Microbiological Testing of the Sawyer Mini Filter

16 December 2013

Summary

The Sawyer Mini Filter was tested for its ability to remove three microorganisms – *Raoultella terrigena*, *Bacillus subtilis*, and *Micrococcus luteus* – using USEPA approved procedures. The organisms were added to test water to reach a 10^7 – 10^8 initial concentration. The test water followed the criteria set forth by the USEPA 1987, following the conditions for "test water #3". All of the three tested filters met the target reduction of 6 log units, or 99.9999% for all runs. The Sawyer Mini filter meets the USEPA standard for bacteria.

Table 1. Mean log removal values (LRV) with standard error for three Sawyer Mini filter tests. Water was collected and microbiologically analyzed after 100, 500 and 900 milliliters passed through the filter.

	Passed through filter		
Organism	100	500	900
M. luteus	7.0927 (0.0239)	7.0927 (0.0239)	7.0927 (0.0239)
B. subtilis	7.407 (0.0188)	7.407 (0.0188)	7.407 (0.0188)
R. terrigena	8.457 (0.2823)	8.616 (0.1312)	8.616 (0.1312)

Introduction

Filtration is "a pressure- or vacuum-driven separation process in which particulate matter larger than 1µm is rejected by an engineered barrier primarily through a size exclusion mechanism and which has a measureable removal efficiency of a target organism that can be verified through the application of a direct integrity test" (40 CFR 141.2). The Sawyer filters underwent challenge testing with specific microorganisms to determine if the filter performed as a barrier. Standard United States Environmental Protection Agency (USEPA) approved procedures were followed.

A minimum of three Sawyer Mini filters were tested in triplicate. The filters were conditioned with a 5% chlorine solution and sterile test water at 20 psi. The challenge microorganism (Table 2) was mixed with test water to obtain a 10^7 cells/100 mL concentration and was forced through the Sawyer filters at 10 psi. 100mL of filtrate was collected in a sterile Whirl pak after 100, 500 and 900 milliliters passed through the Sawyer Mini filter and analyzed for microbial growth using the membrane filtration technique following Standard Methods 9222. (APHA et al., 2012).

Surrogate organisms of similar size, approved by the USEPA, were used in place of the pathogenic target organisms to avoid unnecessary safety hazards.

Table 2. Challenge test organisms and USEPA approved surrogates (USEPA, 2005 and NSF 2005)

Target Organism	**Surrogate**	**Size range (µm)**
Fecal Coliform (bacteria)	*Raoultella terrigena* (ATCC 33628)	2-4
Cryptosporidium	*Bacillus subtilis*	5-7
Giardia	*Micrococcus luteus*	10-12

In the USEPA Guide Standard and Protocol for Testing Microbiological Water Purifiers (1987), it states a minimum reduction for protozoan parasites of log 3 units and a minimum of 6 log units for bacteria. All targeted log reductions for surrogates were set at a 6 log units, or 99.9999% reduction.

Methods

Stock cultures were quadrant streaked onto Trypticase soy agar (TSA) plates and incubated at 32°C for 24 hours. A pure culture was selected from the plate. The pure culture was inoculated into a 250 ml flask containing 100 ml of Trypticase soy broth (TSB). The flask was placed on a multiplatform shaker and incubated at 32°C overnight

to grow the cells to stationary phase. The cells were counted using a Petroff-Hauser counting chamber. The test water was inoculated to obtain a final density in the 10^7-10^8 cells/100 ml range.

Test Water and Solutions:

Test Water: The water used for testing was obtained from the Yellow Breeches Creek, which is the source for municipal drinking water in Cumberland and York Counties in Pennsylvania. Water was collected in a 20 L carboy and autoclaved at 121°C (15 lb pressure) for 35 minutes to obtain sterile test water. 1 L of the test water was aseptically adjusted for the following conditions for "test water #3" (USEPA 1987).

- pH adjusted to 9 by using HCl or NaOH, SM 4500- H^+ B
- Total Organic Carbon minimum of 10 mg/L adjusted with humic acid, SM 5310 C
- Turbidity 30 NTU (Nephelometric Turbidity Unit) or greater, adjusted with Kaolin or Arizona Road dust, SM 2130B/Method 2
- Total dissolved solids were 1,500 mg/L ± 150 mg/L, TDS meter tested
- Temperature of test water was chilled to 4 °C ± 1 °C, SM 2550 B

Standard methods (APHA et al., 2012) were followed to ensure test water conditions.

1.1 L of challenge test water was dispensed into 2L vacuum bottles (Nalgene) and autoclaved at 121 °C (15 lb pressure) for 30 minutes. The final pH was 9.0 ± 0.2, turbidity 100NTU, TOC 15.5 mg/L, and TDS 1400 mg/L. The challenge test water bottles were placed in a refrigerator to attain a temperature of 4 °C prior to testing.

Trypticase Soy Broth (TSB) (BD Diagnostic Systems)

Into 1 L of reagent grade distilled water, dissolved 30g dehydrated TSB. The media was then dispensed in culture tubes and 250 ml flasks, covered with caps/foil and autoclaved at 121 °C (15 lb pressure) for 15 minutes.

Trypticase Soy Agar (TSA) (BD Diagnostic Systems)

To 1 L of reagent grade distilled water, dissolved 40 g dehydrated TSA in a flask and heated to boiling with stirring until the ingredients dissolved. The media was then autoclave at 121 °C (15 lb pressure) for 15 minutes and cooled in a 50 °C water bath. The agar was then aseptically poured agar into 50x9mm petri dishes to 4-5mm depth (7 ml) and allowed to solidify. The plates can be stored for up to two weeks in the refrigerator.

