illustrated that the critical collector diameter in order to create an effective f-sand column is $\sim 175 \mu\text{m}$ given a

flow rate of 1.6mL/min and a column with a 1.5 cm diameter and 10cm length. An additional scale-up was made using glass powder (140-210 μm) with a 5 cm diameter and 10 cm length with an average flow rate of 12 mL/min resulting in a >6.5 LRE for 80 PV with a sharp breakthrough at PV 85.

We also report on the use of biochar pre-filter to remove organic matter from pond water to yield an effective log removal efficiency (LRE) for f-sand columns. Organic matter can block potential bacterial removal sites in f-sand columns. Biochar was used as an inexpensive, readily available and environmentally friendly adsorbent that has been used to treat wastewater effluents. The biochar used was generated from cotton gin waste feedstock with pyrolysis conditions of 700 °C for 2 hours. The biochar f-sand filters used in series produced a >8 LRE.

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Chapter 1

Background: Water Crisis Analysis

The World Health Organization (WHO) has reported that waterborne infectious diseases cause more than 2.2 million deaths in 2016.¹ Acute diarrhea was responsible for 1.3 million deaths and 71.59 million disability adjusted life years globally in 2015 with fecal contamination of water used for drinking, irrigation, and recreational purposes is responsible for waterborne transmission causing 88% of these diarrhea-related deaths.^{3,4,5} It is estimated by 2025, half of the world's population will be living in water-stressed areas.² In 2015, 884 million people worldwide lacked access to a basic drinking water service, while 2.3 billion people lacked even basic sanitation facilities such as toilets or latrines.³ Point of use household technologies are able to significantly reduce diarrheal diseases using boiling, chlorination, bio-sand filters and ceramic filters.⁴ However these technologies are not locally available in many of these water stressed regions, rendering these possible solutions to be unsustainable and thus ineffective. This leads to a need for a water purification system that can be derived from locally available materials to create a sustainable path to clean drinking water.

Moringa oleifera (MO), a deciduous tree, grows widely in many equatorial regions of the world where people are threatened by diarrheal diseases caused by drinking water contamination (Figure 1). The seeds of MO contain cationic proteins. These cationic proteins have antimicrobial, antifungal, and coagulant properties that have allowed MO seeds to be traditionally used as a flocculent.⁸ The challenge of using Moringa as a flocculent is fouling that arises from the organic seed material that is left over. Previous work has shown that proteins will

adsorb onto sand due to electrostatic attractions between naturally negatively charged sand particles and the cationic protein within the Moringa seeds, forming a net positively charge functionalized sand (f-sand) particle that is coated with the cationic proteins.⁸ The sand is then rinsed and the organic matter is removed. This process nullifies the potential fouling challenge but retains the antimicrobial and flocculating capability of the MO seeds.

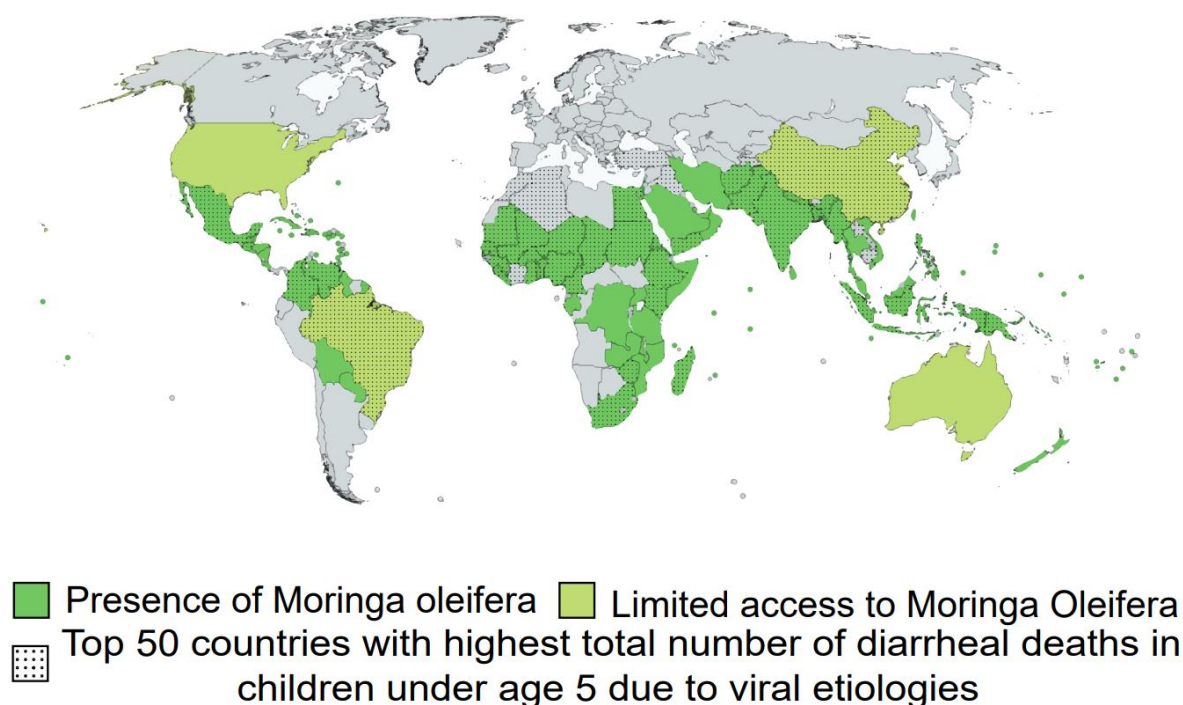


Figure 1 Geographical distribution of MO was plotted based on the data from the Centre of Agriculture and Business International datasheet. (data obtained from Centre of Agriculture and Business International datasheet)

These functionalized sand particles (f-sand) can then be packed into a column to create a functionalized sand filter. Previous work has shown that these f-sand columns can remove greater than 3 log of 1 μm sPSL particles, greater than 8-log of *E. coli* as well as greater than 7-log of MS2 virus.^{5,8} These results are all significantly higher than a bare sand column and the determined bacteria and virus removals both exceed EPA standards.⁵ Previous reports indicate that <1% of the annually produced seeds from one MO tree could suffice to fabricate a

community scale f-sand filter.⁸ The seeds on MO offer a locally available, low energy water purification that is sustainable for developing countries.

In this work, we report high removal of *Escherichia coli* ($> 8 \log_{10}$ or 99.999999%) by using scaled-up f-sand filters coated with a simple MO aqueous seed extract using cheap material. We report on the relative contributions of design parameters during the scale-up process. The use of biochar pre filter to remove TSS from pond water was also investigated.

Chapter 2

Clean Bed Filtration Model

The scale-up of the previous small-scale columns were facilitated using the clean bed filtration theory along with the Yao model for water and waste water filtration.⁶ The following section examines the effects of parameters within the Yao model to explore methods used to improve the total efficiency of a scaled up Moringa coated sand filter.

