Microbial reduction evaluation of Grifaid water filters (model GFF5)

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SUMMARY

Two Grifaid water filtration system units (model GFF5) were evaluated for their ability to remove *Escherichia coli* from water. The units were operated according to the manufacturer's instructions, and challenged with the test microorganisms using general case water and challenge (high organic matter and turbidity) case water. Duplicate samples were collected after passage of 3, 6 and 10 liters of water through the units by hand pumping. Both filter units were highly effective at removing *E. coli* from both the general and challenge case waters. The removal of *E. coli* ranged from 6.17 to >7.89 logs.

Unit 2 performed better than Unit 1 for the removal of *E. coli* from general case waters; the two units performed equally well when tested with challenge case water. Unit 1 performed better during the experiment performed with the challenge case water than it did with the general case water. Comparable reductions in *E. coli* were observed for both the general and challenge case waters for Unit 2.

MATERIALS AND METHODS

Experimental Design

The basic experimental design for evaluating the water purification units was based on the recommendations of the U.S. Environmental Protection Agency's (USEPA) Task Force Report on the *Guide Standard and Protocol for Testing Microbiological Water*

Purifiers (Federal Register, May 26, 1986). This requires that microbiological purifiers be tested with waters of both high and low organic matter, turbidly and dissolved solids. These test waters are referred too as “general case” and “challenge case” waters. These tests require that enteric bacteria are reduced by 6 logs (99.9999%).

Two Grifaid GFF5 filters were provided by Safe Water Trust (Cleadon, Suderland, United Kingdom) and operated according to the manufacturer's instructions. Twenty liters of both general case and challenge case test water was passed through each unit by hand pumping in separate tests. The chemical/physical parameters of the general case and challenge (high organic matter and turbidity) case waters used in the study are shown in Table 1. Tucson tap water was used for the general case test water after dechlorination by passage through activated carbon to remove any chlorine present in the water. Water quality analysis was performed according to Standard Methods for the Examination of Water and Wastewater (APHA, 2005). The challenge water was prepared according to the USEPA protocol (USEPA, 1987) by adding approximately 100 mg/L of AC fine dust (GM, Flint, MI) to obtain a turbidity of ≥ 30 NTU. Total organic carbon (TOC) (≥ 10 mg/L) was obtained by addition of ~23 mg/L of humic acid (Aldrich Chemical Company, WI), and total dissolved solids (TDS) (1,500 mg/L ± 150 mg/L) by addition of sea salts (Sigma Chemical, St. Louis, MO).

Bacterial Analysis

A culture of *Escherichia coli* (ATCC #25922) was prepared on the day before testing by inoculating one colony of the test organism into 100 ml of tryptic soy broth (TSB, Difco,

Sparks, MD) and incubation overnight at 37°C with agitation to obtain the organisms in the stationary growth phase. On the test date, the bacterial cells were washed by pelleting the cells via centrifugation. The supernatant was discarded and the pellet was re-suspended in 0.01 M phosphate-buffered saline (PBS; pH 7.0; Sigma-Alldrich, St. Louis, MO). Three washing steps were performed in total to remove the organic matter present in the broth.

Bacterial assays were conducted by the membrane filtration method on m-Endo Agar LES (Becton Dickinson, Cockeysville, MD). Appropriate dilutions of influent samples were made in sterile PBS. After incubation for 24 hours at 44.5°C, colonies were counted, and the levels of CFU per sample determined. The data were reported as the logarithmic reduction using the formula -log10 (N_{eff} /N_{inf}), where N_{inf} was the concentration of *E. coli* in the influent and N_{eff} was the concentration of *E. coli* in the sample collected after various volumes had been passed through the filter (i.e., after 3, 6, or 10 liters).

RESULTS

Both filters exhibited a reduction in the amount of water flow through the units by the end of the tests conducted with the general case water. The flow rate continued to steadily decline as challenge case water was filtered through the units. This could eventually lead to clogging of the units over time under normal operating conditions. The incubation of the cultured effluent samples at 44.5°C was successful in removing all other bacterial species (originating from either the water or the filter units), allowing for

the accurate quantification of *E. coli* in the effluent samples.

