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SCHREYER HONORS COLLEGE

DEPARTMENT OF CHEMICAL ENGINEERING

CHARACTERIZATION AND SCALE-UP OF WATER TREATMENT VIA ADSORBED *MORINGA OLEIFERA* PROTEIN

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ABSTRACT

Moringa oleifera is a tree that grows across the world in equatorial countries.[1] For thousands of years, people have crushed the seeds, dissolving them in water; the solution was then used to treat water.[2] Moringa has been found to contain a natural cationic protein which, when dissolved, exhibits antimicrobial and flocculant properties.[3] The principal problem with treating water in the developing world is that the seed material left behind acts as biological oxygen demand (BOD), which fosters bacteria growth.[4] One solution for creating potable water in these developing countries is to electrostatically attach the cationic protein to negatively charged sand; this functionalized sand (*f*-sand) could then be utilized to treat water. The abilities and applications of the *f*-sand were studied according to the objectives below. The total turbidity removal capacity was tested over time for aged *f*-sand samples using a model solution of kaolin clay suspended in water. Measurements were taken by correlating absorption with kaolin concentration. It was determined that the total average turbidity removal ranged from 81% to 89%, with 65% to 83% of total clearance being achieved after only 20 minutes. Furthermore, different seed batches were tested for turbidity removal capacity and the total turbidity removal percentage ranged between 47% and 90%; and percentage of total clearance after 20 minutes ranged between 63% and 95%. The next objective was to apply the filter to the community scale that could be used to service an entire village or group of families, thus providing income and occupation as well as safe drinking water. Residence time and filter column dimensions were proposed using preliminary design methodology, and two prototypes were constructed and experimentally tested. Attempts were made at designing a quantification method to measure bacteria removal by *f*-sand.

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ACKNOWLEDGEMENTS

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Chapter 1

Introduction

The Role of the Thesis

The driving force behind the research that follows in this thesis was both to fulfill obligations to the United States Environmental Protection Agency's P3 Grant competition, as well as to take an innovative technology present in the Velegol laboratory and attempt to produce an implementable water treatment technique for areas without access to clean water but with access to *Moringa oleifera* trees (referred hereafter as "Moringa"). Moringa is a native, non-invasive species that is found across equatorial areas of the world. [1] Moringa seed, when crushed in solution, has a cationic protein which dissolves and acts to clarify and disinfect the drinking water. [3] However, the most challenging problem is that the remaining organic seed material acts as biological oxygen demand (BOD), which fosters subsequent bacteria growth. [4] One solution presented is to electrostatically adsorb the *Moringa oleifera* cationic protein (MOCP) to anionic silicon dioxide (sand) particles, and then utilize the functionalized sand (*f*-sand) to treat drinking water. The abilities and applications of the *f*-sand were studied according to the objectives below.

Principal Research Objectives:

- ***Flocculant capacity.*** *Adsorbed cationic Moringa oleifera protein was effectively used to clear model turbid solutions.*
- ***Filter column design.*** *A preliminary filter column was collaboratively designed for community-scale application, and two prototypes were constructed.*
- ***Quantitative bacteria reduction measurement.*** *Two methods were examined to quantify microbial reduction in treated samples; a visual counting technique appeared viable.*

Moringa oleifera as a Point-Of-Use Water Treatment Method

Throughout countries plagued with drinking water contamination issues, point-of use techniques are often used to treat water at the location where it is utilized. Current disinfection techniques include the use of solar radiation and also standard chlorination to kill bacteria, protozoa, etc. [5] In addition to biological contaminants, a common problem with most surface water is the presence of turbidity; thus, flocculation is a requirement for any water treatment source. In developing countries, alum often serves as such a flocculant; however, the sludge left behind is toxic, requiring further treatment. [6] When considering implementation in developing countries, this toxicity issue is non-ideal.

It has been shown and well established in the literature that a protein from the seeds of the *Moringa oleifera* tree functions as both a disinfectant and as a flocculant. [7-11] People in equatorial areas of the world have used Moringa for thousands of years to treat contaminated water, and the Peace Corp utilizes the technology today. [2] It was learned that the design of the protein's structure allows for this antimicrobial action. [3] There is a peptide section that is active in solution, when bacteria are drawn close by the electrostatic interaction with the cationic protein. [12] A helix-turn-helix motif on the protein acts as a molecular knife to disrupt and compromise the cell membrane of the bacteria. [13]

Moringa oleifera offers the solution for purification presented above as well as a solution that promotes environmental sustainability. Moringa is grown around the world in equatorial regions, which often coincide with developing nations, which exhibit exceptional problems with waterborne illness, malnutrition, and poverty. [1, 14] These developing countries do not have the necessary funds to provide widely distributed safe drinking water like the rest of the world; in addition, the resources and technology needed to provide that necessity are not readily available

in these regions. Because *Moringa* is cheap and locally available, the solution is invaluable. The seed exhibits no cytotoxicity, so it is completely safe for consumption in water, unlike some flocculants such as alum. [15] In addition, Moringa is already a regular component of people's lives in those developing nations. The seed contains vitamins and nutrients that fight malnutrition and compliment people's diets, and it can be harvested and pressed to extract oil, which can be converted to biodiesel. [16]

However, one of the greatest barriers to use of Moringa is that treating water with crushed seeds leaves behind much organic plant matter, which then acts as biological oxygen demand (BOD) in solution, which can foster bacteria growth over time, thus contaminating the water further. [4] Much of the research that inspired this thesis was done to develop a technique that eliminated the BOD, which could allow for not only clean, but also potable water.

Egyptian people were reported to rub Moringa seeds on clay pots to wash their drinking water thousands of years ago. Now, we attempt to extrapolate that age old, time-tested technique. Our proposed technique utilized the electrostatic properties of the protein to dissolve it in water and adhere it to negatively charged silicon dioxide particles (sand). The sand would act as carrier particles for the active protein. Moringa has been found to exhibit an isoelectric point between 10 and 11, thus proving its zwitterionic state to be cationic when dissolved in water. [3] Furthermore, Kwaambwa et. al. found that the adsorption isotherm of the protein suggested that a vast amount of multilayer adsorption had occurred, which indicated self adsorption of the protein.[17]

One aspect of water treatment that Moringa doesn't necessarily address is the removal of groundwater contaminants such as metals. These contaminants, such as arsenic or cadmium, would require some sort of redox treatment to be removed.

Since Moringa is a non-invasive species, it could be introduced to new geographic regions which do not naturally exhibit the tree, thus providing the water treatment technology as well as the prosperity aspects that can be derived from this miracle tree. It was hypothesized that the treatment technology could serve as a way for women to gain respect and higher social standing within a community as they take ownership over running something as important as water treatment. If the technology was adapted on the community scale, it could provide an occupation and income for a family. Furthermore, this ownership would ensure the proper and sustained used of the treatment technique. If Moringa was cultivated further, more seeds could be exported, thus presenting the developing nation and people with a profitable crop. The seeds' oils and nutrients are widely used to produce cosmetics, lotions, and dietary supplements, which are presently available and being sold on the market.[18-20]

Background Research on Moringa Protein Functionalized Sand[4]

Although there had been much research surrounding the use of *Moringa oleifera* in solution to clear turbidity and bacteria[4], there had been little research on the protein's effectiveness once adhered to surfaces. Figure 1 visually shows the Velegol laboratory research, where Jerri, et. al. proved that the Moringa protein remained active as a flocculant when adsorbed to sand. [4] *f*-sand was used to clear a model turbid solution produced from dispersed kaolin clay particles.