The Yao model is used along with the clean bed filtration theory in order to scale up our f-sand column for community use. The clean bed filtration theory is widely used to describe mass transport through porous media and correlates the collector efficiency of a single collector to the overall removal/adsorption of a column (Equation 1).⁶ In this equation, d_c is the diameter of the collector, θ is the column porosity, α is the sticking coefficient, L is the length of the column and η is the collector efficiency. The collector efficiency and the sticking coefficient are the two effective controlling parameters for this equation. The sticking coefficient is defined as the probability that when a particle strikes a collector, that the particle sticks. The sticking

coefficient was experimentally back calculated in previous work to be 0.83 for *E. coli* and 0.08 for MS2 virus.⁵

$$(1) \frac{N}{N_0} = \exp\left(-\frac{3}{2d_c}(1-\theta)\alpha\eta L\right)$$

The collector efficiency is defined as the probability that a particle strikes a collector. The value of the collector efficiency can be determined using the Yao model. The Yao model assumes the likelihood for a particle to strike a collector is dependent on three modes of mass transport: interception, sedimentation and diffusion.⁶ Interception is the transport mechanism that describes a particle contacting a collector by virtue of its own size in a constant flow pattern. Sedimentation is the transport process that accounts for different flow trajectories that arises due to differences in density between the particle and collector. Diffusion describes mass transport due to random movement of suspended particles. Each mode of mass transport is calculated separately using Equations 2-4 respectively and then added together to describe to analytically approximate the single-collector efficiency using Equation 5.⁶

$$(2) \eta_I = \frac{3}{2} * \left(\frac{d_p}{d_c}\right)^2$$

$$(3) \eta_G = \frac{(\rho_p - \rho_f)gd_p^2}{18\mu v}$$

$$(4) \eta_D = 4.04 * Pe^{-\frac{2}{3}} = .9 * \left(\frac{kT}{\mu d_p d_c v}\right)^{\frac{2}{3}}$$

$$(5) \eta = \eta_I + \eta_G + \eta_D$$

The article examined the effect that different parameters would have on collector efficiency, but only did so with respect to particle size and used a few select parameters. Our work looked to describe how functionalized parameters will affect the mass transfer to better understand the dependence each parameter has on the overall efficiency of the column. The work focused on the removal of bacteria (*E. coli*) using the given parameters listed in Table 1.

Table 1 List of parameters used for clean bed filtration theory

Parameter	Value	Parameter	Value
k (thermal conductivity)	$1.38 \cdot 10^{-23}$	T (temperature, K)	298
No (# of particles)	10^8	ρ_p (density of particle, kg/m ³)	1050
Dc (collector diameter, μm)	106	ρ_f (density of fluid, kg/m ³)	997.538
DC (column diameter, cm)	1.5	μ (viscosity, Ns/m ²)	$.8904 \cdot 10^{-3}$
θ (porosity)	0.37	Q (flowrate, mL/min)	1.6
L (length, cm)	10	α (sticking coefficient)	.83

Effect of Particle Size

The total collector efficiency along with the contribution from each 3 modes of mass transport is shown in Figure 2 (left). The total corresponding log removal was also plotted shown in Figure 2 (right), along with corresponding log removals for each mode of transport independently. The effect of particle size on collector efficiency was shown in the article, although the results were determined numerically. The results show that for small particle sizes around 2000 nm or less, the efficiency of the collector is heavily dependent on diffusion as the main mode of transport. As particle sizes increase above 3000 nm, the effect of diffusion is severely diminished, and the primary driver of mass transport is through sedimentation and interception. The graph shows a minimum efficiency around 1750 nm where the effect from diffusion is diminishing and the effect of sedimentation and interception starts to become more

significant. The size of *E.coli* used in lab for column experiment is around 1700 nm. Figure 2 shows that the collector efficiency is primarily driven by diffusion, however the effect of sedimentation and interception have some contributions. The particle size of *E.coli* falls directly at the minimum of efficiency in the column, indicating that the removal obtained from *E.coli* experiments would be the minimum possible removal for this set of conditions given a wide distribution of particle sizes.

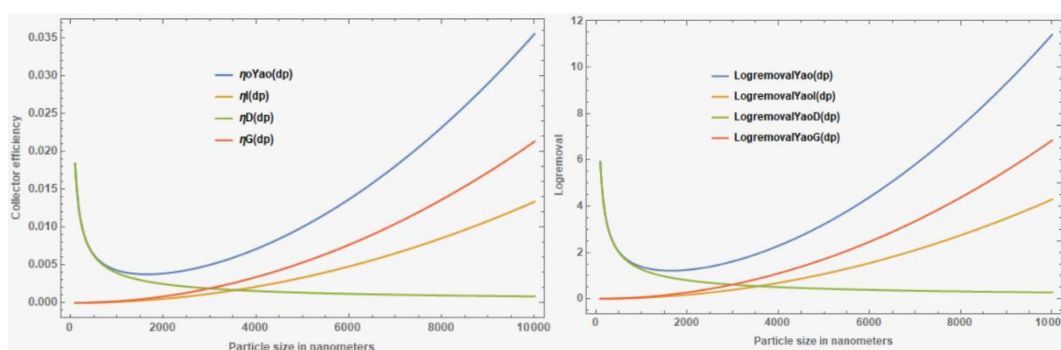


Figure 2 (left) Effect of particle size on collector efficiency (right) Effect of particle size on log removal

Collector Size

The total collector efficiency along with the contribution from each 3 modes of mass transport is shown in Figure 3(left) and the total corresponding log removal was also plotted in Figure 3 (right), along with corresponding log removals for each mode of transport independently. The efficiency due to sedimentation is independent of collector size and remains constant. The collector efficiency, like that of particle size, is driven primarily due to diffusion. The effect of interception is only present in collector sizes below 75 μm and becomes the primary driving force for mass transfer only below extremely small collector sizes below 8 μm . The collector size(sand size) used for lab experiments are <106 μm (mean size around 80 μm).

The graph again shows that diffusion is the main driving force for mass transfer and at this size both sedimentation and interception have very weak effects on the overall efficiency of the collector. The log removal is also inversely dependent on collector size as seen in equation 1, as well as dependent on η . Figure 3 (right) illustrates that the size of the collector has a drastic impact on log removal since increasing collector size not only decreases the single collector efficiency but also decreases the log removal directly within clean bed filtration equation.

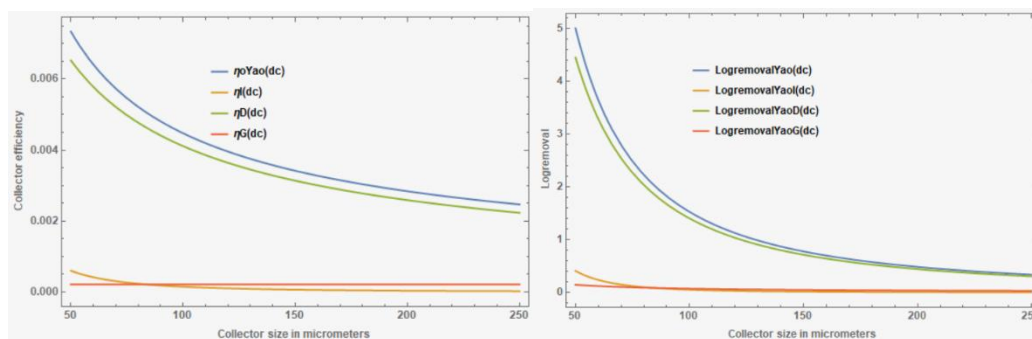


Figure 3 : (left) Effect of collector size on collector efficiency (right) Effect of collector size on log removal

Flow Rate

The total collector efficiency along with the contribution from each 3 modes of mass transport is shown in Figure 4 (left) and the total corresponding log removal was also plotted in Figure 4 (right), along with corresponding log removals for each mode of transport independently. The collector efficiency due to interception remains constant as it does not depend on flowrate. Diffusion is the dominant contributor for collector efficiency throughout the entire range. Even at extreme ends of the spectrum, diffusion remains the prominent mode for mass transport, as shown in the subset graph in Figure 4 (left). The flowrate used for lab experiments is 1.6mL/min. Within this range, the collector efficiency is dominated by the

diffusion mode of transport. The effect of flowrate on log removal is relatively half that compared to that of collector size. If the flowrate is doubled, the log removal drops less than .5 and if the collector size is doubled, the log removal drops a little over 1. Indicating the flowrate should be increased more rather than increasing collector size to maximize the removal during scale-up.

