IRON OXIDE AMENDED BIOSAND FILTERS FOR VIRUS REMOVAL

BY

IAN M. BRADLEY

THESIS

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Adviser:

Assistant Professor Thanh H. (Helen) Nguyen

ABSTRACT

Laboratory studies were performed to determine if the addition of iron oxides throughout biosand filter (BSF) media would increase virus removal due to adsorption. The proposed mechanism is electrostatic adsorption of negatively charged virion particles to positively charged iron oxides formed during the corrosion of zerovalent iron. Initial tests conducted using continuous flow, small-scale glass columns showed high MS2 bacteriophage removal in an iron-amended sand column (5-$\log_{10}$) compared to a sand only column (0.5-$\log_{10}$) over 20 pore volumes. Additionally, two experiments with a column containing iron particles revealed 4 and 5 $\log_{10}$ removal of rotavirus in the presence of 20 mg/L total organic carbon. Full-scale BSFs with iron particles removed >4-$\log_{10}$ MS2 for the duration of the experiment (287 days), while BSF with steel wool removed >4-$\log_{10}$ MS2 for the first 160 days. Plug flow for the BSF was shown to depend on uniformity between the iron oxide material and sand media grains. The results suggest that the duration of effective virus removal by iron-amended biosand filtration depends on source water conditions and the quantity and composition of iron material added. Overall, this study provides evidence that iron-amended BSFs may advance the field of point-of-use technologies and bring relief to millions of people suffering from waterborne diseases.

ACKNOWLEDGMENTS

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CHAPTER 1
INTRODUCTION[1]

An estimated 884 million people - 13% of the world population - lack access to safe drinking water [1]. As a result, 3575 million people die each year from water-related diseases [2]. While it is not possible to quantify the proportion of deaths directly due to unsafe drinking water and not attributed to other fecal-oral transmission routes [3], access to clean drinking water and proper sanitation can provide substantial improvements in health [4, 5]. Point-of-use (POU) water treatments, which allow the purification of water at the point of consumption rather than at a centralized location, allow water quality to be improved at the household scale [6]. Already widespread in their usage, as of 2007, 19 million people are estimated to use POU water treatment, in addition to the 350 million people who boil their water [7]. Studies indicate that the improvement of water quality through the use of POU technologies results in 30-40% reductions in diarrheal disease [7-10]. One of the most promising and widespread POU technologies is the biosand filter (BSF), a household-scale, intermittently-operated slow sand filter, in which the upper layers of sand media remain saturated during operation to allow the formation of a biologically active layer [11].

The BSF consists of a plastic or concrete hollow chamber that tapers slightly towards the bottom [12]. A drainage gravel layer is laid at the bottom of the chamber, covered by a separating gravel layer and a filtration sand layer. Approximately 5 cm above the filtration sand layer sits a diffuser. An outlet tube collects water from bottom of the chamber, passes the water vertically, and discharges the water at the outlet located at a height between the diffuser and the top of the filtration sand layer. During 24-hour

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cycle of filter usage, water is poured into the inlet reservoir. As a result, the hydraulic head pushes the water downwards through the sand filtration layer and into the drainage gravel layer, where it is collected by the outlet tube and ultimately discharged. As the water level drops in the inlet reservoir, the flow rate decreases. Flow ceases when the standing water within the inlet reservoir is at a height equal to the height of the outlet.

The top portion of the filtration sand layer collects the suspended solids, organic constituents, and microorganisms of the source water. Since the filtration sand remains saturated during and in between operation, a biological zone, wherein the sand grains are covered in a biofilm, develops in the top 10-20 cm of the filtration sand layer. The biofilm is credited with the enhanced removal of suspended solids and pathogens through mechanical trapping, adsorption, predation, and natural death. Development of the biofilm results in greater removal of suspended solids and pathogens, but its development also reduces flow rates [13-15].

Dr. David Manz developed the first BSFs in the 1990s at the University of Calgary as a way of improving water quality for low-income families in rural areas with restricted access to safe drinking water [16]. Since then, BSFs have been chosen by hundreds of humanitarian groups as the best method for improving water quality in developing countries and, as of 2009, it is estimated that over 200,000 BSFs have been implemented in over 70 countries [12]. Surveys reveal its wide acceptance by users due to the improved appearance, smell, and taste of the treated water [17]. Considering the criteria of water quantity produced, water quality, ease of use, and ease of access, BSFs have been identified as the point-of-use technology having the most potential to deliver sustainable potable water treatment to the developing world [11].

Both laboratory and field studies have documented improved microbiological water quality through the use of the BSF. BSFs remove greater than 99.9% of *Giardia* cysts and *Cryptosporidium* oocysts [18]. Bacterial concentrations are reduced 70-99.99%, depending on biofilm development and time of sampling [13, 19-21]. The improved water quality has been attributed to at least 20% reductions in frequency of diarrheal illness in studies conducted in the Dominican Republic [20] and Kenya [22]. However, both field and laboratory research has identified a critical shortcoming: BSFs are not highly effective in removing viruses [13].

Viruses cause approximately 40% of diarrheal illnesses in developing countries [23], with rotavirus being the leading cause of childhood diarrhea hospitalizations worldwide [24]. In natural water conditions of pH 6-8, sand and most viruses are negatively charged, causing a net repulsion and reducing virus removal efficacy by sand filtration [25]. Thus, water contaminated with pathogenic viruses is not yet potable after passing through a BSF, and a form of virus removal is required to treat the effluent [12]. The addition of zerovalent iron to the sand media results in filters that more effectively remove viruses from water [26] as corrosion on the iron surface generates a positively charged oxide layer [27] to which the viruses may electrostatically adsorb [28].