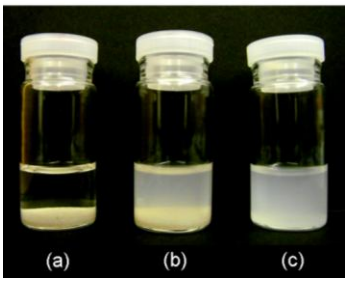


Figure 1: Turbidity removal with *f*-sand (a) was shown relative to treatment with regular sand (b). The stock kaolin was shown (c).[4]

Work was then done to determine the optimal functionalization ratio. Figure 2 shows that sand samples (6 grams) functionalized with varying amounts of Moringa seeds (four, two, and one) exhibited almost no difference in their ability to remove turbidity. The model kaolin solution was cleared to approximately the same concentration at approximately the same rate. However, *f*-sand functionalized with only half of a seed showed less ability to clear turbidity, with respect to both kinetics and capacity. Therefore, it was determined that the optimal seed to sand ratio was between one half and one seed per 6 grams of sand. [4]

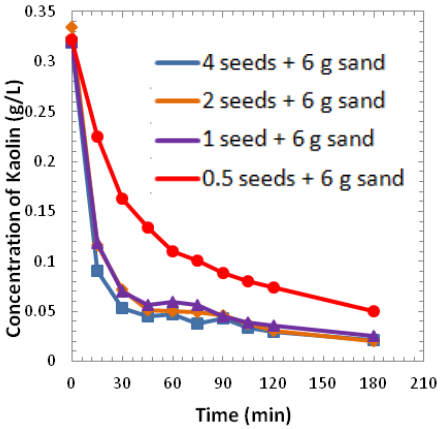


Figure 2: For sustainability and efficiency purposes, the optimum effective seed to sand ratio was determined to be slightly less than 1 seed per 6 g sand. [4]

The method employed to functionalize sand with MOCP left the samples wet upon completion. These samples could be utilized immediately to clear turbidity or they could be allowed to dry (in open air) and stored for later use. Thus, it was necessary to determine if the drying of the sand caused a decrease in effectiveness. Figure 3 shows that the amount of saturation upon usage did not affect the capacity of the *f*-sand sample to remove turbidity; however, the dried samples proved to flocculate the clay slower than the wet samples. It was hypothesized that drying did not limit effectiveness by affecting the protein. Rather, because the protein works in solution, it was believed that the excess time was required to hydrate the protein adhered to the sand surface. [4]

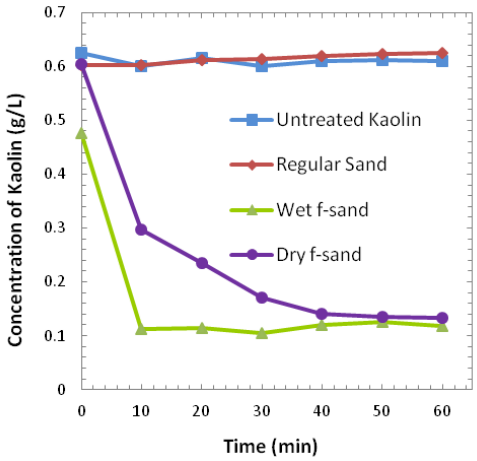


Figure 3: Preliminary kinetics studies showed that *f*-sand remaining hydrated from the time of production to the time of use cleared turbidity faster than sand that was allowed to dry. Total clearance remained constant, however. [4]

Having found that the drying of *f*-sand samples was non-detrimental to total clearing capacity, it was determined that the longevity should be tested to determine the relative effectiveness of aged samples. Figure 4 shows that there was not a significant relationship between sample age and kinetics nor sample age and total turbidity clearing capacity. The implication is that people in the developing world could use this technology to produce *f*-sand in bulk and then consume it over the course of a month, thus utilizing their time efficiently. This short-term study inspired a long-term longevity test which analyzed samples varying from 2 to 7 months in age; results were presented in the body of this thesis. [4]

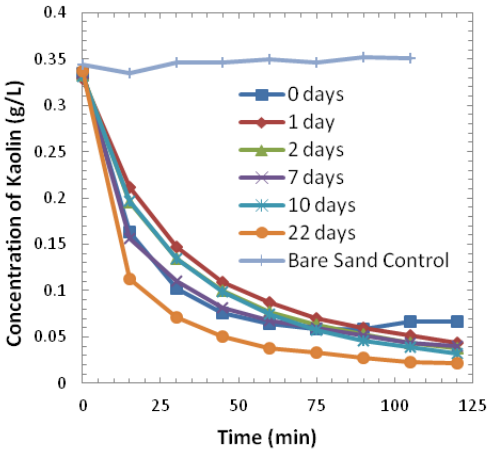


Figure 4: Turbidity removal by sand that was aged up to 22 days relative to a bare sand control. There was no apparent correlation of age and effectiveness. [4]

Lastly, research conducted in our laboratory group clearly showed the ability of *f*-sand to capture and kill bacteria from solution. Figure 5 shows a solution of DH5α *E. coli* treated with regular sand at left and *f*-sand at right. The presence of the red fluorescence in the *f*-sand-treated sample represents bacteria with compromised membranes, thus showing that bacteria are affected by and attached to the functionalized particles. It is still left to be determined whether or not a compromised membrane equates to death and permanent loss of viability. [4]

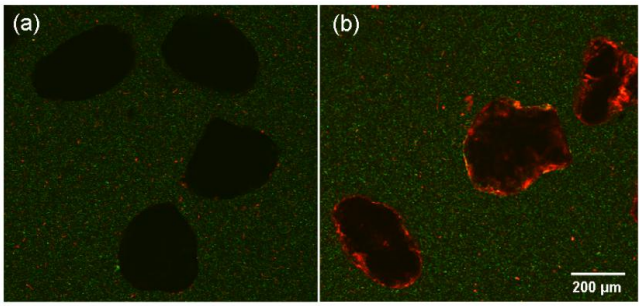


Figure 5: In (a), bacteria remains mostly viable when sand particle are added. However, in (b), sand with adsorbed cationic *Moringa oleifera* protein compromises the membranes of adjacent bacteria, producing the red color. [4]

Thesis Research Focus

While the results of previous research proved that the antimicrobial and flocculant properties of the cationic Moringa protein remain active despite adherence to carrier silicon dioxide particles,[4] many questions still existed regarding the extent of effectiveness and application of the technology. Many variables affect the presence and concentration of the cationic protein in the seeds; therefore, different batches could produce *f*-sand with very different levels of effectiveness. Thus, if experiments were conducted using *f*-sand samples synthesized from various seed stocks, it would be difficult to determine whether variability resulted from the controlled variable or from the variability in seed origin. This thesis explores the variability in *f*-

sand effectiveness between batches of Moringa seed as well as longevity and viability of *f*-sand over the course of 7 months.

Furthermore, while disinfection was previously discussed, filtration is also a method for purifying drinking water. This thesis explores the possibility of applying the *f*-sand technology to a community-scale filter, which could provide occupation for an individual or a family. Hopefully, giving ownership over the process would ensure the proper upkeep and operation of the technology. One filtration method that been proven effective has been the biological slow sand filter, which forms a low permeability scum layer of bacteria, protozoa, and algae; that tightly networked layer acts to filter out many harmful contaminants. Community-scale biological slow sand filters are currently operated according to a similar model; therefore, the functionalized Moringa filter could follow the example or perhaps could be operated in conjunction with the slow sand filter.

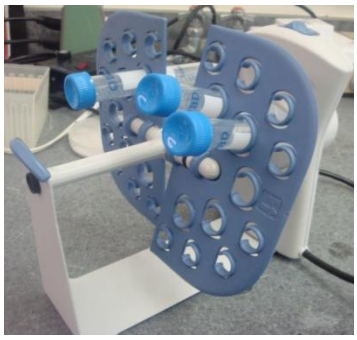
Lastly, much data has been obtained to characterize the turbidity removal capacity of *f*-sand; however, very little research has been done to quantify the bacteria removal effectiveness of *f*-sand. The predominant reason for this was: while absorbance of kaolin clay particles could be correlated with concentration to measure turbidity, no simple technique was available to quantitatively measure bacterial clearance. Therefore, this thesis presents several attempts at designing a cheap, simple bacterial quantification method.