The results for the removal of *E. coli* by the Grifaid GFF5 filter units are shown in Tables 2 and 3. The removal of *E. coli* ranged from 6.17 to >7.89 logs. Unit 2 performed significantly better than Unit 1 for the removal of *E. coli* in the tests using general case water (average reductions of 7.39 logs vs. 6.38 logs, respectively); nevertheless, no difference was observed between the two filter units during the experiments using challenge case water containing high levels of organics and turbidity (average reduction of >7.85 for both). The removal of *E. coli* was comparable for the general vs. challenge case waters for Unit 2 (average of 7.39 logs vs. >7.85 logs, respectively), but was different for the two water types for Unit 1 (average of 6.38 logs vs. >7.85 logs, respectively).

REFERENCES

1. APHA. 2005. American Public Health Association. Standard Methods for the Examination of Water and Wastewater. Washington, DC.
2. USEPA. 1987. United States Environmental Protection Agency. Guide Standard and Protocol for Testing Microbiological Water Purifiers. Federal Register. 54:34067.

Table 1. Characteristics of non-microbiological parameters of test waters

Parameter	General Case Water	Challenge Case Water
Chlorine (mg/L)	None	None
pH	7.94	8.09
Temperature (°C)	24.0	22.5
Turbidity (NTU)	0.64	40.5
Total organic carbon (TOC) (mg/L)	<1	>10
Total dissolved solids (TDS) (mg/L)	341	1460

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Table 2. Reduction of *E. coli* from general case test water by Grifaid GFF5 water filters

FILTER UNIT	INFLUENT	EFFLUENT COLLECTED AFTER 3 LITERS	EFFLUENT COLLECTED AFTER 6 LITERS	EFFLUENT COLLECTED AFTER 10 LITERS
Unit 1	$6.95x10^{8}$*	$4.70x10^{2}$	$3.39x10^{2}$	$1.50x10^{2}$
	LOG REDUCTION	6.17	6.31	6.67
Unit 2	$7.93x10^{8}$	81	< 24.5	< 17.3
	LOG REDUCTION	6.99	> 7.51	> 7.66

*colony forming units

Table 3. Removal of *E. coli* from challenge case test water by Grifaid GFF5 water filters

FILTER UNIT	INFLUENT	EFFLUENT COLLECTED AFTER 3 LITERS	EFFLUENT COLLECTED AFTER 6 LITERS	EFFLUENT COLLECTED AFTER 10 LITERS
Unit 1	$7.13x10^{8}$*	< 10	< 10	< 10
	LOG REDUCTION	> 7.85	> 7.85	> 7.85
Unit 2	$7.83x10^{8}$	10	< 10	14.1
	LOG REDUCTION	7.89	> 7.89	7.75

*colony forming units

Microbial reduction evaluation of Grifaid water filters (model GFF5)

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SUMMARY

Two Grifaid water filtration system units (model GFF5) were evaluated for their ability to remove *Escherichia coli* and MS-2 bacteriophage from water. The units were operated according to the manufacturer's instructions, and challenged with the test microorganisms using general case water and challenge (high organic matter and turbidity) case water. Duplicate samples were collected after passage of 3, 6 and 10 liters of water through the units by hand pumping. The removal of MS-2 bacteriophage ranged from 5.57 to 6.73 logs and *E. coli* from 3.25 to >7.23 logs. Both units yielded comparable reductions in MS-2 for both the general and challenge waters. In contrast, for the removal of E. coli, one filter unit performed far better than the other for both water types.

MATERIALS AND METHODS

Experimental Design

The basic experimental design for evaluating the water purification units was based on the recommendations of the U.S. Environmental Protection Agency's (USEPA) Task Force Report on the *Guide Standard and Protocol for Testing Microbiological Water Purifiers* (Federal Register, May 26, 1986). This requires that microbiological purifiers be tested with waters of both high and low organic matter, turbidly and dissolved solids.

These test waters are referred too as “general case” and “challenge case” waters. These tests require that enteric bacteria are reduced by 6 logs (99.9999%) and viruses by 4 logs (99.99%).