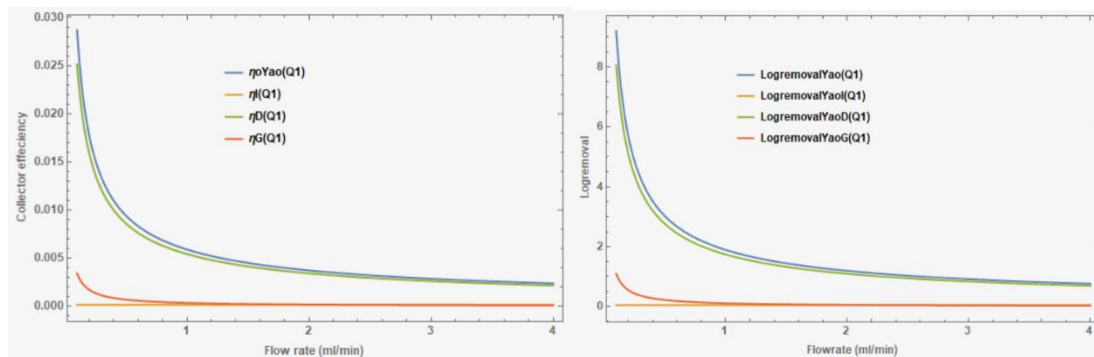


Figure 4 : (left) Effect of flowrate on collector efficiency (right) Effect of flowrate on log removal

Model Conclusion

Yao *et al.* proposed a conceptual model for wastewater filtration to calculate the collector efficiency to predict the removal of a porous packed column. The model assumes three primary modes of mass transport: diffusion, sedimentation and interception. The article examined the effect of different parameters would have on collector efficiency, but only did so with respect to particle size and used a few select parameters. The model is ultimately controlled by the size of the particle, as the size dictates the significance of each mode of transport. Diffusion is the primary mode of mass transport for the particle size of bacteria, with both sedimentation and interception contributions remaining low throughout parameter changes. The collector size and flowrate can be increased at a tradeoff to collector efficiency. This tradeoff can be negated by

coupling the increase in collector size and flowrate with an increase in length. The temperature can also be increased to achieve the desired collector efficiency.

Chapter 3

Procedure

MO seeds

The seeds used in this work were received from Echo Global Farm, Florida. All seeds were stored at room temperature in sealed bag and crushed using a coffee grinder before experimentation.

f-sand preparation (Batch Process)

A batch process was utilized to prepare f-sand. The amount of seed required to functionalize the sand was determined using a seed weight to sand surface area of 5.6 g/m^2 that was determined to be the optimum from previous work. For a lab-scale experiments, 3.1 g of unshelled whole MO seeds were crushed using a coffee grinder and mixed with 610 mL of deionized (DI) water for 5 min. The obtained water extract was filtered through a $1.5 \text{ }\mu\text{m}$ glass fiber filter (Whatman) and $0.22 \text{ }\mu\text{m}$ poly(vinylidene difluoride) (PVDF) filter (Millipore) to remove seed debris. The sand particles were mixed with the seed extract for 5 min followed by settling for 5 min. The supernatant was discarded, and the sand particles were rinsed 3 times with

DI water to remove excess organic matter. The rinsed sand particles were then added into the appropriate column.

f-sand preparation (In-Situ Process)

An in-situ coating procedure was also performed in addition to the batch process. The MO serum is made with the same procedure as the batch process. The serum, instead of a batch mixing, is pumped through the column at 1.6 mL/min with a ratio a seed concentration of 0.005 g seed/mL.

Model Contaminants

E. coli strain TG1 containing plasmids that express red fluorescent protein (pCA24N-rfp-lasR) were used as model pathogens at an influent concentration of 10^8 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. The samples were plated using plates made from Luria-Bertani (LB) broth. *E. coli* was grown using LB liquid media. Chloramphenicol (Cm) antibiotic stock (30 μ g/mL) was used to neutralize bacterial contamination in both plate and culture media. PBS buffer contained NaH_2PO_4 (.58 g/L) and Na_2HPO_4 (2.5 g/L). Sample plates were incubated at 37°C for 24 hours. Bacterial colonies were counted to determine effluents concentrations.

Column Experiments

A variety of different sized column were used to conduct experiments in this work. Glass chromatography columns were used for small scale use with a 1.5 cm diameter and 10 cm in length. Scale-up columns made from PVC piping were used with multiple sizes. These columns were used to perform filtration experiments for quantifying bacterial removal. The porosity of the packed sand column was gravimetrically determined to be 0.37 for <106 μm glass beads. The f-sand slurry coated with MO serum was quickly poured into the glass column and gently mixed by rotation along the length of the column to remove any trapped bubbles. The columns were then packed with DI water overnight and equilibrated with the background electrolyte for 20 pore volumes before switching to appropriate influent solutions prepared in the same background electrolyte. Sterilized water and PBS buffers (10-fold diluted) were used for *E. coli* removal experiments. Once equilibration was completed, the inlet was switched to a $\approx 10^8$ colony-forming unit (CFU)/mL *E. coli* solution in PBS. Effluent samples (1 mL) were collected in sterilized microcentrifuge tubes at 4, 6, and 8 pore volumes. A constant flow rate was achieved using a peristaltic pump (Cole-Parmer) with the feed solution entering from the top of the column. Experimental log removal for particles was calculated using

$$\text{(Eq. 1) } \log_{10}(\text{removal}) = -\log_{10}\left(\frac{N}{N_0}\right)$$

where N and N_0 are the effluent and influent particle concentrations, respectively.

Collector Size experiments

Collectors were boiled and rinsed three times or until no turbulence was seen after 5 minutes of boiling. The boiling ensures that MO serum will adsorb onto the collectors and not onto dirt or smaller clay particles, as well as killing potential contamination sources. The collectors were dried at 35°C overnight before coating and packing.

Gel Electrophoresis

To characterize the protein adsorbed on f-sand, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) evaluation was conducted by loading 12 μ L of f-sand onto a 12% hand-cast SDS-PAGE gel. Coomassie staining was used to visualize the protein bands.