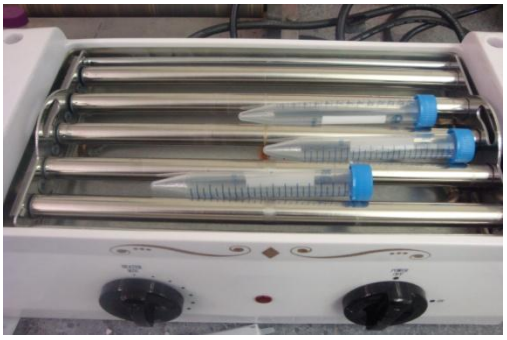
Chapter 2

Materials and Methods

f-Sand Production

When conducting batch optimization and characterization experiments, *f*-sand samples were produced in a batch process as well. Seeds were crushed using a mortar and pestle, and the seed matter was transferred to a plastic tube containing 10 mL of deionized water (DI) for each seed crushed. Using devices shown in figure 7, samples were rolled for approximately one hour and then allowed to settle for 40 minutes. Float seed material was removed and the liquid supernatant, dubbed *Moringa* serum, was removed from the tube. In order to functionalize sand, 2.5 mL of *Moringa* serum and 7.5 mL DI were added to 2 grams of sand in a plastic tube. The mixture was then rolled for approximately 1 hour and then rinsed about 10 times with DI to remove any excess BOD from seed material. Electrostatic interactions between the protein and sand, as well as the protein's self-association, held the protein adsorbed during the rinsing process. The *f*-sand samples were then allowed to air dry and were capped for extended storage.





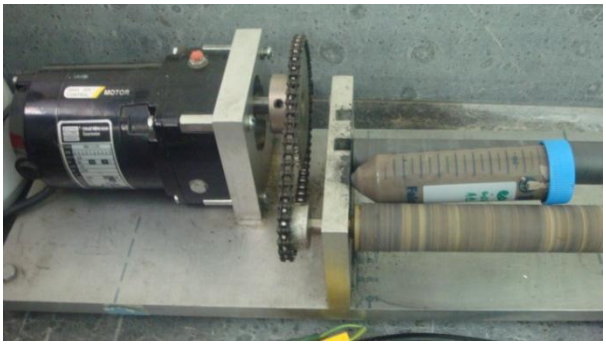


Figure 6: These devices were used to roll plastic tubes during: protein solubilization in water to create Moringa serum, functionalization of sand with Moringa protein, and turbidity removal tests. The device at top left can roll 15 mL and 50mL tubes. The device at top right rolls only 15 mL tubes, and the device at bottom right rolls only 50 mL tubes.

Turbidity Removal Data

f-sand was used to treat a kaolin model turbid solution, in order to characterize the flocculant properties of the technology. During these trials, 15 mL plastic tubes were charged with 6 mL of diluted kaolin stock solution. Samples were rolled using the devices shown in figure 6. A Helios Thermo-Spectronic UV-Vis spectrophotometer was used to measure the absorbance of the solution over time, which decreased when *f*-sand was used. The Beer-Lambert law was used to calibrate the instrument for the specific solution used.[21]

$$A = \varepsilon l c$$

- A = absorbance (at 450 nm)
- ε = absorption coefficient
- l = path length (1 cm)
- c = sample concentration

Two turbidity removal experiments were conducted, and the initial turbidity concentration was determined for each experiment: for the longevity experiment (0.32 g/L) and for the relative batch trail (0.3 g/L). A calibration was conducted for the instrument for each experiment: for the longevity experiments (0.32 g/L = 0.883 AU; ε = 2.76 AU*cm^2/g) and for the relative batch trial (0.3 g/L = 0.951 AU; ε = 3.18 AU*cm^2/g).

Bacteria Removal Techniques

All microscopic imaging was done using a Nikon TE300 microscope. Fluorescent lenses were utilized for fluorescence imaging of live/dead staining. The Virtual Dub software program was used to output the images digitally.

Column Design

A previous filter application produced by colleagues was shown in figure 7. The unique u-shape allowed flow rate to be altered by hydrostatic head differential. Furthermore, fluid could be passed through the filter in reverse to clean the filter, clear any clogs, or regenerate the *f*-sand.

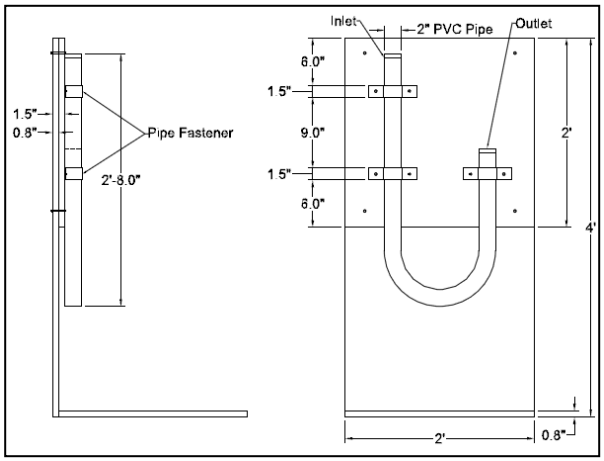


Figure 7: U-shaped filter charged with *f*-sand as the packed media. Filter designed by Lauren McCullough. Figure was drawn by Mary Beth Paskewicz, Bradley Kaley, and Tzonu Tzonev.

In the research of this thesis, two new filter designs are presented, which were derived from fluid flow equations. Materials for those filter columns were purchased at Lowes in State College, PA. The sand used was basic playground sand, which was then sieved to obtain a 60 mesh sample. Upon functionalization, seeds still with shells were crushed and mixed in two large beakers in order to homogenize the *Moringa* serum. Sand was then functionalized by manual mixing with hands and stirring with large objects; it was then added to the filter column as wet slurry. From the point of charging the column with the functionalized sand and during operation, care was taken to ensure that the column remained saturated with water in order to minimize cracking. Empirical flow rates were measured by collecting a measured amount of effluent over a standard time.

Chapter 3

Results and Discussion

Objective 1. Flocculant capacity. *Model turbid solutions were successfully cleared using adsorbed cationic Moringa oleifera protein samples that varied both in age and in seed origin.*

Two control experiments were run to microscopically show the effectiveness of adsorbed *Moringa oleifera* cationic protein (MOCP) acting on a sample of 60 mesh laboratory sand. One sample of sand was functionalized with MOCP and rolled in water, while another sample of sand was simply rinsed and rolled in water. Figure 8 shows that the supernatant of the functionalized sample contained much less debris than the sample rolled in rinsed sand.