The primary objectives of this study were to determine: (1) the efficacy of virus removal during the daily operation of the iron-amended BSF; (2) the efficacy of virus removal using different iron oxide sources; (3) the duration for which iron-amended biosand filtration effectively removed viruses. This study is unique to other studies for two reasons. First, unlike previous iron-amended BSFs, the iron source was added to the top half of the sand media rather than in the diffuser basin to increase the contact time

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between viruses and iron oxides. Second, to our knowledge, this is the longest BSF study conducted examining virus removal in both iron-amended and unmodified BSFs. Both small-scale columns and household-scale BSFs were tested using bacteriophage MS2. Rotavirus was used for select tests with small-scale columns, due to difficulty in propagating the virus.

CHAPTER 2
MATERIALS AND METHODS[2]

2.1. Virus Selection and Assay

Bacteriophage male specific type 2 (MS2) was selected as a model virus because of its structural resemblance to many human enteric viruses and its ease of use. MS2 was replicated and purified as described previously [29-33] with the following modifications . Briefly, *Escherichia coli* (ATCC 15597) grown in tryptic soy broth solution was inoculated with MS2 and incubated, followed by the separation of MS2 via centrifugation. After cell lysis and virus release, debris was removed via microfiltration through 0.2-µm and 0.05-µm low-protein-binding polycarbonate track-etched membranes (Whatman Nucleopore, USA). Virus was concentrated on a 100-kDa membrane surface (Koch Membranes, USA) in a Millipore ultra-microfiltration unit (Whatman Nucleopore, USA). The virus accumulated on the membrane surface was washed extensively with sterilized 1 mM NaCl solution to remove nutrients, microbial products and dissolved organic matter. The final MS2 stock was stored at 4 °C at a concentration of 10^{11} PFU/mL. MS2 was enumerated by the double agar layer procedure USEPA Method 1602. Briefly, plaques formed due to the inoculation of *E. coli* with MS2 at 37 °C for 16 hours, and plates with between 20 and 200 plaques were used for calculating the concentration of MS2.   Any plates containing more than 200 plaques were quantified from a higher dilution plate.

Select experiments were also performed using rotavirus (RV) to verify MS2 results. Group A porcine rotavirus OSU strain was obtained from the American Type Culture Collection (catalog # VR892). Rotavirus was propagated in embryonic African

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green monkey kidney cells (MA-104 cells) and extracted from culture as described by Rolsma et al [34]. The purification protocol was the same as for MS2, except with an additional filtration step in which a 0.05-µm membrane was used. Rotavirus was cleaned and stored in 1 mM sodium chloride (NaCl) plus 0.1 mM calcium chloride ($CaCl_2$) during the 100 kDa ultrafiltration to prevent the dissociation of the outer capsid proteins [35]. The final RV stock was stored at 4 ^{o}C at a concentration of $>10^5$ focus forming unit (FFU) per mL. Rotavirus infectivity assays were carried out following the procedures described by Rolsma et al [36].

2.2. Solution Chemistry

Newmark groundwater (NGW) was used as the background solution. Collected from a natural aquifer underneath the Newmark Civil Engineering Laboratory (205 N. Matthews, Urbana, Illinois, 61801), NGW is greensand-filtered for manganese and iron removal. NGW has been characterized and used in previous studies [37]. Content of total organic carbon (TOC) was measured using a Phoenix 8000 TOC Analyzer (Dohrmann, USA) in previous studies and found to be 2.35 mg/L [29]. Turbidity of the groundwater and the effluent were 0.25 NTU and 0.70 NTU, respectively, as measured by a Hach Turbidity Meter 43900. NGW was chosen over available surface waters for the following reasons. (1) Nearby river water would be impacted by the seasonal runoff from agricultural fields in the surrounding and upstream area, resulting in shifting water chemistry throughout the course of the study. (2) Stagnant water bodies would freeze over in the wintertime, causing inconsistent water collection over the duration of the experiment, which spans multiple months. (3) Newmark groundwater has already been

documented and provides a consistent water quality that better suites the longevity of the project.

Pasteurized primary wastewater effluent from a local wastewater treatment plant was used to establish a biofilm in the MS2 experiments, as previously suggested by Elliott et al. [13]. Treated wastewater effluent from the same treatment plant was used to conduct rotavirus experiments with high levels (20 mg/L TOC) of natural organic matter (NOM). This wastewater treatment plant uses conventional activated sludge treatment.

2.3. Column Experiments

2.3.1. Continuous Flow Test for MS2

Small-scale column tests were conducted to compare results with previous research examining MS2 removal through sand and sand/iron oxide columns [26], and to determine what effect, if any, orientation of iron particles in the sand column has on virus removal. Results were used to determine how household-scale filters would be incorporated with iron as an addition to the sand media. In addition, small-scale columns were used to examine the removal of rotavirus in the proposed design. Due to the difficulty of propagating the virus and the maximum concentrations necessary for use in the BSF, experiments on the household-scale filter with rotavirus were not possible. The glass columns had the following dimensions: 3.8 cm tapered ends, 8.9 cm main body length, 2.5 cm inner diameter, and a total volume between 146.7 cm^3 and 161.5 cm^3 (Figure 1). The control column was dry packed with 224.1 g clean quartz sand. The iron column was packed with a 10% iron by volume mixture with sand (30.4 g iron; 182.7 g sand) and a layer of sand only (42 g) at the influent.

