Biological Testing of an Experimental Slow Sand Filter

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INTRODUCTION

Water is an essential part of life and the importance of clean water cannot be overstated. Since clean water is not available to large numbers of people around the world, water purification is crucial for improving global health. Unfortunately effective water purification systems can be expensive and hard to assemble. Slow sand filters provide an cheap and easy solution to this problem. Normal slow sand filters are helpful at reducing water-borne diseases but they can still be improved. This is where our project began. An experimental slow sand filter from Ethiopia was tested to see if it had an improved clearance of microorganisms that can cause disease. This filter had the same design as a normal slow sand filters but it had copper infused into the filtering media because copper has antimicrobial properties.

TOTAL COLIFORMS AND *E.COLI*

Methods:

- To measure the amounts of bacterial clearance, HB101 *E. coli* was grown and colony forming units (CFUs)/OD600 was quantified. Test water was spiked with 10^7 cells/L and run through the filters. 100mL of test or control water was filtered through sterile 0.45um membrane filters and placed on tryptic soy agar for growth. CFUs were counted and compared to controls. Due to excess bacterial growth during column filtration in initial tests, the columns were subsequently moved to 4 degrees C to slow bacterial growth.

RESULTS:

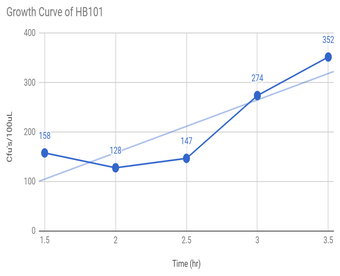
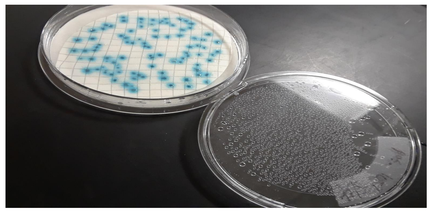


Figure 1. The graph above shows the growth curve of the bacteria in Cfu's/100uL over time. It is from this data that the growth constant for calculating the cells/mL was established.

Bacteria Results 8/4		
Negative control: 0 cfu's		
Dilution of 10^-6	Dilution of 10^-5	Dilution of 10^-4
C1: >1000 cfu's	C1: >1000 cfu's	C1: >1000 cfu's
C2: >1000 cfu's	C2: >1000 cfu's	C2: >1000 cfu's
C3: >1000 cfu's	C3: >1000 cfu's	C3: >1000 cfu's
C4: >1000 cfu's	C4: >1000 cfu's	C4: >1000 cfu's
C5: >1000 cfu's	C5: >1000 cfu's	C5: >1000 cfu's
C6: >1000 cfu's	C6: >1000 cfu's	C6: >1000 cfu's
Positive control (dilution of 10^-8):		
Positive control (dilution of 10^-7): >1000 other cfu's		
Positive control (dilution of 10^-7): 98 E. coli cfu's and >1000 other cfu's ^MI Agar Plate (shows if the bacteria are E. coli or something else)		

Table 2. The results of the first run of experimental test for bacteria were much higher than what was expected and fell into a range that was uncountable. This experiment was a repeat of first experiment. In order to get a countable amount, the column samples needed to be diluted. However, the positive control plated on a MI agar plate showed that most of the cfu's that formed on the regular TSA plates were not E. coli. This led to the realization that the columns were picking up a lot of contamination while the water was slowly being filtered.



EPA Positive Control vs. Field Kit Test for *E. coli*					
EPA (6/15)	Field (6/15)	EPA (6/23)	Field (6/23)	EPA (7/6)	Field (7/6)
Cfu's Added	Cfu's Added	Cfu's Added	Cfu's Added	Cfu's Added	Cfu's Added
1x10^7	1x10^7	1x10^7	1x10^7	1x10^7	1x10^7
% Recovered	% Recovered	% Recovered	% Recovered	% Recovered	% Recovered
95%	3%	0.40%	0%	82%	1.33%

Table 1. Shown here is the amount percentage of HB101 E. coli that was recovered by the EPA method compared to the field kits. These methods were used as the projects controls before testing on the filters began. The field kit had low results constantly because the plate required 24 hours or more of incubation which they did not get.

Bacteria Results(Stored in 4 degree C storage room) 9/28							
Dilution	10^-6	10^-5	10^-4	10^-3	10^-2	10^-1	10^0
C1	7	50	322	>1000	>1000	>1000	>1000
C2	184	150	768	>1000	>1000	>1000	>1000
C3	63	337	>1000	>1000	>1000	>1000	>1000
C4	0	7	63	>300	>1000	>1000	>1000
C5	10	5	48	>300	>1000	>1000	>1000
C6	1	10	70	>1000	>1000	>1000	>1000
Pos. control	2	7	36	>300	>1000	>1000	>1000

Table 3. After the columns andOne last repeat of the bacteria experiment was run with a large dilution serious for each column and the positive control to get a countable amount of cfu's. This way, the data will show if the columns are clearing any of the bacteria. In this case the column filters were not clearing any of the bacteria since the column samples had more cfu's on average than the positive control.