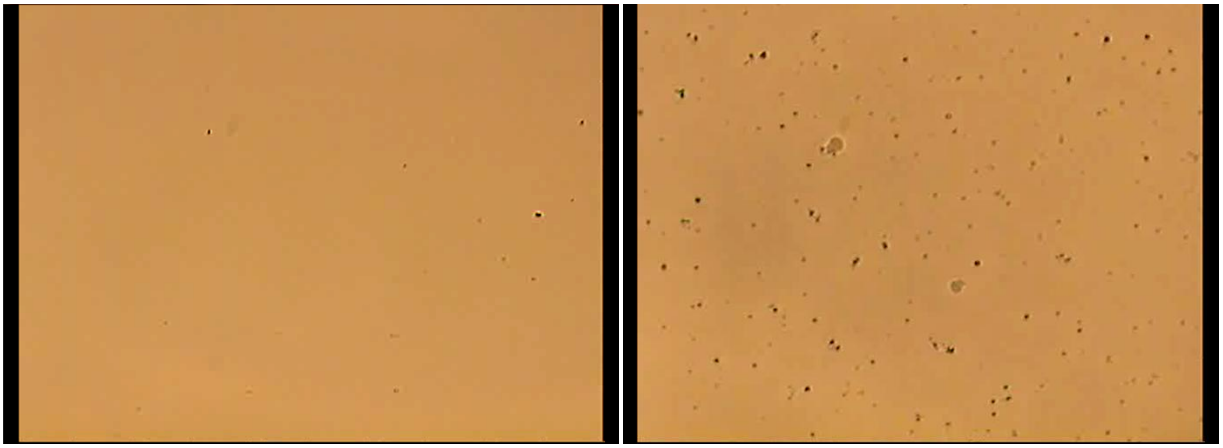


Figure 8: This figure shows the difference between control samples treated with *f*-sand (left) and with regular sand (right). Notice that the sample treated with f-sand displays less particulate than the sample treated with regular sand.

The functionalized sand (*f*-sand) was then used to flocculate kaolin clay suspended in solution, acting as a model turbid mixture. The two most successful studies of this thesis were conducted to characterize the *f*-sand's effectiveness at turbidity removal. First, the longevity of *f*-sand was analyzed study to find the long-term removal effectiveness. Ten samples of f-sand, two from each of five batches, were synthesized and stored dry for extended time periods. Figure 9

shows the samples that were simultaneously tested at their indicated ages. The five data sets represent the average of the two samples of the same age. Having proven earlier that *f*-sand did not lose effectiveness over the course of three weeks, a study was undertaken to examine the viability of the sand at longer time periods. It was expected that deterioration would occur over time; however, it was discovered that at time periods up to seven months, no such weakening was observed. The total average clearances ranged from 81% to 89%, with 65% to 83% of total clearance being achieved after only 20 minutes.

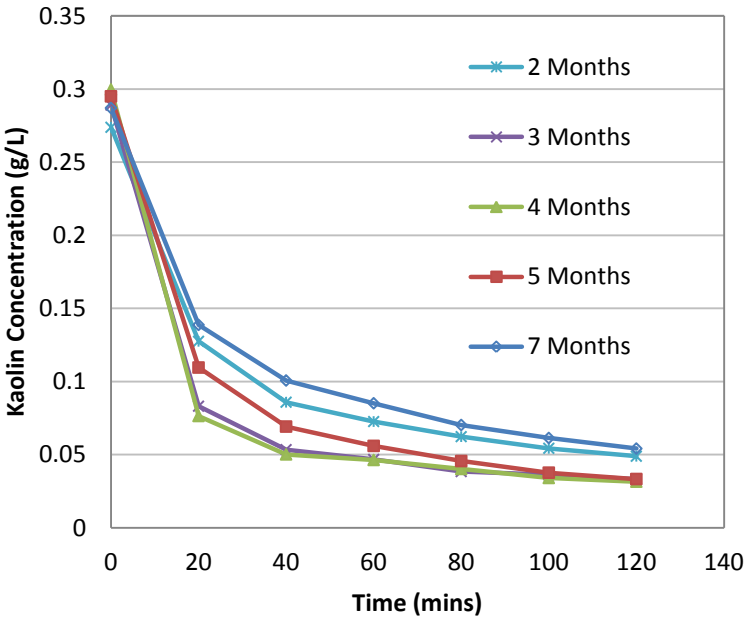


Figure 9: Turbidity removal over time for aged *f*-sand. There is no apparent correlation between effectiveness and age of *f*-sand

It was noted that while the seven month-old samples were least effective, the effectiveness of the other samples were not correlated with age. Furthermore, it is believed that the variability in total clearance was within the tolerance of experimental error, rather than a change in the model deduced from the data, since variation from sample to sample was not monatomic with age.

Turbidity removal experiments were repeated several months after first obtaining initial results; however, it was found that occasionally the samples would be ineffective, sometimes completely. Based upon casual taste tests, it was found that the seed batch, from which the samples were synthesized, seemed to lack the bitterness which ubiquitously characterized the effective seeds. Therefore, it was hypothesized that seed batches were variably effective at turbidity removal.

Six different batches of seeds were used to create samples of *f*-sand, under identical procedures. Furthermore, two control samples were utilized. First, a batch of non-functionalized sand was rolled with the model turbid solution. Second, *f*-sand produced one year prior to the experiments was tested; samples from the same seed batch were believed to contain viable protein, though the degree of effectiveness was unknown.

Figure 10 shows the distinct difference in effectiveness between batches. The total clearance data showed three distinct ranges: the worst sample cleared only 47%, two samples cleared close to 70%, and three more samples cleared approximately 87%. The most effective sample cleared 90% of turbidity, greater than a one-log decrease. Interpreting the kinetic data, the percent of total removal after the standard twenty minute residence time also ranged vastly from 63% to 95%. Interestingly, the three samples which achieved the highest turbidity removal also achieved the highest percent of total removal after the standard twenty minute residence time. Furthermore, the least effective sample also corresponded with the worst kinetic data. Further trials should be conducted to confirm whether or not amount of removal correlates directly with kinetics.

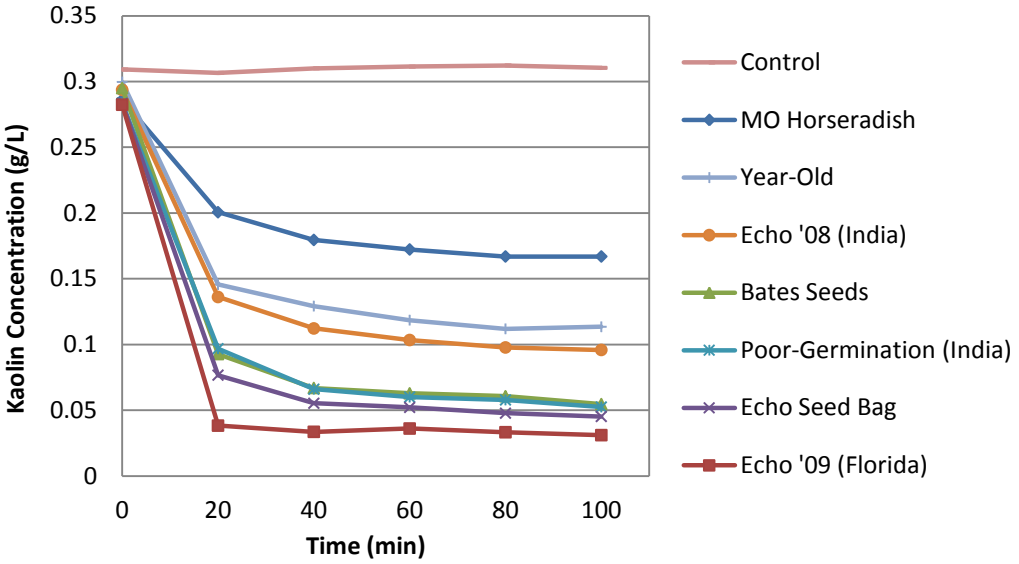


Figure 10: Turbidity removal over time for different batches. There is a large amount of variable effectiveness between individual batches of f-sand; however, all show a minimum clearance of 47%.

Objective 2. Filter column design. *A theoretical filter column was successfully and collaboratively designed for community-scale application, and two prototypes were constructed.*

Throughout the characterization process, it was considered how this water treatment could actually be implemented. Consideration was given to the many variables: cleanliness of water being treated, resources available, community willingness to utilize the technology, etc. The previously discussed methods were all undertaken in a modified batch process, where a bottle would be charged with *f*-sand and dirty water, which could then be cleaned. However, it was noted that this batch process may take more time than the people would be willing to invest, thus likely going unused. Furthermore, people may be unwilling to use the technology without the peer approval of the whole community supporting it. Thus, it was undertaken to determine if, the antimicrobial and flocculant properties aforementioned could be applied to the community-scale. Application to that scale would ensure community support and would keep many individual families from spending time to clean their own water. In addition, one individual or family could run the community filter, thus giving the individual an occupation, while also ensuring invested maintenance and proper use of the filter technology.