Chapter 4

Scale-up Results

The lab scale columns were scaled-up using PVC piping material in order to create a water purification system usable for a community setting with locally accessible and affordable materials. The lab scale column used in previous experiments had a 1.5 cm diameter and 10 cm length with a flowrate of 1.6 mL/cm. The column also utilized $\leq 106 \mu\text{m}$ glass beads as model sand particles. Previous work has shown that with these conditions $>8 \log_{10}$ removal efficiency (LRE) can be obtained. The clean bed filtration model used to scale-up the column only predicted a log removal of 4.7. Since traditional sand sizes in the field are within a range of 0.02-2 mm instead of the $\leq 106 \mu\text{m}$ sand particles previously used, the scale-up column experiments were performed using 212-300 μm glass beads. Due to the underprediction in the model shown in the lab scale column, a predicted log removal was set at 2 LRE for the scale-up column. The scale-up column was scaled-up to a diameter of 2 cm with and length of 20 cm to remain a similar diameter/length ratio and give the predicted log removal with the change in particle size. The flow rate remained the same as the lab scale column at 1.6 ml/mL. The process utilized the batch process of coating to measure *E. coli* removal. The scaled-up f-sand filters achieved a \log_{10} removal efficiency (LRE) of 0.23 ± 0.2 for *E. coli* compared to that of 0.41 ± 0.23 demonstrated by bare sand filters (Figure 5). The results show no enhanced removal using the f-sand columns. The model showed an overprediction with a prediction of 2 LRE compared to 0.23 LRE.

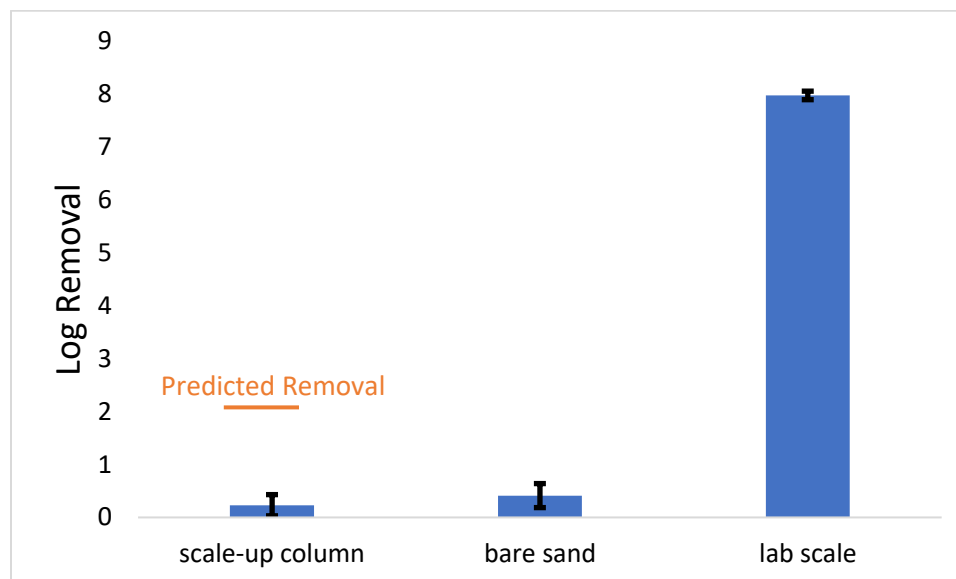


Figure 5 : Scale-up results using PVC column with diameter of 2 cm with and length of 20 cm with a flowrate of 1.6 mL/cm. The column used 212-300 μm glass beads to remove fluorescent *E. coli*. The results show no enhanced removal using the f-sand columns. The model showed an overprediction with a prediction of 2 log removal compared to 0.23 log removal.

An additional column experiment was performed adjusting parameters in order to match the predicted log removal of 4 from the lab scale column to determine if the overprediction found previously still exist at higher predicted log removals. The additional column was made from PVC with a diameter of 3.88 cm and length of 20 cm. The collector was kept at 212-300 μm glass beads, but the flow rate was doubled to 3.2 ml/min. The scaled-up f-sand filters achieved a LRE of 0.86 ± 0.56 for *E. coli* compared to that of 0.1 ± 0.28 demonstrated by bare sand filters (Figure 6). The results show a very slight enhancement using the f-sand columns compared to that of the bare sand. However, there is still an overprediction from the model from the predicted 4 LRE compared to the 0.86 LRE obtained experimentally. The discrepancy within the model can be attributed to the change in collector size from the $\leq 106 \mu\text{m}$ glass beads used in the lab scale to the 212-300 μm glass beads used in the scaled-up columns.

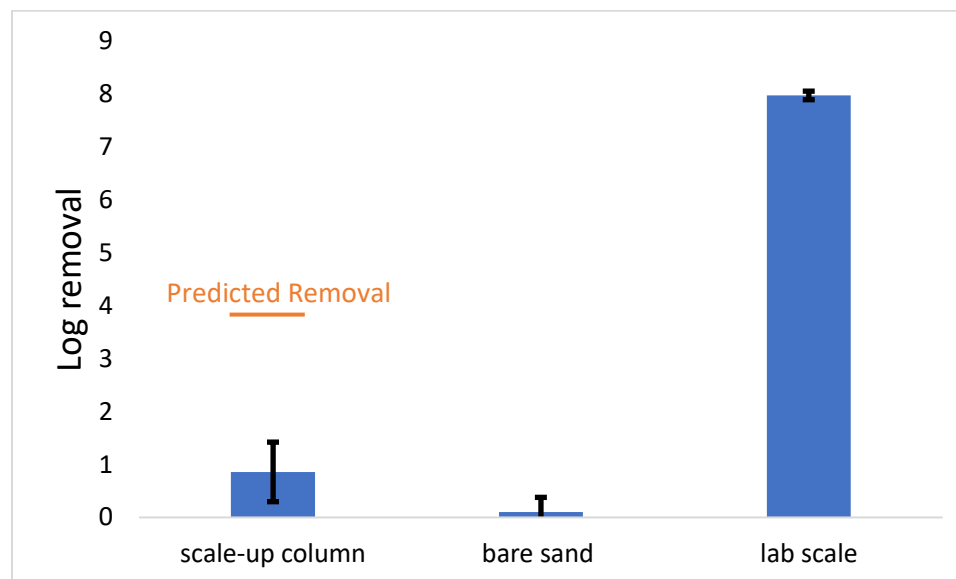


Figure 6: Scale-up results using PVC column with diameter of 3.88 cm with and length of 20 cm with a flowrate of 3.2 mL/cm. The column used 212-300 μm glass beads to remove fluorescent *E. coli*. The results show no enhanced removal using the f-sand columns. An overprediction was observed from the model predicting 4 LRE compared to the 0.86 LRE.

Binary Mixtures of Collector Sizes

The overprediction observed in the model and failure of scale-up columns was due to the increase of collector sizes. A binary mixture of the two collector sizes ($\leq 106 \mu\text{m}$ and 212-300 μm glass beads) was utilized to increase the efficiency of the column. The glass lab scale columns with a 1.5 cm diameter and 10 cm length were used with a flowrate of 1.6 ml/min. A range of the ratio of binary mixtures of the glass beads were used to test the effect of log removal with respect to collector sizes. The results illustrated a distinct change in log removal from ~ 2.0 LRE to >8 log removal at a ratio of 55% $\leq 106 \mu\text{m}$ to 45% 212-300 μm glass beads (Figure 7). The log removal of 55% $\leq 106 \mu\text{m}$ glass beads is maintain at higher ratios and the ~ 2.0 LRE is maintained at lower ratios. The results illustrate the need for a critical percentage of small collector sizes for f-sand columns to see enhancement from that of a bare sand column. The

porosity of each binary mixture was taken to determine a relationship between the effectiveness of the column and porosity. There is a minimum located around the 50:50 mixture that would indicate the potential change in controlling flow ways that could cause the distinct jump in LRE after the 50:50 mixture.