Two Grifaid GFF5 filters were provided by Safe Water Trust (Cleadon, Suderland, United Kingdom) and operated according to the manufacturer's instructions. Twenty liters of both general case and challenge case test water was passed through each unit by hand pumping. The chemical/physical parameters of the general case and challenge (high organic matter and turbidity) case waters used in the study are shown in Table 1. Tucson tap water was used for the general case test water after dechlorination by passage through activated carbon to remove any chlorine present in the water. Water quality analysis was performed according to Standard Methods for the Examination of Water and Wastewater (APHA, 2005). The challenge water was prepared according to the USEPA protocol (USEPA, 1987) by adding approximately 100 mg/L of AC fine dust (GM, Flint, MI) to obtain a turbidity of ≥ 30 NTU. Total organic carbon (TOC) (≥ 10 mg/L) was obtained by addition of ~23 mg/L of humic acid (Aldrich Chemical Company, WI), and total dissolved solids (TDS) (1,500 mg/L ± 150 mg/L) by addition of sea salts (Sigma Chemical, St. Louis, MO).

Bacterial Analysis

A culture of *Escherichia coli* (ATCC #25922) was prepared on the day before testing by inoculating one colony of the test organism into 100 ml of tryptic soy broth (TSB, Difco, Sparks, MD) and incubation overnight at 37°C to obtain the organisms in the stationary

growth phase. On the test date, the bacterial cells were washed by pelleting the cells via centrifugation. The supernatant was discarded and the pellet was re-suspended in 0.01 M phosphate-buffered saline (PBS; pH 7.0; Sigma-Alldrich, St. Louis, MO). Three washing steps were performed in total to remove the organic matter present in the broth.

Bacterial assays were conducted by the membrane filtration method on m-Endo Agar LES (Becton Dickinson, Cockeysville, MD). Appropriate dilutions of influent samples were made in sterile PBS. A 100-ml sample of undiluted unit effluent was also assayed. After incubation for 24 hours at 37°C, colonies were counted, and the levels of CFU per sample determined. The data were reported as the logarithmic reduction using the formula $-\log10 (N_{eff} / N_{inf})$, where N_{inf} was the concentration of *E. coli* in the influent and N_{eff} was the concentration of *E. coli* in the sample collected after various volumes had been passed through the filter (i.e., after 3, 6, or 10 liters).

MS-2 Analysis

Influent and effluent samples from the filters were kept at 4°C until assayed for MS-2 bacteriophage. Approximately 0.5 ml of a log-phase culture [3-4 hours growth in liquid tryptic soy broth (TSB; Difco, Sparks, MD, USA) medium with agitation at 37°C] of host *E. coli* bacterium (ATCC# 15597) was added to 5 ml of molten tryptic soy agar (TSA; containing 1% agar; Difco, Sparks, MD) in a test tube. Next, 0.1 ml of each dilution (10-fold serial dilutions in PBS were tested) of the virus samples were added to separate tubes. The tubes were then vortexed gently to mix the cultures and poured onto the surfaces of separate TSA plates. The plates were then swirled gently to cover the entire

surface of the plates with the agar overlays. The overlays were then allowed to solidify at room temperature and then the plates were incubated (inverted) in an incubator for 24 hours at 37°C.

The MS-2 coliphages were enumerated by counting plaques (circular clearing of the bacterial growth in the agar overlays) to determine the number of plaque-forming units (PFU) of virus per milliliter of sample. The data were reported as the logarithmic reduction using the formula -log10 (N_{eff} /N_{inf}), where N_{inf} was the concentration of MS-2 in the influent and N_{eff} was the concentration of MS-2 in the sample collected after various volumes had been passed through the filter (i.e., after 3, 6, or 10 liters).

RESULTS

The results for the microbial removal are shown in Tables 2 through 5. The removal of MS-2 bacteriophage ranged from 5.57 to 6.73 logs and *E. coli* from 3.25 to >7.23 logs. Both units yielded comparable reductions in MS-2 for both the general and challenge waters. For instance, the average removal of MS-2 for Unit 1 was 6.32 logs for the general case water and 6.66 logs for the challenge case water. The average removal of MS-2 for Unit 2 was 5.81 logs for the general case water and 5.80 logs for the challenge case water. In addition, the average log reduction observed between the two units were also similar (6.59 logs vs. 5.81 logs).