Bacterial Test Water Preparation

Saturated cultures of each bacterial strain were prepared by inoculating 10 mL of TSB with the test organisms and incubated on a rollodrum overnight at 32 ^{o}C. The following day the cultures were counted using a Petroff Hausser counting chamber and appropriately diluted so that the final concentration of bacteria in the 1.1 L testing sample was 1 x 10^{7} – 10^{8} cells/L.

Pressurizing device

All tubing, bottles, caps, and glassware were washed and autoclaved prior to each trial. Initial conditioning of the Sawyer mini filter was attained by passing 1 L of 5% bleach solution followed by 2 L of test water (without organisms) through the filter at 20psi (Fig. 1). The last liter of test water was collected as negative controls at 100, 500 and 900 milliliters in sterile Whirl paks. Challenge test water (with organisms) were forced through the Sawyer Mini filter at 10 psi. Collection of filtrate was performed at 100, 500, and 900 milliliters in sterile Whirl paks.

Figure 1. Pressurizing device for forcing challenge water through the Sawyer filter.

Microbiological analysis

Standard Methods 9222 (APHA et al. 2012) were followed, the following description is abbreviated. The 100 ml sample was vigorously shook and poured into the vacuum funnel. A vacuum was applied to filter the sample through the 0.45µm filter paper. The funnel walls were rinsed three times with 20-30 ml sterile deionized distilled water. Using sterile forceps the filter was transferred to the prepared petri dish grid side up. The petri dish was incubated at 32 ± 0.5°C for 48 hours, count colonies at 24 and 48 hours.

Initial seed counts were confirmed by serial dilution, using 99ml sterile deionized distilled water blanks. The final dilution for plating was 10^{-6} and 10^{-7}.

Calculations:
Colony forming units (cfu)
Cfu/100ml = 100 x (number of colonies)/ volume of sample filtered in mL
Log removal value (LRV), target is 6 log unit reduction.

LRV= log (C_f) – log (C_p)
C_f =feed concentration (cfu/100ml)
C_p= filtrate concentration (cfu/100ml)

Results

All trials had comparable outcomes of zero or minimal cfu/100ml (Table 3). All trials attained 6 log unit reduction or higher. (Table 4)

Table 3. Challenge filtration test trials on the Sawyer Mini HFM. Filtrate collected at 100, 500, and 900 milliliters underwent microbiological membrane filtration, values are expressed as colony forming units per 100 milliliters (cfu/100ml).

Trial	Organism	Initial seed	100	500	900
1	*M. luteus*	1.17×10^7	0	0	0
	B. subtilis	2.38×10^7	0	1	0
	R. terrigena	6.87×10^8	0	0	0
	Negative control		0	1	0
2	*M. luteus*	1.18×10^7	0	0	0
	B. subtilis	2.58×10^7	0	0	0
	R. terrigena	2.41×10^8	3	0	0
	Negative control		0	0	0
3	*M. luteus*	1.38×10^7	0	0	0
	B. subtilis	2.74×10^7	0	0	0
	R. terrigena	4.28×10^8	0	0	0
	Negative control		0	0	0

Table 4. Log removal values (LRV) on the Sawyer Mini HFM test trials. 6 log unit reduction or greater was the target range.

Trial	Organism	100	500	900
1	*M. luteus*	7.068	7.068	7.068
	B. subtilis	7.373	7.373	7.373
	R. terrigena	8.836	8.836	8.836
2	*M. luteus*	7.07	7.07	7.07
	B. subtilis	7.41	7.41	7.41
	R. terrigena	7.905	8.382	8.382
3	*M. luteus*	7.14	7.14	7.14
	B. subtilis	7.438	7.438	7.438
	R. terrigena	8.63	8.63	8.63

Back flushing was performed to demonstrate the effectiveness of the filter performing as a barrier. Back flush recovery showed two log reduction after 1 liter of test water passed through the filter (Table 5). This back flush test demonstrated that the Sawyer Mini HFM truly is a barrier.

Table 5. Back flush recovery test trials on the Sawyer Mini HFM. Filtrate collected at 100, 500, and 900 milliliters underwent microbiological membrane filtration, values are expressed as colony forming units per 100 milliliters (cfu/100ml).

		Back flush		
Trial	Initial	100	500	900
1	2.225×10^8	TNTC	1.68×10^7	3.5×10^6
2	3.786×10^8	TNTC	2.85×10^7	3.6×10^6
3	2.858×10^8	TNTC	1.94×10^7	3.4×10^6

Discussion

All of the three Mini Filters tested showed a 6 fold or greater reduction of the test organisms, indicating the filters successfully remove the organisms from the challenge water. If the surrogate organisms, *M. luteus* and *B. subtilis*, were held to the USEPA standard for *Giardia* and *Cryptosporidium*, respectively, then only a 3 log reduction would be required. Thus, the filter would have met the USEPA standard for both bacteria and protozoans. These tests show that the Sawyer Mini filter meets the USEPA standard for bacterial removal.

References

American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF) 2012. Standard Methods for the Examination of Water and Wastewater. 22nd ed. American Water Works Association.

Federal Register 2012. National Primary Drinking Water Standards. 40 CFR 141.2

NSF International. 2005. EPA/NSF ETV Equipment Verification Testing Plan for the Removal of Microbiological and Particulate Contaminants by Membrane filtration. Ann Arbor, MI.

USEPA 1987. Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources Appendix O: Guide Standards and Protocol for Testing Microbiological Water Purifiers. Contract No. 68-01-6989 U. S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, DC.

USEPA 2005. Membrane Filtration Guidance Manual. EPA 815-R-06-009 U. S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, DC.