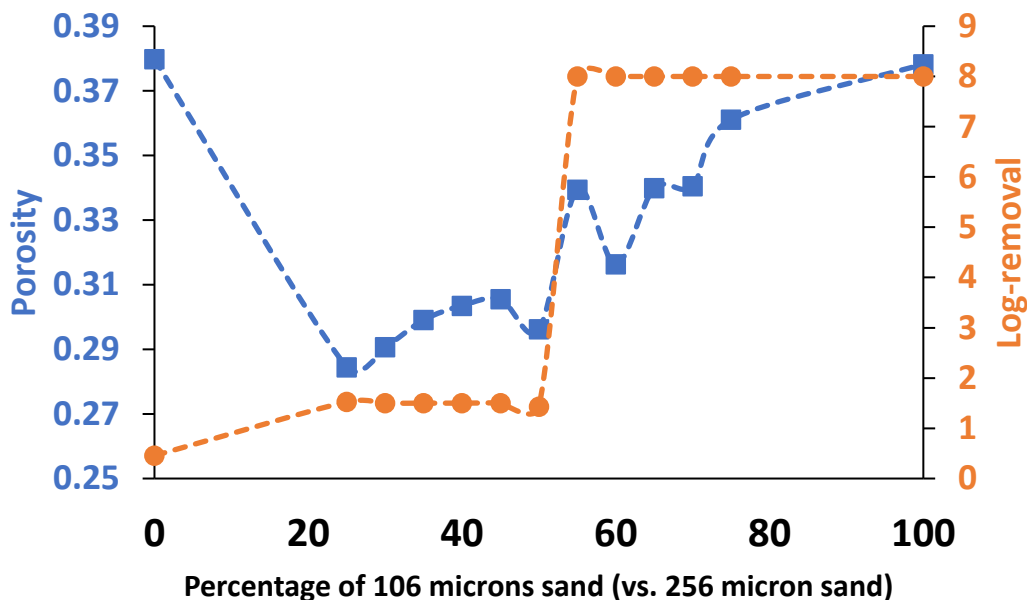


Figure 7: The glass lab scale columns with a 1.5 cm diameter and 10 cm length were used with a flowrate of 1.6 ml/min. The column used a binary mixture of 106 and 256 μm glass beads to remove fluorescent *E. coli*. At greater than a 55:45 ratio of a binary mixture of 106 and 256 μm glass beads, LRE of f-sand columns exceed that of bare sand removal.

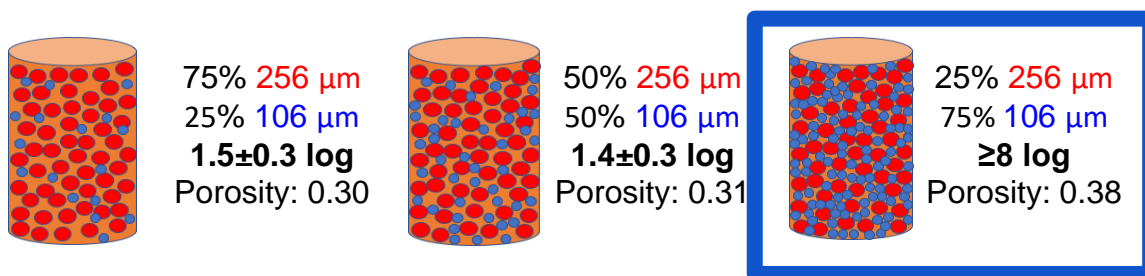


Figure 8: ≥ 8 LRE can be seen at a greater binary mixture of 106 and 256 μm glass beads

Different Collector Sizes

Column experiments were performed using a variety of different size and material collectors. Table 2 shows the size of all the collectors used in addition to the glass beads used previously. Figure 9 showed that variation within collector size fits with predictions of clean bed filtration model. The figure illustrates a critical value close to the 175 μm collector size. This can be seen in the disparity in LRE between the sand received from Echo Global farms and the glass powder which have different LRE but the same range in sand size. The $\leq 106 \mu\text{m}$ glass beads and the $\leq 106 \mu\text{m}$ silica sand both had LRE close to 8. The 212-300 μm glass beads and Fisher sand both had LRE resembling that of bare sand. Such observations indicate the critical collector size in order to create an effective f-sand column to be $\sim 175 \mu\text{m}$ at the selected parameters.

Table 2: collector sizes for all material used in column experiments

Materials	Sizes of the collectors
Silica sand	< 106 microns
echo sand	140-210 microns
Glass powder	140-210 microns
Sand	210-300 microns

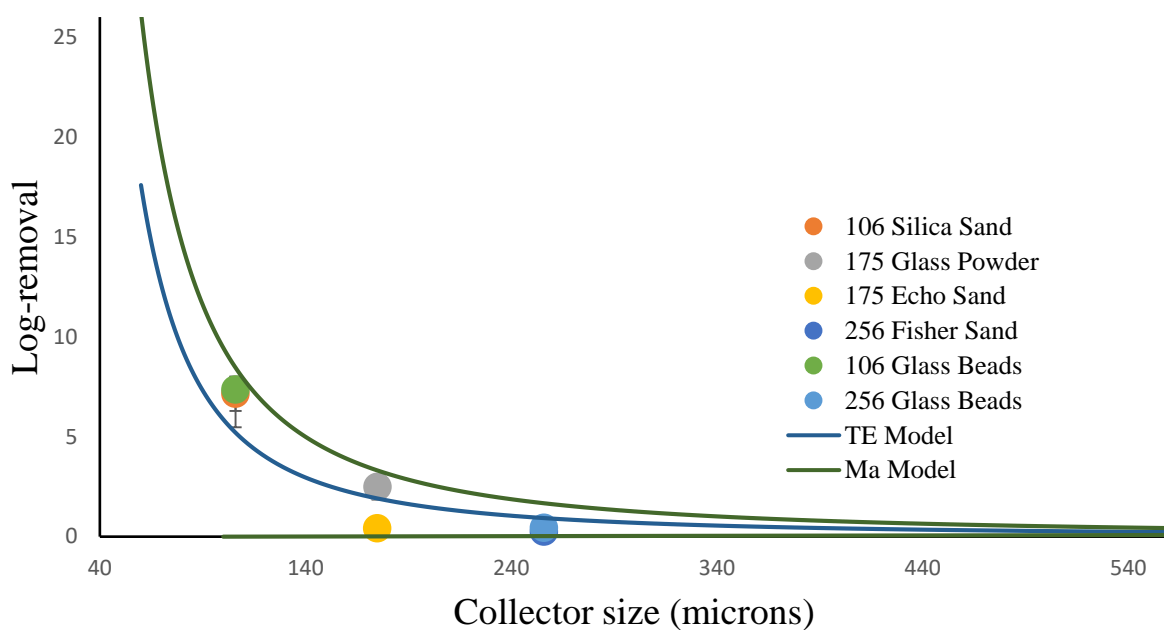


Figure 9: Column experiments with a 1.5 cm diameter and 10 cm length and a flowrate of 1.6 mL/cm were used to remove fluorescent *E. coli* using different collector sizes. Collector size reach a critical value $\sim 175 \mu\text{m}$ where LRE becomes greater than that of bare sand.