In contrast, Unit 1 performed significantly better than Unit 2 for the removal of *E.*

coli (average reductions of 6.47 logs vs. 3.85 logs, respectively); nevertheless, the removal of *E. coli* was comparable for the general vs. challenge case water for each individual unit (> 6.15 logs vs. 6.79 logs for Unit 1; 4.25 logs vs. 3.45 logs for Unit 2).

A complication arose during the assay of the *E. coli* from the filter effluents. Contaminating bacteria coming off of the filter in high numbers made it difficult to count the recovered *E. coli*, particularly in the undiluted samples. For instance for Unit 1 for the general case water, the membrane filters for the 100-ml, 10-ml, and 1-ml effluent volumes were overgrown by the contaminating bacteria and therefore could not be counted. Thus, the reductions reported in Table 3 could be underestimated.

REFERENCES

APHA. 2005. American Public Health Association. Standard Methods for the Examination of Water and Wastewater. Washington, DC.

USEPA. 1987. United States Environmental Protection Agency. Guide Standard and Protocol for Testing Microbiological Water Purifiers. Federal Register. 54:34067.

Table 1. Characteristics of non-microbiological parameters of test waters

Parameter	General Case Water	Challenge Case Water
Chlorine (mg/L)	None	None
pH	8.0	7.90
Temperature (°C)	23.6	24.2
Turbidity (NTU)	0.2	31
Total organic carbon (TOC) (mg/L)	<1	>10
Total dissolved solids (TDS) (mg/L)	475	1470

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Table 2. Reduction of MS-2 bacteriophage from general case test water by Grifaid GFF5 water filters

FILTER UNIT	INFLUENT	EFFLUENT COLLECTED AFTER 3 LITERS	EFFLUENT COLLECTED AFTER 6 LITERS	EFFLUENT COLLECTED AFTER 10 LITERS
Unit 1	8.65×10^{8}*	2.35×10^{2}	7.35×10^{2}	3.92×10^{2}
	LOG REDUCTION	6.56	6.07	6.34
Unit 2	8.20×10^{8}	1.03×10^{3}	9.03×10^{2}	2.21×10^{3}
	LOG REDUCTION	5.90	5.96	5.57

*plaque forming units

Table 3. Reduction of *E. coli* from general case test water by Grifaid GFF5 water filters

FILTER UNIT	INFLUENT	EFFLUENT COLLECTED AFTER 3 LITERS	EFFLUENT COLLECTED AFTER 6 LITERS	EFFLUENT COLLECTED AFTER 10 LITERS
Unit 1	8.43×10^{8}*	6.12×10^{3}	7.07×10^{2}	1.58×10^{2}
	LOG REDUCTION	> 5.13	> 6.08	> 7.23

Unit 2	$8.08x10^8$	$5.29x10^4$	$4.24x10^4$	$4.06x10^4$
	LOG REDUCTION	4.18	4.28	4.30

*colony forming units

Table 4. Reduction of MS-2 bacteriophage from challenge case test water by Grifaid GFF5 water filters

FILTER UNIT	INFLUENT	EFFLUENT COLLECTED AFTER 3 LITERS	EFFLUENT COLLECTED AFTER 6 LITERS	EFFLUENT COLLECTED AFTER 10 LITERS
Unit 1	$2.78x10^{9}$*	$5.30x10^2$	$8.10x10^2$	$5.20x10^2$
	LOG REDUCTION	6.72	6.54	6.73
Unit 2	$1.99x10^9$	$2.74x10^3$	$2.94x10^3$	$3.87x10^3$
	LOG REDUCTION	5.86	5.83	5.71

*plaque forming units

Table 5. Removal of *E. coli* from challenge case test water by Grifaid GFF5 water filters

FILTER UNIT	INFLUENT	EFFLUENT COLLECTED AFTER 3 LITERS	EFFLUENT COLLECTED AFTER 6 LITERS	EFFLUENT COLLECTED AFTER 10 LITERS
Unit 1	$7.70x10^{8}$*	$1.90x10^2$	$9.00x10^1$	$1.20x10^2$
	LOG REDUCTION	6.62	6.95	6.80

Unit 2	$8.00x10^8$	$4.55x10^5$	$3.10x10^5$	$1.66x10^5$
	LOG REDUCTION	3.25	3.41	3.68

*colony forming units