ANALYTICAL SERVICES, INC. (ASI)
Microbiological Testing, Research and Consulting

13 October 2013

Jim Shepherd
Berkey Water
PO Box 201411
Arlington, TX 76006

Subject: Microbiological Challenge Study; ASI Report 47068-2E

Dear Jim,

This report pertains to microbiological challenge testing of your "Travel Berkey" unit equipped with one Earth Filter Element, performed as initially described in ASI proposal 2013-0424-01P (dated 24 April 2013).

Project Summary

This testing was exploratory in nature and the objectives of the project were to generate original performance data regarding the Berkey Earth elements.

Types of challenge organisms included used; *Bacillus atrophaeus*, *Salmonella enterica*, and *Escherichia coli*,. One filter element of each type was challenged with each test organism type; no replicate testing was performed. Testing was performed using only Test Water 1 prepared as described in NSF P248, Emergency Military Operations Microbiological Water Purifiers (Dec. 2008).

We appreciate your selection of ASI as a microbiological testing/consulting resource. If you have any questions about the testing described herein, please contact me at anytime.

Sincerely,
ANALYTICAL SERVICES, INC. (ASI)

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Project Description

The objective of this testing was to generate microbial reduction performance data for the Berkey Water Earth Element Travel Berkey systems were provided for testing. It is understood the filter elements contain complex, proprietary filter matrices that treat water from the 1.5 gallon (5.7L) reservoir, and that no true integrity test procedure is currently available to verify the condition of the filter media and proper installation.

The Travel Berkey units were operated as per manufacturer's instructions. For efficiency, the challenge organisms were cocktailed and trials were performed as summarized below (using a new filter element for each trial);

- P248 Test Water 1 with *E. coli* ATCC 15597 and *Salmonella enterica* ATCC 14028
- P248 Test Water 1 with *B. atrophaeus* (ATCC 9372, spores).

The log reduction goals for this project were established by the client as follow:

- Vegetative bacteria: 4 log reduction
- Bacterial spores: 3 log reduction.

Organisms with the exception of *B. atrophaeus* were propagated as per ASI's standard procedures and stock samples collected prior to use as positive control samples. A suspension of *B. atrophaeus* spores (ATCC 9372) was purchased commercially from Sterilator Company, Inc. (Cuba, NY). The challenge water for the initial trial was prepared as per NSF P248 Phase I General Test water using DI water.

Prior to testing, each filter element (with the exception of the Earth elements) was primed using priming buttons provided and laboratory deionized water according to client's instructions. Each filter element was then installed in a Travel Berkey unit and the units were then filled with sterile laboratory deionized (DI) water for collection of negative control samples. Each negative control was analyzed for all four test organisms. Once negative controls were collected, the units were emptied of any remaining sterile DI water and the reservoirs were filled with approximately 2.5 L of test water. The test water was seeded with challenge organism stock prior to addition to the travel units, to achieve pretreatment concentrations of approximately 10e5 spores/mL (*B. atrophaeus*) and 10e7 CFU/mL (*E. coli* and *S. enterica*). One influent sample was collected from the test water prior to adding to the test units. The results from the analysis of this sample established the pretreatment concentrations and were used to calculation log reductions of each organism type.

After the majority of the seeded reservoir volume had been processed, effluent samples were collected from the treated water chamber tap. The treated water samples were composites of all the water treated up to that point in the test. Data from these analyses were used to determine the log reduction achieved.

All samples were analyzed quantitatively as per ASI's standard procedures. Log reductions for each organism type were calculated as follows:

$$\text{Log Reduction} = \text{Log}_{10}\left(\frac{\text{Influent concentration}}{\text{Effluent concentration}} \right)$$

Results Earth Filter Elements

Description	*S. enterica*	*E. coli*	*B. atrophaeus* spores
Negative Control	None Detected	None Detected	None Detected
Influent (CFU/mL)	5.8×10^7	4.8×10^7	3.3×10^5
Effluent (CFU/mL)	<2	<2	9.9×10^2
Log Reduction	>7.46	>7.38	2.52
Log Reduction Goal	6 log	6 log	3 log
Goal Achieved?	Yes	Yes	No

Discussion

Influent challenge concentrations were appropriate for the project log reduction goals.

All control samples yielded expected results and the test results were accepted as valid.

Under the preliminary testing described above, the Berkey Earth element met log reduction criteria for bacteria *E. coli* and *S. enterica*, however, not for *B. atrophaeus* spores. While ASI is not aware of the details of the Earth Element filters, the performance differential between *Salmonella* and *E.coli* as compared to *Bacillus* spores seems surprising. All ports (filter and plugged) between the influent reservoir and the effluent chamber were monitored repeatedly during the test period and no evidence of leakage/by-pass was observed during this trial.

For future testing, considerations should include discrete samples of the effluent at different time intervals, performance of the elements under challenging test water conditions and replicate tests of a single filter type.