Characterization of protein adsorption

Gel electrophoresis was performed on glass powder (140-210 μm) and $\leq 106 \mu\text{m}$ glass beads to understand the characterization of the proteins adsorbed onto the surface of each collector. Both collectors show a distinct clear band at 15 kDa, showing that both collectors have the same proteins adsorbed onto the sand from MO seeds extract.

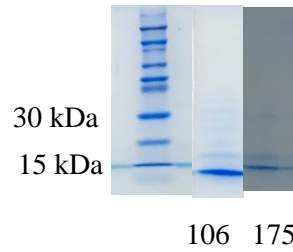


Figure 10: Gel Electrophoresis spectrum of <106 μm 175 μm glass beads show similar protein absorption.

Revisit Scale-up

A scale-up column was made with a diameter of 5 cm and length of 10 cm. The column was run using in-situ coating with glass powder (140-210 μm). As previous results showed, glass powder had a high log removal compared to bare sand under lab scale settings. This study looked to determine the longevity of the scaled-up column. In order to estimate the capacity of this column, the fraction (f) defined as the sand surface area covered by *E. coli* was experimentally determined using the breakthrough of the column.

$$f = \frac{J_p * V_b * A_p}{A_s N_s}$$

where A_s is the surface area of one sand particle, N_s is the total number of sand particles in the column, J_p is the flux of influent particles, V_b is the volume filtered at breakthrough and A_p is the cross-section area of an influent particle. The A_p value used an approximate average *E. coli* diameter of 1.7 μm which was previously used in longevity calculations done in in our lab.⁸ The f value for a 1.5 cm diameter and 10 cm length with a flowrate of 1.6 mL/cm and ≤106 μm glass beads was previously calculated to be $4.1 \pm 2\%$.⁸ This would correspond to a predicted breakthrough for this scale-up column to be 300 PV or 1.9 L. Experiments were done running

the 5-diameter scale-up column for 110 PV (700mL) to determine the breakthrough point and f-value. The average flowrate was ~ 12 mL/min, with the flow rate slowly decreasing throughout the experiment (started at 12.5 mL/min and ended at 11.5 mL/min). The flow rate was created via a constant head tank to simulate settings experienced in field testing. The flow rate was chosen to be over 10 mL/min as this value is estimated to be able to sustain a family of 5 for a day. The results show a >6.5 LRE for only 80 PV, which corresponds to 500 mL, as compared to the predicted 300 PV. The results showed a sharp breakthrough at PV 85 resembling LRE similar to that of bare sand (Figure 11). The glass powder is effectively able to be scaled-up to purify bacterial contaminated water for community use, but at a lower breakthrough point than initially calculated. The f value for the scaled-up column was calculated through the experimental breakthrough point of 80 PV to be 1.1%, which is significantly lower than the 4.1% found previously. This difference was expected as the increase in collector size used in the scale-up column reduced the overall collector efficiency, which would trend toward a quicker breakthrough time and a lower f value than that of smaller collector sizes. This f-value allows for a more accurate prediction of the breakthrough for columns using 140-210 μm collector sizes.

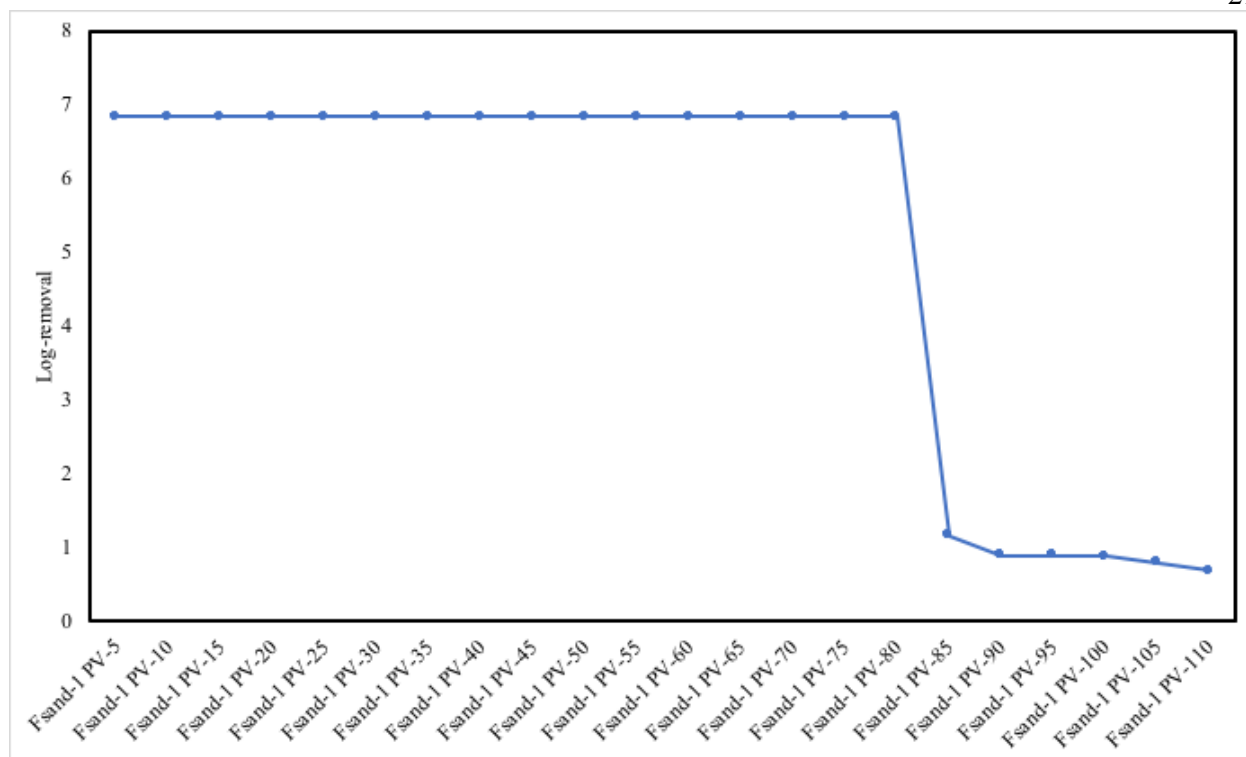


Figure 11: Breakthrough experiment for scaled-up PVC column with a 5 cm diameter and 10 cm length. The column was ran with an average flowrate of ~12 mL/min with glass powder (140-210 μm) to remove fluorescent *E. coli*. The results show a breakthrough after 80 PV or 700mL.

Chapter 5

Biochar Prefilter

Introduction

A potential problem of f-sand filters is the presence of organic matter contained within contaminated drinking water. The presence of organic matter can be quantified using TOC (Total Organic Carbon) measurements. Organic matter along with small dirt particles can block potential bacterial removal sites in f-sand columns. Activated carbon (AC) has been explored as potential materials for the adsorption of TOC. ACs are versatile adsorbent that are particularly effective in the adsorption of organic and inorganic pollutants from aqueous solutions.¹¹ However, adsorption using activated carbon poses several problems including the need for high-energy requirements during processing, and a substantial negative environmental footprint.¹¹ To create a locally sustainable process, the introduction of ACs can be impractical in many settings.