Zerovalent iron particles (ETI8/50) used in the column studies were obtained from Peerless Metal Powders & Abrasive (Detroit, MI). The iron was used without pretreatment. Sand used in the column studies was an industrial quartz obtained from Unimin Corporation (Le Sueur, MN) described as 5020 (20% retained on 50 mesh or coarser) and 0.15 mm effective size in filtration. The sand was washed with deionized water until the supernatant was no longer cloudy.

Each column was flushed using a peristaltic pump for 10 pore volumes (PVs) of NGW at a flow rate of 1.36 mL/min to establish a steady-state flow. Held vertically, the bottom end of the column was used as the influent entrance (Figure 1). Plug flow condition was verified by conducting tracer tests using 3 pore volumes of a 0.1 M NaCl solution. Each column was then flushed with another 10 pore volumes of NGW. A solution containing ~10^9 PFU/mL of MS2 was introduced and samples were taken in 1.5 mL centrifuge tubes until breakthrough was well-established. Samples were taken from the control column every 5 minutes for 7 pore volumes, while samples were taken from the iron column every 15 minutes for 20 pore volumes. The effluent concentrations of MS2 were determined by plaque assay.

2.3.2. Non-Continuous Flow Test for MS2

To simulate the normal operation of a BSF, 4 additional columns (with the same dimensions and packing procedure as described in Section 2.3.1) were charged daily with 1 pore volume NGW containing ~10^8 PFU/mL of MS2. Primary effluent (PE) obtained from the local wastewater treatment plant, was also added to the solution (2.5% PE) to stimulate biofilm growth at the influent sand surface, located at the bottom of the column during charges. The columns were stored between charges in an inverted position, so that

the influent entrance was located on top and exposed to air (Figure 1). Three of the columns were packed with different orientations of iron particles (10% iron by total volume): an even mixture throughout the sand, a middle layer, and a layer at the influent. One column was packed with only sand.

2.3.3. Non-Continuous Flow Test for Rotavirus

Two different columns (sand only and 10% iron by total volume) were then flushed with aquifer water according to the procedures described in Section 2.3.2. Columns were charged with NGW and 2.5% PE for 6 weeks to establish a biofilm. Two sets of experiments were conducted. For the first set, the influent water was Newmark groundwater with 2.35 mg/L TOC. About 30 days after the first set of experiments, the second set of experiments was conducted, for which the influent water was effluent from a local wastewater treatment plant. The treated wastewater effluent was not disinfected and had a TOC of 20 mg/L. For each set of experiments, influent water was seeded with rotavirus (~10^4 FFU/mL or ~10^5 FFU/mL) and each column was charged with 1 pore volume of solution. After 24 hours, effluent samples were collected and determined by rotavirus infectivity tests.

2.4. Biosand Filter Experiments

2.4.1. Filter and Media Preparation

Two 60 L capacity plastic BSFs were obtained from HydrAid (Grand Rapids, MI). The filters were packed according to the four layer system established by Dr. Manz [38]. Each filter contained 5 cm of under drain gravel (6.25 mm-12.5 mm), 5 cm of medium sized support gravel (3.125 mm-6.25 mm) to separate the drainage layer from the filtration sand, and 40 cm of filter media (effective size 0.4 mm) with a 5 cm top layer

of fine sand (effective size 0.15 mm). Sand was manually sieved using the appropriate meshes. The effective sand size was similar to that used previously in research and practice [12, 13]. One filter was amended with iron by adding 5.54 kg of a mild steel nail (40 mm length, 2 mm diameter, "bright" finish) mixed evenly throughout the top 20 cm of the filter media, excluding the layer of fine sand.

The initial maximum flow rates for the unmodified plastic BSF and iron-amended plastic BSF following the first 20 L charges were 0.64 L/min (0.70 m/h) and 0.93 L/min (1.01 m/h), respectively. Every 24 hours each filter was charged with 20 L of a solution containing $\sim 10^8$PFU/mL MS2 and 2.5% PE to establish biofilm growth. PE was not added after day 20, when biofilm activity was well-established.

Three concrete BSFs were constructed using a steel mold built to specifications [39] provided by the Centre for Affordable Water and Sanitation Technology (CAWST) and were representative of BSFs in use worldwide [12, 40]. Each concrete filter was packed using the same specifications provided for the plastic filter. One filter was amended with iron by adding 5.54 kg of zerovalent iron particles (ETI8/50, same as used in column experiments) mixed evenly throughout the top 20 cm of the filter media, excluding the layer of fine sand. Another filter was packed with extra fine steel wool (Red Devil, Inc. #0000), which was mixed evenly throughout the top 20 cm of filter media. Due to the steel wool's low weight and large volume, only 0.26 kg of steel wool was used.

The initial maximum flow rates for the unmodified concrete BSF, iron particle amended concrete BSF, and steel wool amended BSF following the first 20 L charges were 0.64 mL/min (0.70 m/h), 0.59 mL/min (0.64m/h), 0.50 mL/min (0.54 m/h),

respectively. Every 24 hours each filter was charged with 20 L of a solution containing $\sim 10^7$ PFU/mL MS2 and 2.5% PE to establish biofilm growth. PE was not added after day 20, when biofilm activity was well-established.

X-ray diffraction (XRD) experiments were performed on rusted iron particle samples to determine the mineral composition of the rust. A Rigaku D/Max-b diffractometer with a copper X-ray source controlled by MDI's DataScan was used. The following parameters were used: 45 kV and 20 mA, the scanning angle range (2θ) was 15-80 degrees, and the scanning rate was 0.6 degree/min with a step increment of 0.05.

