The filter 2 system, consisting of a molded carbon block filter (top) and housing (bottom), functioned on the principle of electrostatic attraction of negatively charged microorganisms to positively charged surfaces.

Because of their small size, *Cryptosporidium* oocysts cannot be easily filtered from water. This study evaluated the use of surrogates for measuring the microbial treatment performance of two point-of-use devices incorporating filtration with electrostatic charge interaction mechanisms. Both systems were challenged with *Cryptosporidium* oocysts (4–6 µm), *Bacillus subtilis* (*B. subtilis*) spores (~1.2 µm), polystyrene latex (PSL) beads (~ 3 µm), *Escherichia coli,* and MS2 bacteriophage. The target biological contaminants were more effectively removed than the PSL beads, and the smaller *B. subtilis* spores mimicked *Cryptosporidium* oocyst removal more closely than the ~ 3-µm PSL beads. Thus, surface charge appears to be an important factor for microorganism attachment, and *B. subtilis* spores should be considered a more appropriate surrogate than PSL beads for evaluating *Cryptosporidium* movement through charged media. For noncharged devices, PSL beads may still be useful as a surrogate for *Cryptosporidium.*

Evaluating surrogates for *Cryptosporidium* removal in point-of-use systems

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A point-of-use (POU) device can be defined as a plumbed-in or faucet-mounted system used to treat water at a single tap or multiple taps, in contrast to a point-of-entry (POE) system, which is used to treat all of the water entering a residence or building. POU systems are usually installed at the kitchen sink or faucet and treat water used only for drinking or cooking. POU devices may use one or more of the commonly available water treatment options such as adsorption, filtration, chemical disinfection, ultraviolet treatment, reverse osmosis (RO), ion exchange softening, or distillation. A single POU device may use more than one treatment methodology to treat source water.

Between 1997 and 2001, the use of POU devices in US households increased from 32 to 41% (Lau et al, 2005). The extent of the demand for POU devices is shown by the fact that in 1998, 2 million units were sold, generating revenue of $2.2 billion (Davis et al, 2001). Many of these POU devices are aimed at chlorine or organic chemical reduction, but some are designed and certified to ANSI/NSF standards to remove microbial contaminants. The use of submicron POU water filters reduced the risk of infection during the 1993 waterborne outbreak of cryptosporidiosis in Milwaukee, Wis. (Addis et al, 1996). It has been

The filter 1 system incorporated two housings—one containing a pleated prefilter (far left) to remove coarse particulate matter and a second containing an extruded activated carbon filter (middle). The photo on the right shows the second filter in the housing.

suggested that in some communities, approximately 35% of reported gastrointestinal illnesses may be water-related and preventable by a POU RO unit (Lau et al, 2005; Payment et al, 1991). On the other hand, RO devices remove almost all ions, including potentially beneficial minerals (WHO, 2008; Cotruvo, 2006). Some types of POU treatment devices have generated interest for protection against intentional or accidental contamination of drinking water supplies. The events of Sept. 11, 2001, have further emphasized the importance of appropriately designed POU or POE devices in this regard.

BACKGROUND

The US Environmental Protection Agency (USEPA) initiated a research program to evaluate the performance of various POU devices for treating tap water in homes, either routinely or as an emergency measure during accidental or intentional contamination. This study evaluated the performance of two POU devices in removing biological contaminants including protozoa, represented by *Cryptosporidium* and appropriately sized polystyrene latex (PSL) beads or *Bacillus subtilis* (*B. subtilis*) spores as surrogates for *Cryptosporidium*; bacteria, represented by *Escherichia coli* (*E. coli*); and viruses, represented by MS2 bacteriophage. This study also evaluated the appropriateness of PSL beads and *B. subtilis* as conservative surrogates for *Cryptosporidium* removal in high-dose challenge testing of devices that function by adsorptive and cationic surface attachment and by filtration.

Microspheres and aerobic spores as a surrogates for *Cryptosporidium*. The difficulty in accurately enumerating *Cryptosporidium* from large-volume samples has made it impractical to implement and enforce regulatory requirements for this pathogen (Clancy et al, 1999; Nieminski et al, 1995). As a result, the USEPA's Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) allows utilities that require additional treatment for pathogen removal and/or inactivation to choose from a variety of options, including determinations of source water contamination potential, bin classifications that determine required log removals of treatment processes, and "demonstration of performance," which requires studies that reliably quantify *Cryptosporidium* log removals (USEPA, 2006; Emelko & Huck, 2004). Considering the cost, analytical difficulty, and health risks associated with live infectious oocysts, it is desirable to establish a quantitatively reliable surrogate for *Cryptosporidium* for use in demonstration of performance of both POU and larger water treatment systems.