Beginning the design process, a theoretical model was created as a platform for designing a prototype. It should be noted that any prototype designed would be specific to a certain set of conditions; therefore, it would be important to have a design methodology to derive modifications based upon the conditions of the filter's locale.

The Kozeny-Carman equation, which gave the hydrostatic head loss relative to media depth, was used to derive theoretical design parameters.[21]

$$\frac{h_L}{L} = \left(\frac{Vk\mu}{\rho_w g}\right)\left(\frac{(1-\varepsilon)^2}{\varepsilon^3}\right)\left(\frac{S}{d_{particle}}\right)^2$$

- h_L = frictional head loss (ft)
- L = depth of media in filter (ft)
- V = superficial velocity of water (ft/s)
- k = Kozeny constant (assume 5)
- μ_w =viscocity of water (lb/s/ft^2) (at assumed 80°F) [21]
- ρ = density of water (lb/ft^3) (at assumed 80°F) [21]
- g = gravitational constant (32.2 ft/s^2) [21]
- ε = porosity
- S=particle sphericity (assume 6)
- $d_{particle}$ = particle diameter (ft)

Interpreting the equation, several constants were assumed, thus leaving three variables from which head loss could be determined: filter length (L), media particle diameter ($d_{particle}$), and superficial velocity. One design specification made was to assume a flow rate of 8 oz/min (0.24 L/min); at that flow rate it was hoped that the filter would be able to produce enough for a village. This seemed to be a reasonable rate at which people would be willing to wait for water; any longer and perhaps people would forsake the life-saving technology for convenience sake. Specifying the flow rate then allowed the superficial velocity to be determined by the cross-sectional area, which is directly proportional to the filter diameter. Using a sand density of 1.6 g/mL, the porosity of 250 um 60 mesh sand was calculated to be 30 ± 7.3%, which was assumed to be approximately constant for all mesh sizes.[22] This, however, was not effective porosity, which could be slightly lower, thus increasing the hydrostatic head loss. In addition, hydraulic conductivity for the sand was calculated to be (5.25 ± 0.72) x 10^{-4} ft/s, or (1.60 ± 0.22) x 10^{-4} m/s.

Media size was a crucial factor, since it could drastically affect the filter's performance, thus it was first to be determined. Figure 11 was produced by the Kozeny-Carman equation and shows a graph of hydrostatic head loss per unit length relative to the mesh size (represented as particle diameter) of the sand media. Ideally, a smaller mesh size would be better for filtering out contaminants, especially larger protozoa such as cryptosporidium and giardia;[23, 24] however that factor must be balanced against the head loss. This graph illustrates that at three different filter diameters, 250 µm (60 mesh) sand yielded the most efficient balance between particle diameter and head loss. Below a particle diameter of 250 µm, the head loss through the filter drastically increased, thus requiring the physical height of the liquid to be very large. This would necessitate a very tall column which was impractical at this stage of the design. If future implementable designs exhibited a need for a finer particle mesh, the feed tank could be placed at higher elevation, a rooftop for example.

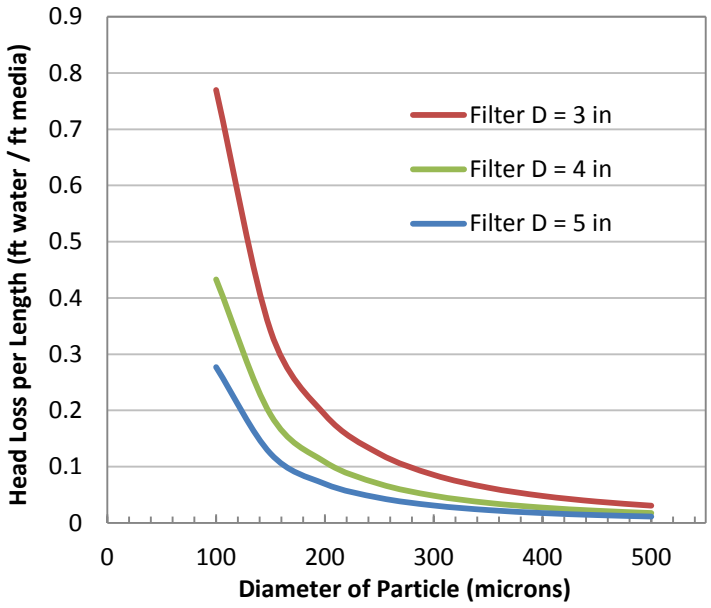


Figure 11: Hydrostatic head loss versus media particle size. The "knee of the curve" occurs where head loss spikes as particle diameter decreases; it would appear that the design point is close to 250 um.

It was necessary to determine a specification of a residence time for our filter design. Figure 3 showed that wet *f*-sand achieved almost 100% of total clearance after only 10 minutes. Furthermore, the majority of total clearance in dry *f*-sand trials occurred over the first 20 minutes. Based upon that batch optimization data and considering that the *f*-sand could dehydrate at some point, the ideal conservative estimate for residence time was approximately 20 minutes. Calculations of residence time were then conducted based upon the equation below, where V_{filter} represents volume, which is directly proportional to both the filter diameter and length.

$$Residence\ time = \frac{V_{filter}\varepsilon}{Q}$$

Assuming the constant grain size of 60 mesh, and a constant flow rate of 8 ounces per minute (0.24 L/min), figure 12 was produced to illustrate the residence time relative to filter diameter for varying filter length, according to the equation above.

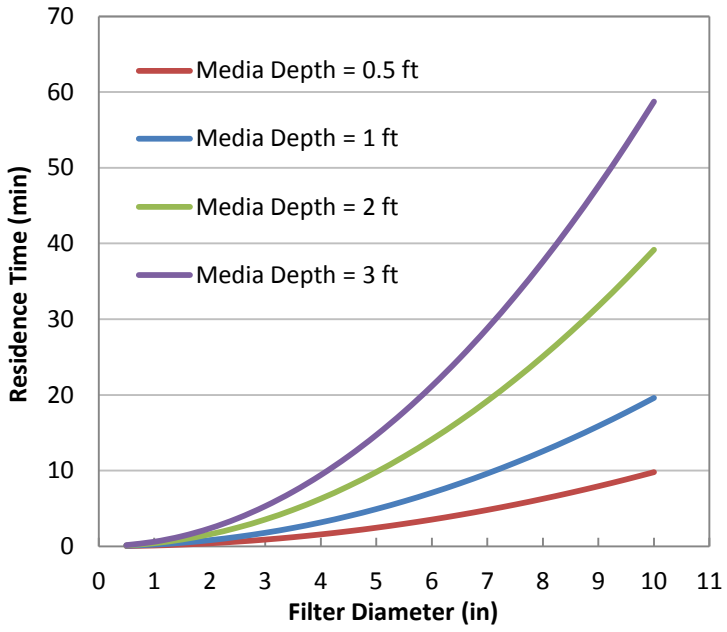


Figure 12: Residence time vs. filter diameter, varying media depth, at a constant mesh size (60). A large filter diameter and media depth are required to achieve the necessary 20 minute residence time.

One consideration for future research regarding residence time would be the implication of mass transfer on the system. It has previously been assumed that adsorption was kinetically limited; however, in a filter column, the bulk concentration of turbidity would constantly be replenished by the continuous flow, thus increasing diffusion. Furthermore, the falling liquid would increase the settling effect of mass transfer. Thus, if the adsorption process was mass transfer limited, the application of the treatment technology to the filter column could greatly decrease the required theoretical residence time. Future research, perhaps applying the *f*-sand technology to a small-scale filter column, should explore the adsorption limitations.