2.4.2. Water Chemistry

Influent and effluent samples were collected in 15 mL tubes for analysis of pH, dissolved oxygen (DO) content, alkalinity, NO_3^-, NH_4^+, Cl^-. Samples for MS2 were taken in 1.5 mL sterilized centrifuge tubes and held in a 4 ^{o}C refrigerator until analysis within 24 hours. Trace metal concentrations were determined using inductively coupled plasma mass spectrometry (ICP-MS) with an ELAN Dynamic Reaction Cell instrument (PerkinElmer, Norwalk, USA). Samples were diluted to a total dissolved solid concentration of 0.25%, and the light wavelength intensity from excited atom species was used to determine analyte concentrations. Turbidity of the groundwater and the final effluent was0.25 and 0.7 NTU as measured by a Hach Turbidity Meter 43900.

3.1. Tracer Tests for Column Experiments with Non-Continuous Flow

Four NaCl breakthrough curves through sand and iron columns with varying orientations of zerovalent iron are plotted in Figure 1. The three zerovalent iron orientations (10% iron by volume) were an even mixture throughout the sand ('mixed'), a middle layer ('band'), and a layer at the influent ('top'). Independent estimates of the pore volumes (sand only/top placement: 50.0 ± 0.1 ml, mixed/band: 54.9 ± 0.1 ml) were found by measuring the water volume necessary to fill each column completely. All columns had a resulting porosity of 34%. The NaCl tracer response suggests uniform plug flow with a sharp incline in conductivity after one pore volume and a sharp decline after a negative step input was introduced.

The Morrill Dispersion Index (MDI) was calculated for all four tracer tests using the method defined by Tchobanoglous et al. to confirm plug flow [41]. The MDI for each test was approximately 1.4. The USEPA classifies flow with an MDI of less than 2.0 as effective plug flow, while an ideal plug flow reactor has an MDI of 1.0 [42].

3.2. Column Experiments

3.2.1. Short Term Removal Using Continuous Flow (MS2)

The $\log_{10}$ reductions obtained by continuous flow through clean quartz sand with no iron particles and sand mixed with iron (10% iron by volume) are shown in Figure 2. For the sand with no iron particles, the breakthrough concentration of MS2 was achieved after one pore volume, with approximately half of the MS2 being retained (49.9%). MS2 concentrations in the effluent of the column of sand mixed with iron particles were under
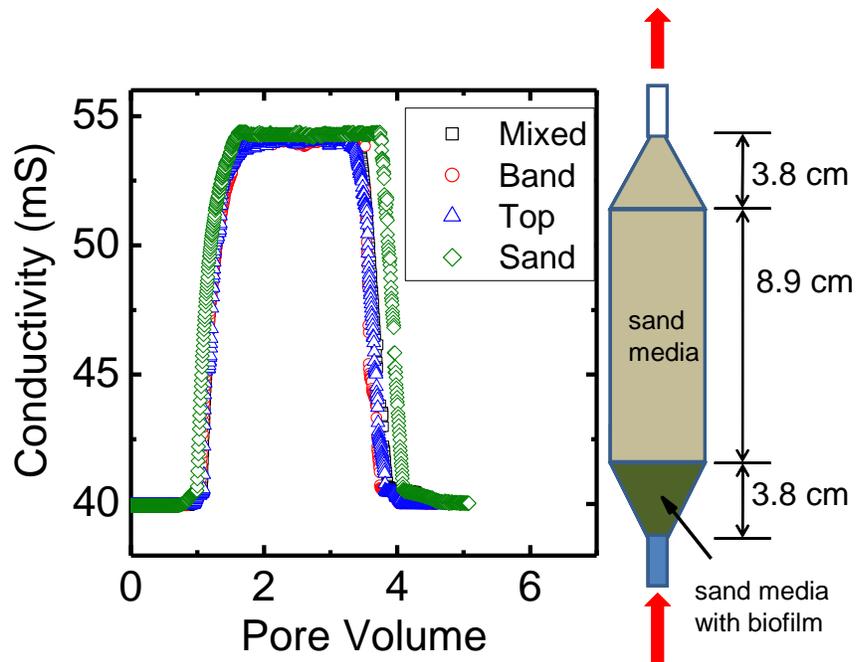

Figure 1. (Left) Breakthrough curves of NaCl tracer from columns packed with sand and zerovalentiron. (Right) Diagram of glass columns used for all small-scale experiments. For non-continuous tests, the column was inverted after each daily charge and the influent exposed to air to allow biofilm growth.. The sketch for the column is not made to scale.

the limit of quantification (20 PFU/plate of undiluted sample) until 6 pore volumes continuous flow had passed, with the breakthrough concentration around 5-$\log_{10}$ removal (99.999%). In addition, overnight (i.e. 18- hour) batch experiments using 15 mL tubes containing 10 mL of solution and 5 mL of iron particles or rusted iron nails revealed complete removal of 4×10^6 PFU/mL of MS2. Thus, MS2 removal was due to adsorption on iron oxides.

Sorption of MS2 in the iron column most likely occurred due to electrostatic interactions between positively charged iron oxides formed during iron corrosion (e.g. hematite and magnetite) and negatively charged virion particles. A number of studies have also reported the adsorption and inactivation of viruses by iron oxides [29, 43-45].