Biochar is a stable carbon (C)-rich, energy dense by-product synthesized through the pyrolysis of waste biomass in the absence of oxygen, and at relatively low temperatures (<700°C).¹⁰ Biochar is inexpensive, readily available and environmentally friendly adsorbent that has been used to treat wastewater effluents.¹¹ Biochar particle size and distribution heavily depend on the feedstock used as well as the temperature used in the pyrolysis process. The temperature used to create biochar has a great impact on the size and amount of biochar produced.¹¹ In general, the increased of the temperature would decrease the biochar productions. An increase in burn temperature leads to a decreased of the solid yield and an increase of the liquid and gases.¹¹

Biochar is expected to have excellent potential as an adsorbent or filter given its large surface area and microporous structure. Biochar has been shown to effectively be used in the filtration for of TSS, heavy metals and polycyclic aromatic hydrocarbons storm water. However, biochar was not efficient in removing *E. coli* from storm water.¹² The introduction of a biochar prefilter for the removal TOC could be utilized for effective removal of bacteria using a f-sand filter with a high turbidity influent sample.

Procedure

A biochar prefilter was constructed using a 1 cm diameter and 20 cm length glass column. A mixture of 1:1 biochar-to-sand ratio was obtained, and 10 g of the mixture was packed into the column with a 1 cm of sand placed before and after the biochar mixture. The sand used in the biochar column was 212-300 μm glass beads. A constant flowrate of 1.0 mL/min was maintained using a peristaltic pump and the pH of the effluent samples were measured. Influent samples were collected from a local pond and spiked with 10^8 CFU/mL *E. coli* strain TG1 containing plasmids that express red fluorescent protein (pCA24N-rfp-lasR) (same as scale-up experiments). The biochar effluent was collected and ran through f-sand filter (see column experiment procedure. The f-sand column had a 1.5 cm diameter and 10 cm in length and used 40:60 ≤ 106 μm glass beads to 212-300 μm glass beads. The f-sand column used 1.6 mL/min flow rate. Effluent samples (1 mL) were collected in sterilized microcentrifuge tubes at 4, 6, and 8 pore volumes.

Biochar Characterization

Biochar was made from cotton gin waste feedstock. The pyrolysis process was performed for 2 hours at 700 °C. The surface was determined to be 16.33 m²/g with total pore volume of 0.0115 cm³/g. The average pore diameter was 3.59 nm with a pH of 10.93.¹³

Results and conclusion

Experiments were performed to test the effectiveness of the biochar prefilter. Results showed that the LRE of the biochar column effluent had a log removal of 1.03. The biochar effluent was then fed through a f-sand column to yield >8 LRE. The pond water influent was fed directly into a f-sand column without the present of a biochar prefilter to yield a LRE of 0.99 (Figure 12). The results show that the biochar prefilter is capable of removal of TSS and TOC that otherwise would render the f-sand column to be ineffective as shown by the low LRE observed without the presence of the biochar prefilter.

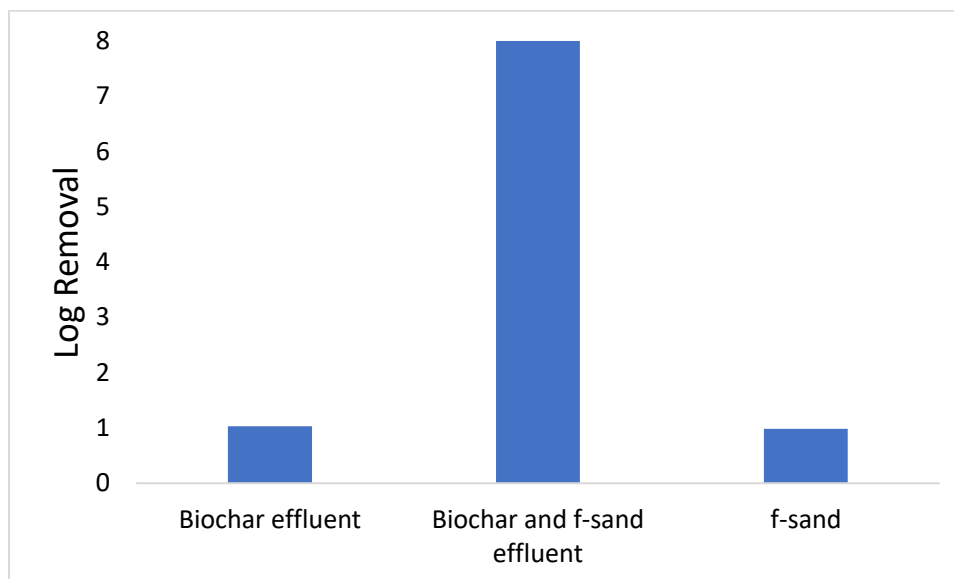


Figure 12: A biochar prefilter of 10g of 1:1 biochar-to-sand was used in a 1.5 cm diameter and 20 cm length glass column. The f-sand column had a 1.5 cm diameter and 10 cm in length and used 40:60 $\leq 106 \mu\text{m}$ glass beads to 212-300 μm glass beads. The results show that the biochar prefilter in series with a f-sand filter can remove >8 LRE of *E. coli* from spiked pond water.

Chapter 6

Conclusion

In summary, the use of clean bed filtration model led to the scaled-up design of a lab-scale f-sand column. Initial scale-up results showed no enhanced removal using the f-sand columns. The use of binary mixtures of collector sizes ($\leq 106 \mu\text{m}$ and $212\text{-}300 \mu\text{m}$ glass beads) showed the need for a critical percentage of $>55\%$ small collector sizes for f-sand columns to see enhancement from that of a bare sand column. Experiments were performed using different ranges of collector sizes and illustrated a critical collector size in order to create an effective f-sand column to be $\sim 175 \mu\text{m}$ at the selected parameters. An additional scale-up was made using glass powder ($140\text{-}210 \mu\text{m}$) with an average flow rate of 10 mL/min resulting in a >6.5 LRE for 80 PV with a sharp breakthrough at PV 85. The f value for the scaled-up column was calculated through the experimental breakthrough point of 80 PV to be 1.1% .

We also showed the use of biochar pre filter to remove TOC from pond water to yield an effective LRE for f-sand columns. Organic matter along with small dirt particles can block potential bacterial removal sites in f-sand columns. Biochar was used as an inexpensive, readily available and environmentally friendly adsorbent that has been used to treat wastewater effluents. The biochar used was generated from cotton gin waste feedstock with pyrolysis conditions of $700 \text{ }^\circ\text{C}$ for 2 hours. The biochar f-sand filters used in series produced a >8 LRE, compared to 0.99 LRE for a f-sand column without the use of biochar prefilter.