Based upon the results in figure 12, a large volume filter, either in terms of length or diameter, would be required to achieve the specified 20 minute residence time. At a media depth of 1 foot (0.3 m), a diameter of 10 inches (0.25 m) was required; increasing the length to 2 feet, decreased the necessary diameter to 7 inches (0.18 m). However, an immediate problem with creating a large filter is that many seeds are required to functionalize the column. It was determined from the optimization data, referenced in the introduction, that a ratio of 1 seed per 6 grams of sand would be the functionalization specification. The number of seeds could be calculated by multiplying the seed to sand ratio by filter volume and sand density, according to the equation below.

$$\#\,Seeds = V_{filter} * (1 - \varepsilon) * \frac{N_{seed}}{M_{sand}} * \rho_{sand}$$

Figure 13 shows the number of seeds required for functionalization relative to the filter diameter for varying media depths, according to the calculation above.

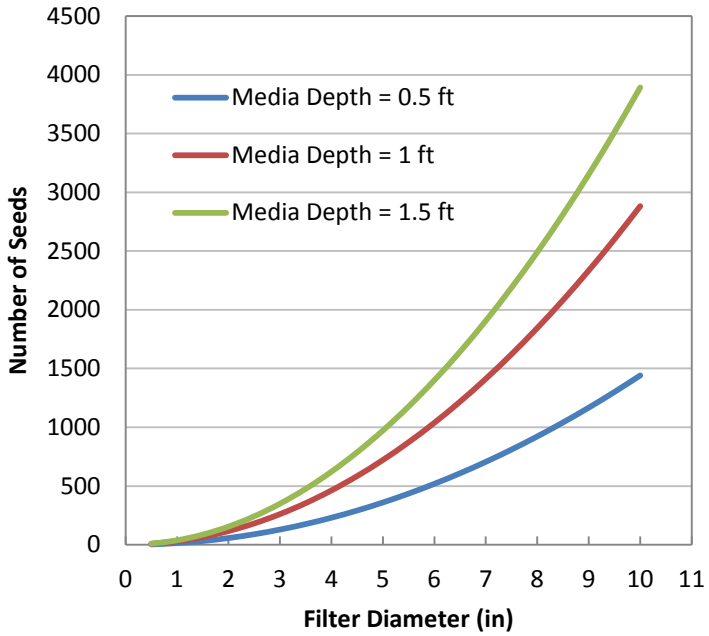


Figure 13: Seeds required to functionalize the filter column relative to filter diameters for various media depths. It was assumed that a ratio of 1 seed per 6 g sand would be used to functionalize the theoretical column.

Achieving the necessary 20 minute residence time at the 1 foot depth (0.3 m) and 10 inch (0.25 m) diameter, greater than 2500 seeds would be needed, which would be readily available to villages in equatorial climates. For a community with abundant access to *Moringa oleifera*, the theoretical design point would specify:

- Filter residence time of 20 minutes
- Filter flow rate of 8 ounces per minute (0.24 L/min)
- Particle diameter of 250 microns (60 mesh)
- The filter diameter and length can be varied to adjust the amount of hydrostatic head required. An aspect ratio > 2:1 is recommended to minimize the need for horizontal spreading.

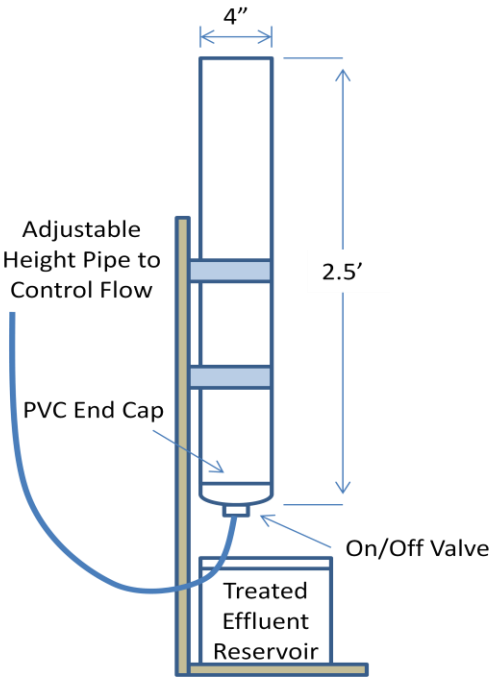
The design proposed above is a theoretical estimate that does not account for the communities' access (or lack thereof) to specific types of sand and materials. For instance, it

would be difficult for villages to obtain media of homogeneous mesh size. Additionally, producing *f*-sand with contaminated water may decrease the effectiveness; and the turbidity removal ability at different starting concentrations has yet to be examined. Therefore, a more comprehensive study of the design parameters should be undertaken, to account for manipulation of the variables based upon locality.

While the theoretical design may be possible in a village with access to many trees, it was not ideal for the laboratory scale; therefore, for the prototype design, less seeds were used and a shorter residence time was employed. Furthermore, upon initial trials, the flow rates were much greater than predicted, thus it became clear that channeling was the biggest concern. This issue decreased both the residence time as well as the contact time of the contaminated water with the adsorbed protein throughout the column. To mitigate the issue, gravel was added to the top of the bed to act as a dispersant and the hydrostatic head was drastically reduced. The first filter was thus designed with a diameter of 4 in. (0.1 m), a media depth of 1 ft. (0.3 m), and a gravel top of 8 in. (0.2 m). The operational hydrostatic head was specified to be 1.5 in. (0.038 m) of water. From Darcy's law, below, those specifications yielded a theoretical flow rate of 4.7 oz/min (0.14 L/min), which was still very reasonable, and a residence time of 5.3 minutes, which was approximately 27% of the specification.[21] Figure 14 shows the modified filter design.

$$Q = KA\frac{dh}{dl} = KA\left(\frac{depth\ of\ filter\ media + depth\ of\ excess\ water}{depth\ of\ sand}\right)$$

- Q = flow rate
- dh/dl = hydraulic gradient
- K = hydraulic conductivity
- A = filter cross sectional area



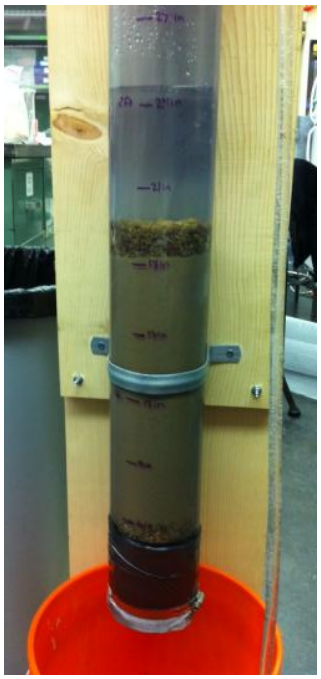


Figure 14: Modified filter design to include gravel top dispersant. Hydrostatic head shown is not representative of operating conditions.

The residence time proved a problematic specification to achieve, despite the addition of the gravel top. It was hypothesized that channeling was still occurring, possibly caused by either forceful water addition or drying and cracking of the filter media. Therefore, to improve the residence time, it was decided that the filter should employ the ability to be charged with contaminated water and then retain the water until the specified residence time was reached. Thus it could be operated in either batch or continuous modes. This second filter, shown in figure 15, was designed with a diameter of 6 in. (0.15 m), a media depth of 15 in. (0.38 m), and a gravel cap of 2 in. (0.05 m).