You et al. found complete break-through of MS2 by sand columns in a similar study [26]. Although 49.9% removal is higher than observed in previous studies, overall removal by the quartz sand was limited. Differences in sand composition may have led to the increased reduction. The quartz sand in this study was cleaned and dried, but it was not treated with a citrate solution to remove existing metal ions and oxides. Trace levels of metal oxides may have been present, leading to heightened MS2 removal. You et al. used a solution containing sodium citrate and citric acid to decrease iron levels in the sand columns from 32.5 mg iron/kg sand to below the detection limit (0.02 mg iron/kg sand) before testing [26].

The higher reduction may also be due to the pH of the Newmark groundwater (pH 6.2), which is lower than that of artificial groundwater (AGW; pH 7.5) used in other experiments. The isoelectric point (IEP), the pH at which the surface charge of the virus is neutral or zero, is 3.5-3.9 for MS2 [29, 46-48]. As the pH of the aqueous solution

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decreases and approaches the IEP, the net negative charge of the MS2 decreases, resulting in the reduction of electrostatic repulsive interactions between the negatively charged virion particles and the negatively charged sand particles [4]. A lower pH in the source water would result in less repulsion and, therefore, greater virus removal by filtration.

3.2.2. Long Term Removal of MS2 in Columns with Biofilm Growth

The long term (72 day) $\log_{10}$ removal for four glass columns simulating a daily, 1 pore volume charged BSF is shown in Figure 3. While the sand column averaged only 1-$\log_{10}$ (90%) removal, all three iron columns had greater than 5-$\log_{10}$ removal for the duration of the experiment. Reduction of MS2 by the iron columns ranged from 5-$\log_{10}$ to complete removal (>8-$\log_{10}$). In comparison, the virus reduction by the sand column ranged from no removal to 3-$\log_{10}$ (99.9%). After about 1 week, biofilm growth could be seen in all 4 columns, but removal rates did not increase as the biofilm developed. Biofilm formation occurred only at the influent entrance, which was tapered down significantly from the maximum diameter of the column. Since biofilm formed only on a relatively small area, it made minimal contribution to virus removal. In contrast, Elliot et al. found that the reduction of bacteriophages MS2 and PRD1 in BSFs increased from 0 to 1.3-$\log_{10}$ as the biofilm developed [13].

Previous studies have also shown that bacteriophage adsorption (MS2) is reduced in the presence of natural organic matter (NOM) and phosphates that compete for and fill iron oxide adsorption sites [49]. Influent and effluent total organic carbon levels for all four columns were measured as an indicator of NOM. The influent concentration of TOC for each column was approximately 3.1 mg/L. Although each iron column retained
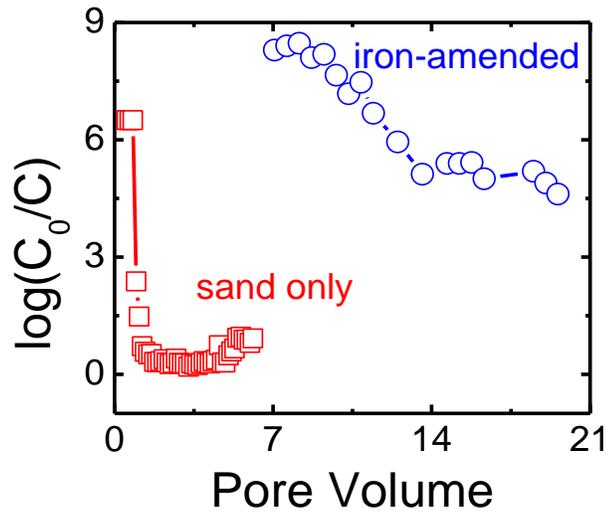
Figure 2. Log₁₀reduction of bacteriophage MS2 by column packed with sand only and sand mixed with zerovalent iron particles (10% by volume).
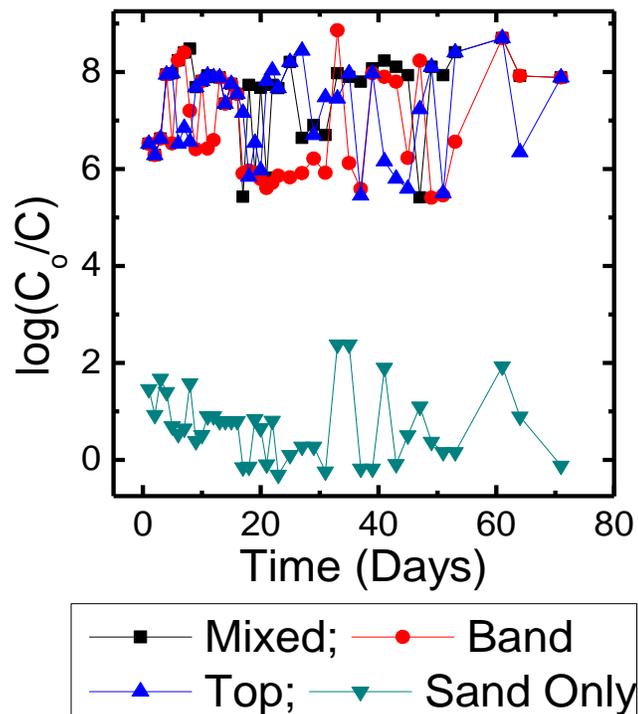

Figure 3. Log₁₀ reductions of MS2 by three columns packed with sand only and with sand mixed with zerovalent iron. Three columns were packed with different orientations of iron particles (10% iron by total volume): an even mixture throughout the sand, a middle layer, and a layer near the influent.

more TOC (0.5-0.3 mg/L) than the sand column (0.1 mg/L), virus removal was not adversely affected. The extended contact time (24 hours) most likely contributed to the increased removal, combating any negative effects caused by the introduction of NOM. It was expected that the iron column with a layer of iron particles at the influent entrance (the "top" column) would experience reduced adsorption because the absence of sand media and established biofilm above it would lead to increased exposure to NOM. However, as discussed previously, relatively small biofilm development occurred. Therefore, no significant differences in removal rates between columns of different iron particle orientations were observed.