Oocyst-sized PSL microspheres have been used as a nonbiological surrogate for determining oocyst removal by several researchers (Emelko & Huck, 2004; Emelko et al, 2003; USEPA, 2003b; Amburgey et al, 2001; Swertfeger et al, 1999; Li et al, 1997). A nearly perfect linear correlation was observed between log removals of 4- to 6-µm PSL microspheres and *Cryptosporidium parvum* (*C. parvum*) oocysts in field-scale bag filtration devices (Li et al, 1997). The relationship between *C. parvum* and PSL beads' removal has been shown to be filter-specific and affected by system operating conditions (Emelko et al, 2003). The PSL microspheres used in this study had a mean diameter of 2.83 µm.

Several studies (Brown & Cornwell, 2007; Cornwell et al, 2003; Dugan et al, 2001; Yates et al, 1998) have demonstrated that *B. subtilis* spores are a conservative

In this back view of the point-of-use/point-of-entry manifold system, the two housings of the filter 1 system are shown at the left and the filter 2 system is on the right.

surrogate for *Cryptosporidium* oocysts for systems required to demonstrate LT2ESWTR compliance. This noninfectious aerobic spore is almost always present in surface water in significantly greater concentrations than *Cryptosporidium* (USEPA, 2006; USEPA, 2003a; Nieminski & Bellamy, 2000; Rice et al, 1996), thus allowing for ease of detection and determination of treatment system removal efficiency. *B. subtilis* spores have a mean size of ~ 1.2 µm (Westphal et al, 2003) versus 4–6 µm for *Cryptosporidium* oocysts (WHO, 2004), and their surface charges and zeta potentials are very similar at the operating pH range (6.5–8) of this study (Lytle et al, 2002). Furthermore, the analytical methods for enumeration of aerobic spores are simpler, faster, much less expensive, and more reliable than the enumeration techniques for *Cryptosporidium* (Brown & Cornwell, 2007). Finally, *Cryptosporidium* oocysts are typically removed more readily than aerobic spores during physical filtration and clarification treatment processes (Brown & Cornwell, 2007); thus, demonstration of removal of *B. subtilis* before disinfection would be expected to provide a conservative estimate of *Cryptosporidium* removal.

MATERIALS AND METHODS

System description. A POU test apparatus was designed and constructed at the USEPA Test and Evaluation Facility in Cincinnati, Ohio, to allow different POU devices to be plumbed in for testing their effectiveness in removing biological contaminants. The main components of the POU test apparatus were feed tank, carbon filters and deionization filters to remove chlorine and other impurities from source water, feed pump, contaminant injection pump, inline mixer, various POU devices, kitchen sink and faucet, and the associated valves and electronic control devices necessary to operate the system. The test apparatus was also equipped with sensors that continuously measured basic parameters, such as flow rate, pressure, turbidity, and conductivity.

Two POU devices were tested in this study. In the system configuration tested, the POU filter 1 system[1] incorporated two housings. The first housing contains a pleated prefilter designed to remove coarse particulate matter. The second housing contains an extruded activated carbon filter consisting of carbon, a high-molecular-weight cationic polymer, a cationic silver complex, a thermoplastic binder, and a pH-altering material (US patents 6,630,016 and 6,770,204). The cationic material, in combination with the biologically active metal complex, enhances microbiological interception of the activated carbon block filter medium. The pH-altering material adjusts surface charge to enhance performance.

The POU filter 2 system[2] featured a patented technology developed for the removal of microorganisms from contaminated water that relies on the principle of electrostatic attraction of negatively charged microorganisms to positively charged surfaces. The device consists of a molded carbon block filter containing an insoluble, inorganic material with a high isoelectric point, a binder with a low melt index, and an optional component containing silver to suppress bacterial growth when used over an extended period of time. The isoelectric point of the material refers to the pH level of an aqueous environment at which the material has a net zero surface charge. Below this point, the material has a net positive surface charge; above it, the material has a net negative surface charge. For this purpose, magnesium hydroxide was determined to be the material of choice because of its relatively high isoelectric point (~ 10.5), its relative high abundance, and low cost. A low-melt-index binder is required to prevent the binder from flowing under elevated temperatures and pressures but to remain tacky so as to bind the particles together.

The filters are formed by mixing the magnesium hydroxide with activated carbon and a binder, heating

This front view shows the setup for the point-of-use/point-of-entry manifold system at the US Environmental protection Agency Test and Evaluation Facility in Cincinnati, Ohio.

the mixture in a mold, and compressing the fine particles together to form a solid carbon block filter. Compression of the filter medium helps to control the pore size within the filter and thus control the flow rate of the water through the system. The filter as tested contained 40% magnesium hydroxide, 30% activated carbon, 25% binder,[3] and 5% of a material containing silver to help control bacterial growth within the filter.