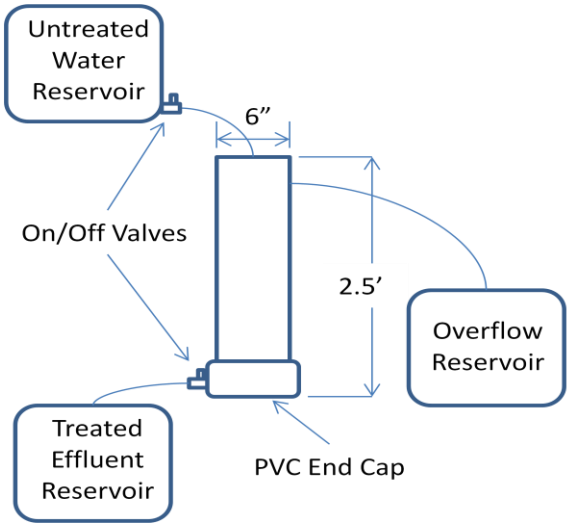


Figure 15: Third design, shown with gravel top acting as dispersant. The filter was designed such that the flow could be stopped to allow for increased residence times.

The filter was built with plastic piping, a PVC base, and a simple valve; these materials would likely be available in applicable equatorial countries. Furthermore, when operated in the batch mode, this design eliminated the need for exact particle size, volume, and flow rate specifications. Therefore, it would not be necessary to closely match the size or material specifications. The most critical parameter would be the seed to sand functionalization ratio, to ensure effective use.

The design, calculations, and prototyping of the filter were done in full collaboration with civil engineering students: Bradley Kaley, Mary Beth Paskewicz, and Tzonu Tzonev.

Objective 3. Quantitative bacteria reduction measurement. *Two methods were examined to quantify microbial reduction in treated samples; a visual counting technique appeared viable.*

It had previously been proven that *f*-sand compromised the cell membranes of DH5α *E. coli* bacteria, essentially eliminating viability. Furthermore, Jerri, et. al. showed that *f*-sand acted as a flocculant for the bacteria as well.[4] Therefore, the next step was to quantify those results to determine if the concentration of microbial contamination could be reduced to safe drinking levels. Visual microscopy, the most readily available imaging technology, was first used to derive a possible technique.

A proportion was set of the pixels of the digital visualization screen relative to the dimensions of the lens, which yielded an x to y ratio of 0.75, giving the dimensions of the viewing area to be 140 µm by 105 µm (x:y). Knowing the depth of the slide to be 200 µm, gave the visualized volume to be 2.94×10^{-6} mL. At any given count of cells on the screen after setting has occurred, the concentration of solution could be determined.

A sample of DH5α *E. coli* bacteria was obtained with a concentration ranging between 10^7 and 10^8 cells per mL. Such large concentrations were used in order to clearly see reduction; changes at lower concentrations could be lost in experimental noise. After visualization, approximately 225 cells were counted on the screen; dividing by viewing volume gave a concentration of 7.65×10^7 cells per mL. This number was accurately within the range of the known concentration.

Limitations of the technique were discovered. At low concentrations, the bacteria are highly spread out, thus counting did not necessarily give a representative sample of the total concentration. However, this technique could be used to confirm the concentration of an

untreated sample. Perhaps, the viewing volume could be recalculated for a different lens. Furthermore, this technique is very useful when observing the flocculant effects of *f*-sand at removing bacteria from solution; however, it is very difficult to determine the viability of the bacteria using only visual microscopy. Ultimately, combining this technique with live/dead fluorescent staining could account for this problem.

A widely accepted technique in biology is dilution plating, where a sample is diluted to a know ratio in order to determine the CFU's per volume, which can be re-scaled to find the concentration in the original sample. Dilution plating was employed for an initial filter test run. It was hypothesized that over time, effluent bacteria concentration would increase, as the adsorptive saturation capacity was reached at the breakthrough time. Figure 16 shows the results of the trial, where samples were taken over time and labeled in an increasing order. A lack of any CFU's at samples 6 and 12 was shown, as was a severely inconsistent elevation of CFU's at samples 3, 5 and 19. Based upon those results, it was determined that the technique was ineffective.

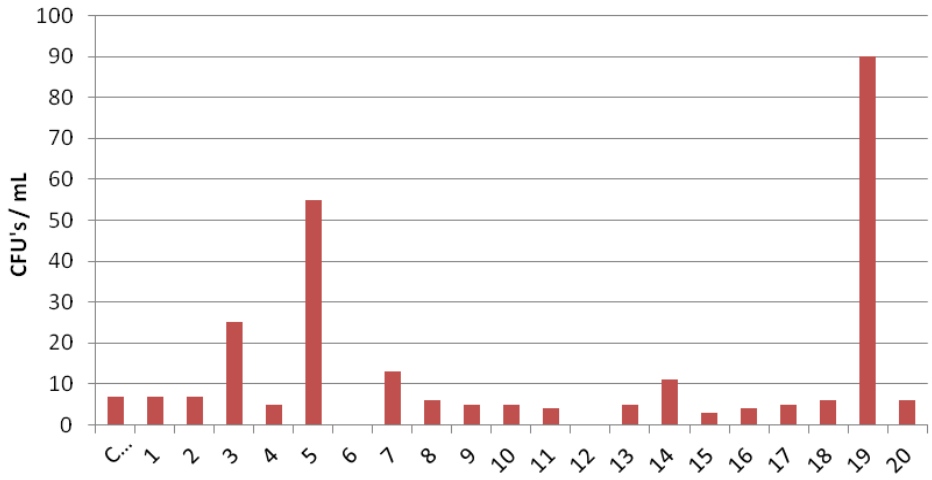


Figure 16: CFU/mL from functionalized filter column run. Bacteria removal was measured for contaminated water (10^5 cell/mL) passed through a functionalized filter. The inconsistent results show that the either the quantifying technique or the filter were ineffective.

To confirm that the technique was inconsistent, rather than the filter operation, several bacteria removal trials were conducted using a batch method. This method had successfully reduced the concentration of a model turbid kaolin solution by approximately one log. Dilution plating the results of that method produced erroneous results, similar to those of the filter trial; therefore, it was determined that the capabilities of the laboratory and experimenters precluded the use of this technique. Perhaps UV-spectroscopy could be used to measure the changing optical density of samples, which could be correlated with concentration. Lastly, fluorescence intensity tests with live/dead staining would show higher values at higher concentrations and could thus be correlated to determine concentration. However, these tests were also beyond the capability of the experimenters and the capacity of the laboratory.

Chapter 4

Conclusion

Conclusions

Moringa oleifera, which grows naturally around the world in equatorial regions,[1] has been shown to contain a protein which, when dissolved in solution, acts as a flocculant and disinfectant.[3] With an isoelectric point between 10 and 12, the protein is cationic in water, thus allowing it to electrostatically adhere to negatively charged silicon dioxide carrier particles.[3] The functionalized sand (*f*-sand) can then be used to treat contaminated water without leaving behind problematic amounts of organic matter to serve as biological oxygen demand (BOD). It was determined that the *f*-sand could be dehydrated and stored for at least seven months without losing any ability to remove turbidity from a kaolin clay solution, serving as a model for turbid surface water. Within the first 20 minutes, 65% to 83% of the total clearance had been achieved, and the total percentage of turbidity removal ranged from 81% to 89%. Furthermore, *f*-sand samples synthesized using various batches of seeds were tested to determine relative effectiveness. It was shown that the percentage of total clearance after 20 minutes varied between 63% and 95%, and the total turbidity removal ranged drastically from 47% to 90%.

A preliminary design of a community-scale filter column was proposed, and two prototypes were built and tested to determine major limitations. Several methods to quantify bacteria removal were proposed and tested, with one technique appearing feasible, a visual counting method.