3.2.3. Removal of Rotavirus in Columns with Biofilm Growth

For the first set of rotavirus experiments (described in Section 2.3.3), the iron column was charged on two separate days with $\sim10^4$ FFU/mL of rotavirus and obtained removal below the detection limit on each daily charge. The second set of experiments run on the same iron and sand columns used treated wastewater effluent with 10 times higher TOC to evaluate the effect of NOM on virus removal. With the treated wastewater effluent, we obtained 5.2-$\log_{10}$ removal of rotavirus by the iron column and 1.1-$\log_{10}$ removal by the sand only column. Note that even with higher TOC, the iron column still allowed substantial rotavirus removal.

The results are consistent with the MS2 experiments performed in these studies and rotavirus experiments performed by Gutierrez et al. [29]. In a study using flow-through columns packed with hematite coated glass fibers, it was found that 3×10^4 FFU of rotavirus was removed per gram of hematite nanoparticles. Furthermore, Gutierrez et al. found that only 2% of adsorbed rotavirus remained infectious after attachment [29].

Previous research suggests that electrostatic interactions between viruses and iron oxides are so strong that they can cause viruses to disintegrate [29, 50]. With an IEP of 4.5 [29], the highly negative potential of rotavirus in source water leads to favorable conditions for viruses to adsorb and be rendered non-infectious.

3.3 Biosand Filter Experiments

3.3.1. Head-Loss Development Over Time

As the BSF accumulates bacteria and organic particulate matter to develop a biofilm, the head-loss of the system increases, and users will notice the effluent flow rate decreasing over time as the BSF is used repeatedly. The buildup of organic matter and development of the biological layers (known as "ripening") results in a more effective filter [13, 20]. The flow rates for the two plastic filters are plotted in Figure 4. To promote biofilm growth, 2.5% primary effluent (PE) was added to both filters until day 20.

The BSF without iron nails behaved as expected: as time progressed and the filter ripened, the flow rate decreased and removal efficacy increased. The iron nail filter, however, behaved differently. The flow rate did not decrease as expected, indicating a problem in establishing uniform flow across the entire cross-sectional area of the filter. Although the filter with iron nails distributed evenly throughout the sand established uniform flow early in the experiment, independent flow paths eventually developed. Water bypassed the iron nails at an elevated flow rate, despite the continued growth of biofilm.

Figure 4 also shows the flow rate of the concrete filter packed with iron particles compared to the flow rate of the plastic BSF packed with iron nails. Flow rate problems

were effectively eliminated by using an iron material closer in size to the filtration sand media grain size, and the flow rate for the iron particle BSF behaved as desired. As organic matter accumulated, the filter ripened and the flow rate decreased. The steel wool filter displayed similar results.

3.3.2. Water Characteristics

Analytical parameters including pH, DO, alkalinity, and NO_3^- remained consistent throughout each BSF experiment. Influent and effluent pH was between 7.1 and 8.2 with an average alkalinity of 22.5 mg/L as $CaCO_3$. DO was reduced in the filters but always remained above 1.01 mg/L. Iron oxides formed during iron corrosion were filtered out by the sand media, and iron was not present in the effluent (ICP-MS detection limit of 0.0059 mg/L). Leaching of other trace metals from the iron sources was not seen, and heavy metals such as chromium, lead, cadmium, and arsenic were not present in the effluent.

3.3.3. MS2 Removal

Results for the removal of MS2 through the sand/iron nail BSF is shown in Figure 5. The iron nail filter started with a 6.5-$\log_{10}$ (99.99997%) removal, but adsorption of virus particles quickly declined as flow paths short-circuited around the iron nails. Subsequent filter charges did not achieve plug flow, causing reduced contact time with iron particles. After 10 days, removal for the iron nail BSF was less than 4-$\log_{10}$ (99.99%), and successive samples were between 3 and 4-$\log_{10}$ (99.9-99.99%) These results indicate that the iron nails used are not appropriate for use in the BSF; smaller iron material must be used to obtain a more evenly graded media that will promote uniform flow and not detract from the original (without iron) efficacy of the BSF. The flow paths
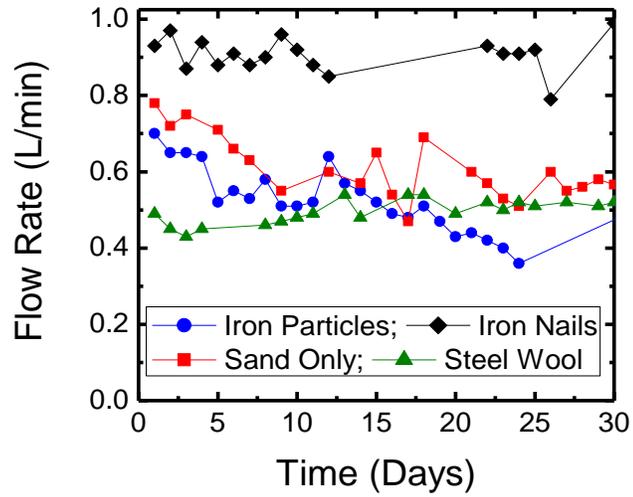
Figure 4. Flow rates for sand BSF (plastic), iron nail BSF (plastic), steel wool BSF (concrete), and iron particle BSF (concrete).
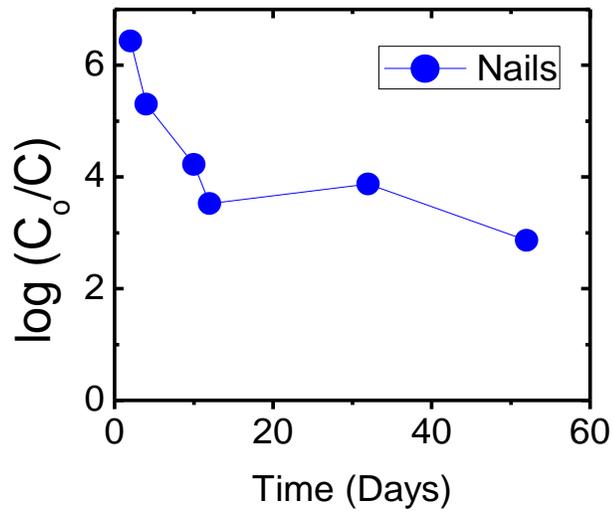