BIBLIOGRAPHY

- (1) Efstratiou, A.; Ongerth, J. E.; Karanis, P. Waterborne transmission of protozoan parasites: Review of worldwide outbreaks - an update 2011–2016. *Water Res.* 2017, 114, 14–22.
- (2) Drinking water, World Health Organization: Geneva, February 7, 2018.
- (3) Troeger, C.; Forouzanfar, M.; Rao, P. C.; Khalil, I.; Brown, A.; Reiner, R. C., Jr; Fullman, N.; Thompson, R. L.; Abajobir, A.; Ahmed, M. Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Infect. Dis.* 2017, 17, 909–948.
- (4) Black, R. E.; Morris, S. S.; Bryce, J. Where and why are 10 million children dying every year? *Lancet* 2003, 361, 2226–2234.
- (5) UNICEF. Progress for Children: A Report Card on Water and Sanitation; UNICEF, 2006.
- (6) Water quality and health. Drinking water chlorination: A review of disinfection practices and issues. Technical Report; World Health Organization: Geneva, 2014.
- (7) Clasen, T.; Schmidt, W.-P.; Rabie, T.; Roberts, I.; Cairncross, S. Interventions to improve water quality for preventing diarrhoea: Systematic review and meta-analysis. *BMJ.* 2007, 334 (7597), 782.
- (8) Xiong, B.; Piechowicz, B.; Wang, Z.; Marinaro, R.; Clement, E.; Carlin, T.; Uliana, A.; Kumar, M.; Velegol, S. B. Moringa oleifera f-sand Filters for Sustainable Water Purification. *Environ. Sci. Technol.* 2018, 5, 38-42.
- (9) Yao, Kuan-Mu; Habibian, Mohammad; O'Melia, Charles. Water and Waste Water Filtration: Concepts and Applications. *Environ. Sci. Technol.*, 1971, 5, 1105-1112.
- (10) Lehmann, J., Joseph, S. (2009). "Biochar for Environmental Management: Science and Technology." Earthscan Press, ISBN 978-1-84407-658-1.
- (11) Yanyan, L., Kurniawan, T. A., Zhu, M., Ouyang, T., Avtar, R., Dzarfan Othman, M. H. Albadarin, A. B. (2018). "Removal of acetaminophen from synthetic wastewater in a fixed-bed column adsorption using low-cost coconut shell waste pretreated with NaOH, HNO₃, ozone, and/or chitosan." *J. of Environ Mang.*, 226, 365–376
- (12) Reddy, K.R., Xie, T., Dastgheibi, S. (2014). "Evaluation of biochar as a potential filter media for the removal of mixed contaminants from urban storm water runoff." *J. Environ. Eng.*, 140.
- (13) Ndoun, Carla. "Biochar as a filter media for the adsorption of pharmaceuticals from wastewater effluent irrigation water" The Graduate School at Pennsylvania State University, Department of Agricultural and Biological Engineering. 2019.

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Permanent Address:

Education:

The Pennsylvania State University

University Park, PA

Schreyer Honors College | Bachelor of Science in Chemical Engineering

College of Engineering | Minor in Environmental Engineering

Class of 2020

GPA: ---/4.0

Professional Experience:

REU Summer Program

Researcher under Dr. Stephanie Velegol

University Park, PA

May 2018 - Aug 2018

- Published author in the Environmental Science & Technology letters
 - Successfully achieved >7 log removal of MS2 virus using a *Moringa Oleifera* coated sand filters
- Worked to scale-up the *Moringa Oleifera* coated sand filters for community use with cheaper materials
 - Presented this work at the national AIChE conference in Pittsburgh
- Performed, constructed and analyzed tracer tests to determine packing efficiencies within columns
 - Created and developed tools to analyze tracer test data using Mathematica

Research Experience

Researcher under Dr. Stephanie Velegol

University Park, PA

Jan 2018 – present

- Successfully remove low concentration *E.coli* (10^3 CFU/mL) using a *Moringa Oleifera* coated sand filters
- Performed *E. coli* culture protocol and double layer agar plaque assay protocol for virus removal quantification
- Conducted nanoparticle removal and zeta potential experiments using DLS
- Lead group testing different possible substitutes for *Moringa Oleifera* seeds to create alternative sand filters
 - Designed and created test for possible removal of *E. coli* using rape seeds and egg whites
 - Learned and conducted gel electrophoresis and fluorometric assays

EcoLab

Supply Chain Engineer

Beloit, WI

May 2019 - Aug 2019

- Created and designed an app to easily gain hourly updates on each manufacturing line
 - Used Microsoft Power Series to create individual dashboard reports that update in real time
 - Allowed for easy identification of inefficient trends and errors in processes
- Conducted time studies to find and eliminate non-value adding tasks and cut losses
 - Eliminated over 100 hours of work/per year
- Redesigned group meeting boards to better display and track metrics and improve meeting efficiencies

Leadership Experience:

QXE Chemical Engineering Honors Society

Member

University Park, PA

Jan 2018 - Present

- Volunteering service to the chemical engineering department in such areas such as tutoring and outreach programs
- Focus on the investigation and innovation in chemical engineering, service, comradeship and professionalism

- Developed professional skills needed in the current job climate such as building resumes, participating mock interviews, and conducting productive project management

AIChE American Institute of Chemical Engineering

Member

University Park, PA

Jan 2018 – Present

- Provide professional and personal growth, and chemical engineering expertise in meeting societal needs

The National Society of Leadership and Success

Member

University Park, PA

Jan 2018 - Present

- Offered unique lectures from the nation's leading presenters to teach better group and leadership skills

Mentor Experience:

LA Organic Chemistry

Learning Assistant

University Park, PA

Aug 2018 – May 2019

- Help facilitate learning, teach students and bridge the gap between the professor and students during lectures
- Hold office hours throughout the week to help tutor students outside of lectures

IA Phase and Chemical Equilibrium

Instructional Aide

University Park, PA

Aug 2019 - Present

- Create homework assignment and answer keys
- Conduct office hours twice a week along with review sessions for exams

Community Involvement:

Springfield Special Interest THON Organization

Member

University Park, PA

Aug 2016 - Present

- Attend weekly meetings to organize efforts to raise funds and awareness for the fight against pediatric cancer
- Help in raising \$192,883.78 as an organization

Thon Dancer Relations Committee

Member

University Park, PA

Aug 2016 – Mar 2017

- Worked with a small committee to help individual dancers through the 46 hour dance marathon
- Manage different activities and events throughout the weekend to keep the dancers in the best health and spirit possible

Community Outreach:

Art's Fest Kid's Engineering Day

Organizer

University Park, PA

July 2018

- Help organize and set up fun activities with the intent to help kids get involved and interested in science and engineering
- Ran and oversaw different tents to ensure activities were running properly

Penn State Water Heroes

Organizer

University Park, PA

Mar 2018

- Worked with kids in a variety of different activities to help get them interested in science and engineering
- Set-up a station for kids to see bacteria removal using different filters

Honors, Skills, and Interests

Honors: Schreyer Honors College, Dean's List (FA 16, SP 17, FA 17, SP 18, FA 18, SP 19)

Scholarships/Awards: Duda Student Research Award, McTurk Honors Scholarship, L. Riess Scholarship, Reppert

Endow Scholarship, LT Harry Wagner Scholarship, Bio-fellowship Endowment

Publication: *7 Log Virus Removal in a Simple Functionalized Sand Filter*, Environmental Science & Technology

Presentation: 2018 AIChE national conference poster presentation, 2018 REU poster and verbal presentation

Skills: Mathematica, Microsoft Office, HTML, Microsoft Power Series

Interests: College Football, Country and Rock Music, Fantasy Sports