METHODS

- Columns were set up in two different locations. Initial tests were performed at room temperature. Subsequent tests were performed at 4 degrees Celsius.
- For each experiment, the columns were assembled and suspended above sterile collection bottles.
- Once the columns were set up, water was poured into the top, with fresh test water added daily to maintain flow rate.

MALE SPECIFIC COLIPHAGE

Methods:

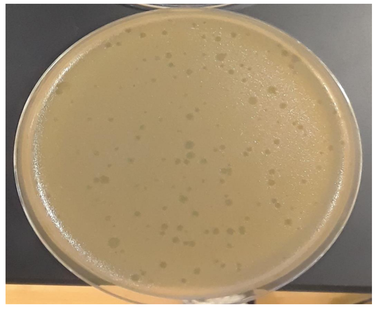
- MS2 bacteriophage is often used as a surrogate for enteroviruses in water filter testing. Famp *E. coli (*an F^+ strain) was used as a host for the MS2 phage. Infected *E. coli* are killed and present as "plaques" in a lawn of bacterial cells. The number of plaques is directly related to the number of infective phage units, called plaque forming units (pfus). Phage quantification was based on the number of pfus present in test water compared to controls.
- **RESULTS:**

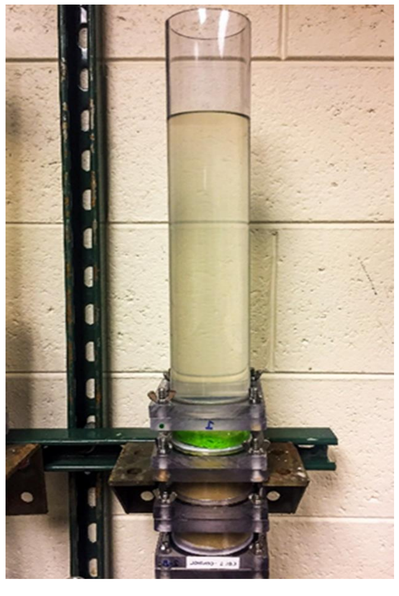
Phage Results (7/31)	
Test Sample	Log. Reduction of Phage
Positive Control	X
Negative Control	7.24
Column 1	6.39
Column 2	5.96
Column 3 (#1)	7.24
Column 3 (#2)	5.94
Column 4 (#1)	6.76
Column4 (#2)	7.24
Column 5	7.24
Column 6	7.24

Table 4. The table above shows the results of the first run of the experiment. The positive control gave the starting number of phage inside of the test water so that the log reduction of each column could be calculated. Also the experimental design originally had each column being tested in duplicates but due to the slow flow rate only columns 3 and 4 had enough water to be tested twice.

Phage Results (8/2)	
Test Sample	Log. Reduction of Phage
Positive Control	X
Negative Control	6.79
Column 1	4.37
Column 2	6.79
Column 3	6.79
Column 4	6.79
Column 5	3.85
Column 6	X

Table 5. The table above shows the results of the second run of the experiment. This time there was only enough water for single tests to be run on each column and column 6 was slipped before it was tested.





CONCLUSIONS

- **Coliform Results:** In initial tests, bacterial contamination precluded quantifying bacterial log reduction. In subsequent test, no statistically significant reduction of *E. coli* was observed for any of the columns. Further testing may help determine the reasons for this lack of clearance and if any sources of contamination affected results.
- **Coliphage Results:** For each type of media, a log. reduction of 3.85 or greater was shown, which is above the EPA 99.9% purification standard for viral clearance. This data leads to the conclusion that the biosand filters with or without copper added can clear bacteriophage, and therefore enteroviruses, from water.
- **Cysts Result:** For cysts, the EPA standard of purification is a 3 log. reduction. This is equal to a 99.9% reduction of cysts. The beads assay showed a high log. reduction for each of the column filters expect column 3. This reduction was higher than expected but is still shows strong evidence that each type of column filter is very effective at clearing out cysts based on the very successful data from the beads assay. To further improve this study, live cysts could be cultured and used for future experiments.

CYSTS

Methods:

- Fluorescent beads (4 micron diameter) are an acceptable surrogate for parasitic cysts in water filter testing. 10^6 beads per liter (20uL) were added to test water. 100 mL samples were collected and filtered through black 0.2 micron filters. Fluorescent beads were counted in three fields of view using a Lionheart FX automated microscope and the average was used to count the beads present in a sample. Test water was compared to controls to calculate the logarithmic reduction.

RESULTS:

Logarithmic Reduction of Cysts	
Test Samples	Log Reduction
Negative Control	8.61
Column 1	6.88
Column 2	6.88
Column 3	8.61
Column 4	7.79
Column 5	5.91
Column 6	7.79

Table 6. Fluorescent beads in three random fields of view were counted and the average was used to calculate the number of beads/sample. Column 5 and the positive control were the only samples for which an autocounting program on the LionHeart FX microscope was used. The remaining samples were counted manually due to the low number of beads present.