Figure 5. MS2 reduction in the BSF containing iron nails.

created also highlight the importance of contact time, a point further illustrated in Figure 6. Water that has been allowed to sit overnight in the filter (24 hours) achieves additional removal of ~1-$\log_{10}$ (90%).

The concrete sand filter achieved between 4-$\log_{10}$ removal and no removal, with an average of about 2-$\log_{10}$ (99%) removal (Figure 7) for the first 150 days. In comparison, the two filters packed with iron (particles and steel wool) achieved between 7-$\log_{10}$ (99.99999%) and >5-$\log_{10}$ removal, with an average of 6-$\log_{10}$ (99.9999%) reduction of virion particles for the first 170 days of filter use. The iron particles and steel wool resulted in filters with a more even media grade than in the filters containing nails. This promoted uniform, steady flow throughout the entire cross-sectional area of the filter, allowing sufficient contact time between each filter charge and the iron media. The corresponding increase in MS2 removal shows that iron-amended BSFs can effectively remove select viruses, with removal rates that exceed U.S. Environmental Protection Agency (USEPA) drinking standards (4-$\log_{10}$ removal) [51].

However, removal in the steel wool filter decreased significantly after day 170, dropping to an average of 2-$\log_{10}$ removal. Sorption of viruses to the steel wool stopped once its adsorption sites were exhausted, and remaining removal of viruses was due to the sand media. The reduction in removal capacity to levels below those observed in the sand-only filter may indicate that the addition of steel wool to the sand media adversely affected the traditional filter's removal efficacy.

The sand only BSF saw a steady increase in removal from 2-$\log_{10}$ to >4-$\log_{10}$. Previous research has shown that biofilm ripening and media aging contribute significantly to the MS2 removal capacity of the traditional sand BSF [13], but long term

studies (>2 months) have not been previously conducted. However, it is well-known that filter ripening plays a crucial role in particle removal during granular filtration [52-54]. The results from this study suggest that traditional BSFs may experience significant virus removal ($>2 \log_{10}$) once filters have been in use for several months. If this is the case, filters would only need to be amended with an appropriate iron source for the initial ripening period.

3.3.4 Iron Oxide Formation

Iron samples from the iron particle BSF were taken after the study ended. Iron particle composition after corrosion was 48% iron, 40% magnetite, 9% hematite, and 3% other. With 48% of the particles still present in the form of iron, further iron oxide generation was possible. In contrast, the steel wool present in the other iron-amended BSF was completely oxidized. Enhanced virus removal by iron oxide adsorption stopped after day 170 when adsorption sites became filled and no further sites could be generated.
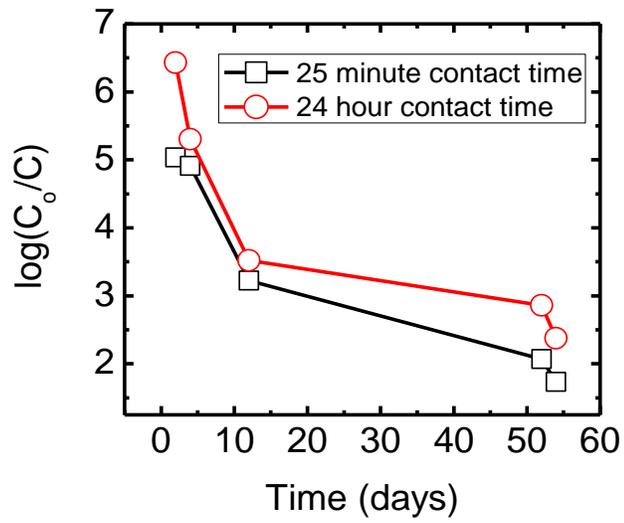
Figure 6. Comparison of MS2 reduction at two different pause periods for the BSF containing iron nails.
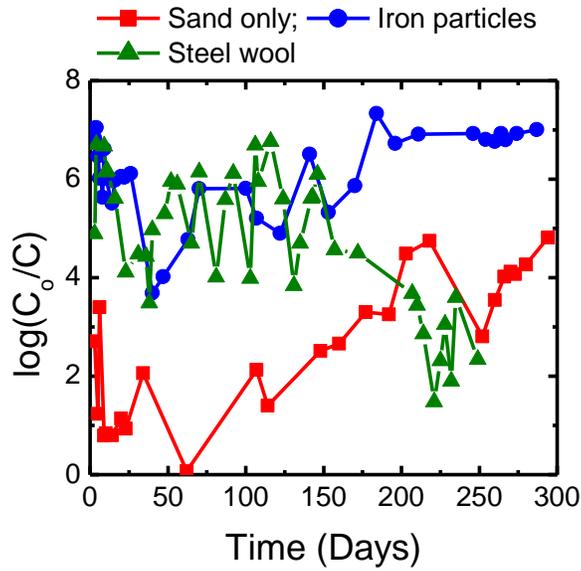

Figure 7. Long termMS2 reduction by three different concrete filters: sand, steel wool, and iron particles.

