Microbial Reduction Evaluation of the AQUAFILTER FAMILY Water Filtration System

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SUMMARY

Two Aquafilter Family filtration system units 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 after 3, 6 and 10 liters of water through the units by hand pumping. The removal of MS-2 bacteriophage ranged from 4.90 to 5.75 logs and *E. coli* from 6.00 to >8.23 logs.

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 Aquafilter Family filtration 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 thought activated carbon to remove any chlorine present in the water. Water quality analysis was 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) by addition of

sea salts (Sigma Chemical, St. Louis, MO)

Bacterial Analysis

Escherichia coli (ATCC-25922) was grown overnight in Trypticase Soy broth (Difco, Detroit, MI) at 37^{o} C to obtain the organisms in the stationary growth phase. The bacterial cells were pelleted by centrifugation and resuspended in phosphate buffered saline. This procedure was repeated three times to remove 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 0.025 M phosphate buffered saline (PBS) at pH 7.0. A 100 mL sample of undiluted unit effluent was also assayed.

Bacteriophages Assay

MS-2 (ATCC 15597-B1) was grown in its host *Escherichia coli* (ATCC 15597). A 18 to 24 hour culture of host bacteria was grown in Tryptic Soy Broth (TSB; Difco, Detroit, MI) was transferred (0.1 ml) to fresh TSB and grown for 3 hours at 37^{o}C with continuous shaking. Stock phage was serially diluted in Tris buffer saline (Sigma Chemical, St. Louis, MO) to approximately 10^{5} plaque forming units (PFU)/ml. A 1 ml suspension of host and 0.1 ml of the phage dilution were mixed in molten overlay agar (top agar; TSB with 1% Bacto agar; Difco, Detroit, MI) and poured onto a pre-solidified Tryptic Soy Agar (TSA, 1,5% agar; Difco, Detroit, MI) petri dishes. After 18 to 24 hours incubation at 37^{o}C, 6 to 7 ml of sterile Tris buffered saline was added to petri dishes with confluent plaques and allowed to sit for a maximum of one hour to allow the phage

to diffuse from the agar surface. The liquid fraction was recovered from the plates with aid of a pipette, centrifuged and the resulting supernatant filter sterilized and tittered and stored at 4^{o}C.

Influent and effluent samples from the filters were kept at 4^{o}C until assayed for bacteriophage. Serial dilutions (1:10) of the samples were in Tris buffered saline, added to test tubes containing 3 ml of molten 1% TSB top agar and 1 ml of a 3 hour host culture and then poured onto solid TSA (1.5% agar). The plates were incubated for 24 hours at 37^{o}C, after which the plaques were enumerated.

Results

The results of microbial removal are shown in Tables 2 to 5. The test microorganisms were removed effectively from both types of water. The average MS-2 exceeded 5.45 logs and for *Escherichia coli* was greater than 7.34 logs.

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	Worst Case Water
Chlorine	None	None
pH	7.85	7.90
Total organic carbon (TOC)(mg/L)	<1	10
Turbidity (NTU)	0.1	30
Temperature (°C)	20 +/- 5	20+/- 5
Total dissolved solids (TDS)	300 mg/L	1500

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Table 2. Reduction of MS-2 bacteriophage from general case test water

UNIT LAB CODE	INFLUENT	SAMPLE COLLECTED AFTER 3 LITERS	SAMPLE COLLECTED AFTER 6 LITERS	SAMPLE COLLECTED AFTER 10 LITERS
AQUA 1	4.8E9	6.00E4	4.75E4	5.75E4
	LOG REDUCTION	4.90	5.00	4.92
AQUA 2	3.60E9	1.12E4	1.25E4	7.50E3
	LOG REDUCTION	5.50	5.42	5.68

*plaque forming units

Table 3. Reduction of *E. coli* from general case test water

UNIT LAB CODE	INFLUENT	SAMPLE COLLECTED AFTER 3 LITERS	SAMPLE COLLECTED AFTER 6 LITERS	SAMPLE COLLECTED AFTER 10 LITERS
AQUA 1	1.69e9*	<10	<10	<10
	LOG REDUCTION	>8.23	>8.23	>8.23
AQUA 2	1.00E9	9.50e2	1.00e3	8.50e2
	LOG REDUCTION	6.05	6.00	6.07

*colony forming units

Table 4. Reduction of MS-2 bacteriophage from challenge case test water

UNIT LAB CODE	INFLUENT	SAMPLE COLLECTED AFTER 3 LITERS	SAMPLE COLLECTED AFTER 6 LITERS	SAMPLE COLLECTED AFTER 10 LITERS
AQUA 1	2.50E9*	5.50E3	4.25E3	4.50E3
	LOG REDUCTION	5.66	5.77	5.75
AQUA 2	1.10E9	3.25E3	2.50E3	2.75E3
	LOG REDUCTION	5.53	5.64	5.60

*plaque forming units

Table 5. Removal of *E. coli* from challenge case test water

UNIT LAB CODE	INFLUENT	SAMPLE COLLECTED AFTER 3 LITERS	SAMPLE COLLECTED AFTER 6 LITERS	SAMPLE COLLECTED AFTER 10 LITERS
AQUA 1	1.50E9*	<10	<10	<10
	LOG REDUCTION	>8.17	>8.17	>8.17
AQUA 2	4.00E9	6.00E2	4.00E2	4.50E2
	LOG REDUCTION	6.82	7.00	6.95

*colony forming units