In addition to providing safe, healthy drinking water, Moringa could also foster prosperity among people. If either a batch technique or a continuous filter design were employed in developing countries, people could have improved health and lifestyles. Furthermore, the

operation of the community-filter could provide profitable occupation for an individual or family and could also potentially empower women with a viable livelihood. In addition, if the technology were to become wide-spread, more Moringa trees could be grown, thus leading to a greater amount of seeds that could be exported for ingredients in dietary supplements, cosmetic products, or even biodiesel, thus generating revenue for the developing nation.[16, 18-20] The usage of this technique could clearly provide for both the safety and prosperity of developing people groups around the world.

Future Research

While no significant difference appeared to exist between the total clearance measurements of the longevity study, it is recommended that further trials be conducted so that a statistical analysis can be used to confirm that the variance is based upon experimental limitations. In addition, it is recommended that further trials be conducted to determine the implications of the variable clearance measurements between *f*-sand samples synthesized from different seed stocks. Further research should be done to confirm that the results are consistently and statistically different. Our research group has already isolated the protein that is believed to exhibit the flocculant and antimicrobial effects; therefore, it would be interesting to quantify the amount of protein per seed for each batch to see how it correlates with clearance ability. Furthermore, basic turbidity removal trials should be conducted while varying the starting concentration of the model turbid kaolin solution to see whether one log removal can still be achieved; the model turbid solution being used as an adsorbate should be varied as well.

The most successful application of the *f*-sand technique thus far has been the batch-type method, rather than the continuous flow filter. However, the utilization of a filter column offers many benefits such as the removal of larger protozoa; thus, if improvements could be made, the

solution could be very viable. The limiting design specification was that a large amount of seeds would be required to functionalize a large column; therefore, further laboratory research should be scaled down. Furthermore, scaling down would assist in limiting channeling, which proved to be a major issue during column operation. In this scaled-down system, the limitations of adsorption could be explored as well.

While much work has been done to successfully quantify the turbidity removal capacity, perhaps the largest hurdle to overcome is the development of a technique to quantify bacteria removal. It is recommended that several quantification methods be designed and tested on batch-style bacteria removal trials using DH5α *E. coli* solution treated with *f*-sand. The suggested visual counting method is limited to observing less than one order of magnitude of change; plating methods and fluorescence intensity measurements could be explored further to obtain more precise results. These results should then be compared to a range of *f*-sand samples synthesized from various seed types as well as the isolated protein.

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Appendix A. Research Presentation for U.S. EPA P3 Grant

The aforementioned research was directed towards fulfilling the grant provided by the United States Environmental Protection Agency's P3 Grant. The Velegol laboratory had previously obtained $10,000 from phase 1 of the grant competition: to optimize batch turbidity removal, to quantitatively analyze results, and to build a working prototype. These topics were explored (as partially detailed in this thesis) and were used to produce a grant proposal for phase 2 of the competition. Furthermore, three days were spent presenting the research in Washington D.C. at the U.S. EPA's Sustainable Design Exposition. The audience ranged from relevant field professionals to other students and the general public. The poster shown in figure 17 was produced to aid in the presentation at the exposition.

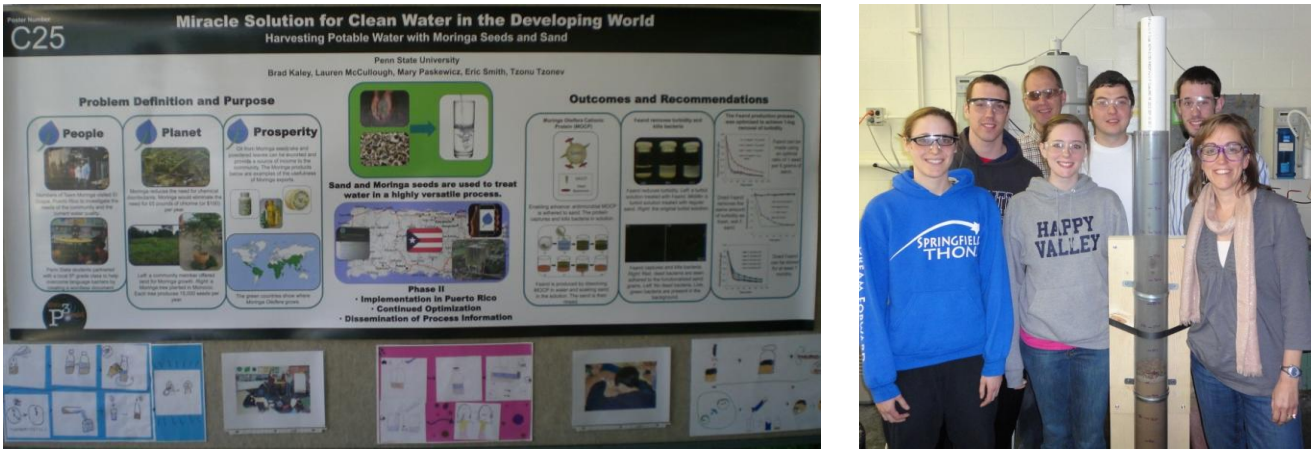

Figure 17: (Left) The poster representing the *Moringa oleifera f*-sand research at the EPA sustainability design exposition in Washington, D.C. (Right) The research team responsible for the collaborative effort also included: Dr. Darrell Velegol, Dr. Stephanie Velegol, Bradley Kaley, Lauren McCullough, Mary Beth Paskewicz, and Tzonu Tzonev.

Appendix B. Academic Vita for Eric Thomas Smith

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eric.th.smith@gmail.com Fowler, OH 44418

Objective: To obtain a full-time assignment in Chemical Engineering upon graduation in May 2012

Education - **The Pennsylvania State University**, University Park, PA May 2012

- Schreyer Honors College
- Bachelor of Science in Chemical Engineering
- Minor in Environmental Engineering

Work Experience

Dow Corning Corporation: Manufacturing Engineering Intern - Hemlock, MI Summer 2011

- Fluids & Adhesives Department at Healthcare Industries Materials Site
- Contributed to 2 capital projects and various other process improvement initiatives
  - Installation and validation of a new ~$ 4M clean room used for medical packaging
  - Redesign and installation of a flammable chemical pour-down process

Lafarge North America: Process Engineering Intern: Cement Division - Ravena, NY Summer 2010

- Conducted performance inspections on process equipment, including mills, separators, & kilns
- Responsible for: 1) Leading a power conservation initiative
  2) Upgrading plant-wide safety features and documentation

Research Experience

The Laboratory of Dr. Darrell Velegol – The Pennsylvania State University Fall 2010 - Present

- Purifying H_2O in the developing world using seeds from the locally-grown *Moringa* tree
  - Collaborated on writing a grant proposal for the EPA $75,000 P3 Award
- Analyzed colloidal micro-particles acting as motors when exposed to H_2O_2 or UV light

Research Presentations

U.S. EPA National Sustainable Design Expo – Washington, D.C. Spring 2011

- *Moringa* water treatment research was presented in competition for the EPA P3 Award
- Research reviewed by relevant field experts and public

International Engineering Experience

Industry Tour in France - IUT Béthune, Université d'Ártois Summer 2009

- Toured: **LVM** – polyvinyl chloride chemicals, **Jokey France** – plastics manufacturing;
  Herta – food processing, and **ArcelorMittal** – steel
- Gained an international perspective on engineering, business, and education

Selected Leadership Positions & Activities - The Pennsylvania State University

- **Teaching Assistant:** *Honors Leadership Jumpstart* Summer 2009 - Present
  - Instruct first-year Schreyer honors students in the principles of leadership theory
- **President**: Alliance Christian Fellowship (~150 Student Members) Spring 2010 - Present
- **Executive Team Member:** Alliance Christian Fellowship Spring 2008 - Spring 2010
- **Language Partner:** A program for international students to practice English skills Fall 2010 - Present

Selected Honors and Awards

- Boy Scouts of America -- Eagle Scout Award January 2005