CHAPTER 4
DISCUSSION[4]

Previous testing in small scale columns has shown that MS2 has minimal sorption to sand media. In particular, studies have demonstrated lower adsorption of MS2 when compared to bacteriophages (*phi*X-174) and human pathogenic viruses (rotavirus, echovirus-12, and poliovirus). Data from small scale saturated flow studies showed that MS2 had no sorption compared to *phi*X-174 with about 80% removal [55]. Higher removal of *phi*X-174 was attributed to a higher isoelectric point (IEP = 6.6) than that of MS2 (IEP = 3.5-3.9). Virus sorption is largely governed by electrostatic interactions and van der Waal's forces. Because quartz sand is negatively charged in source water (IEP = ~2.2), and MS2 is more negatively charged than *phi*X-174, MS2 experiences higher repulsion at pH = 7. Bales et al. [56, 57] also demonstrated that *phi*X-174 and poliovirus exhibit similar removal through sand columns due to their shared IEP of 6.6. Additionally, Bales et al. [57] showed that poliovirus sorbed to silica beads much more readily than MS2. Small-scale studies with sand columns have also shown higher adsorption of rotavirus (IEP = 4.5) than MS2 [29]. It is also important to note that hydrophobic interactions may be important for bacteriophage adsorption to hydrophobic surface due to the presence of hydrophobic areas on the surface of bacteriophage such as MS2 and PRD-1 [56].

In large-scale studies using BSFs similar to those used in this study, Elliot el al. [13] saw greater removal of echovirus-12 (>2-log) than bacteriophages MS2 and PRD-1 (~1-log). These differences were also attributed to echovirus-12 having a higher IEP

(echoviruses have IEP of 5-6.4) than MS2 and PRD-1, which have similar IEPs of 3.5-3.9 and 4.2, respectively [58].

When positively charged iron oxides are added throughout the sand media, it is expected that all viruses that are negatively charged at pH = 7 will be able to readily adsorb to iron oxide surfaces. At the same time, MS2 experiences high repulsion from the sand particles due to its low isoelectric point, and based on past studies, is a more difficult virus, compared to echovirus type 12, to remove through conventional sand filtration [13]. Having a low IEP would make a virus relatively negative at pHs near neutral. This charge leads to less removal in a sand-only BSF, due to the repulsion of negatively charged sand particles, and greater removal in an iron-amended BSF, due to stronger electrostatic attraction to positively charged iron oxides. While MS2 bacteriophage can be used as a surrogate for rotavirus because these two virus have similar interfacial properties [29, 30, 59, 60], other enteric viruses may require different surrogates.

When a virus attaches to particles in water, the overall size of the aggregate is greater than that of a monodispersed virus. As a result, virus-particle aggregates would be easier to remove by filtration [61, 62]. Experiments presented here were conducted as a worst case scenario for monodispersed viruses. In addition, a recent publication showed that norovirus, which has a similar size as the studied virus MS2, associated with 0.45-180 μm particles, and attachment and settling was not an effective removal mechanism for viruses in waste stabilization ponds [63]. Similarly, recent study with bacteriophage MS2 and rotavirus has found that steric repulsion prevents viruses from aggregating and adsorbing to silica and organic matter surfaces [30, 60].

It is expected that biofilm development is highly dependent on source water characteristics, including concentration and identity of native microorganisms that attach to the sand, nutrients from which the microbes derive an energy source, NOM that could shelter or cover the microbes, DO levels, and even water temperature. In our household-scale studies, MS2 removal in the sand-only BSF increased with biofilm development in a trend that matches previous studies [13], despite the utilization of different water sources. While investigating biofilms developed from different water sources was not a focus during this phase of the research, the currently on-going research involves collecting data from BSFs installed and operating at different locations in Guatemala.

CHAPTER 5
CONCLUSIONS[5]

The following conclusions resulting from this study indicate a potential advancement in household water treatment technologies by amending the BSF with iron materials:

- Both MS2 and rotavirus were treated to USEPA standards for virus removal, greater than 99.99% removal, through the adsorption to positively charged iron oxides in small-scale studies.

- Untreated iron material distributed uniformly in sand media of a BSF will oxidize and effectively remove >4 $\log_{10}$ MS2 and rotavirus from a natural source water.

- The duration of effective virus removal by iron-amended biosand filtration depends on both source water conditions and quantity and composition of iron material added, all of which need to be researched further.

- After 200 days in operation, the unmodified BSF underwent significant ripening and was able to provide more than 4 $\log_{10}$ of MS2 virus removal.

- Further research is needed to determine the effects of competitive adsorption with other water constituents on the efficacy of virus removal by iron-amended biosand filtration.

- For relatively little cost (approximately 4 USD per filter based on initial cost estimates), BSFs can be amended with local iron materials, thereby providing a substantially improved barrier against waterborne viruses and, hopefully, bringing relief to millions of lives in the process.

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[5] Ian Bradley, Anthony Straub, Peter Maraccini, Sheila Markazi, Thanh H. Nguyen. Iron Oxide Amended Biosand Filters for Virus Removal. *Water Research* (2011).

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