Experimental test runs. To evaluate specific bacteria removal, the POU devices were challenged with two species: *B. subtilis,* a predominant aerobic spore, and *E. coli,* a bacterium common in the lower intestinal tracts of mammals. *B. subtilis* test runs were also used to evaluate the potential value of the aerobic spore as a surrogate for *Cryptosporidium* removal. MS2 bacteriophage, a bacterial virus that is a commonly accepted water treatment surrogate for pathogenic enteric viruses (Harrington et al, 2003) was used to challenge the POU devices to evaluate the performance in removing viruses. *C. parvum* oocysts were used to challenge the systems to evaluate the performance in removing protozoa. PSL beads with a mean size of 2.83 µm were used to challenge the devices as a nonbiological surrogate for *C. parvum* in accordance with the LT2ESWTR, which dictates that a surrogate with an effective size of 3 µm or smaller must be used to demonstrate *Cryptosporidium* removal (USEPA, 2005). The results from the PSL bead tests were compared with *B. subtilis* results to evaluate the relative suitability of these two surrogates for *Cryptosporidium* in measuring system performance.

Between 1997 and 2001, the use of point-of-use devices in US households increased from 32 to 41 percent.

For each contaminant, a new filter was used and triplicate tests were conducted to evaluate the performance of the system. All tests were performed using dechlorinated potable water from the municipal supply of Cincinnati at a flow rate of 0.5 gpm.

Injection, sampling, and analysis. For *B. subtilis, E. coli,* and MS2 bacteriophage challenges, 1 mL of stock suspension at an approximate concentration of 10^9 cells/mL was mixed with 500 mL of 0.01% polysorbate surfactant[4] in a 1-L glass beaker. A subsample was collected to determine the actual concentration of the injection suspension. A peristaltic pump was used to inject the 500-mL suspension into the influent stream of the filtration system. When the injection was completed, the beaker was filled with an additional 500 mL of 0.01% polysorbate surfactant and injected into the feed stream. The total injection time for the suspension and the rinseate was approximately 60 min. Grab samples (100 mL) from the influent and effluent stream were collected at 0, 5, 10, 20, 30, and 60 min after the start of the injection. Duplicate samples were collected 10 min after the start of injection of the organism. The collection of multiple effluent samples during the entire time of injection of the contaminant effectively minimized the possibility of not detecting a contaminant in the effluent even with expected low effluent contaminant concentration.

B. subtilis[5] and MS2[6] bacteriophage stocks were obtained from laboratories. *E. coli*[7] stock was prepared by culturing with nutrient broth. Grab samples (100 mL) for *B. subtilis* were analyzed following methods described by other researchers (Rice et al, 1994). Grab samples (100 mL) for *E. coli* were analyzed following method

10029 (Hach Co., 1999). Grab samples for MS2 bacteriophage were analyzed following method 1602 (USEPA, 2001a). The analytical techniques used for enumerating the microorganisms are capable of detecting the presence of a single cell in a collected sample.

For PSL beads and *Cryptosporidium* challenges, the procedure for preparing and injecting the test suspension was identical to that used for *B. subtilis, E. coli,* and MS2 bacteriophage challenges. The total injection time for the bead suspension and the rinseate was 30 min. The system was run for 4–5 h after completion of the injection. It is conventional to divert a slipstream of the effluent from the system through a 1-µm membrane in a manifold membrane system during the test period to collect the beads or oocysts from the effluent. However, the relatively low flow rate for the tested POU systems made it possible to divert the whole effluent stream through the collection membrane. The beads or oocysts were extracted from the membrane with a squeegee, followed by rinsing with 0.01% polysorbate surfactant. The final volume of the effluent sample was ~ 1 L.

Samples were analyzed according to method 1622 (USEPA, 2001b) using a hemacytometer. Because a hemacytometer was used for enumerating PSL beads, this instrument was also used to enumerate *Cryptosporidium* oocysts to ensure consistency between the analytical techniques. A 500-µL subsample was also collected and analyzed to determine the total beads or oocysts in the influent. PSL beads[8] (3.0-µm microspheres) were used as stock suspension for this challenge. The Iowa strain *Cryptosporidium* oocysts stored in antibiotic solution were obtained from the Sterling Parasitology Laboratory at the University of Arizona in Tucson and the USEPA's Microbial Contaminants Control Branch in Cincinnati. The analytical techniques used for enumerating *Cryptosporidium* oocysts and PSL beads can detect the presence of a single oocyst and/or bead in a collected sample.

An additional single *B. subtilis* challenge test was conducted using the postinjection membrane sampling method for direct comparison with *Cryptosporidium* and PSL beads. A 0.40-µm membrane was used in the manifold membrane unit (instead of the 1-µm membrane used for PSL beads and *Cryptosporidium* oocysts) to collect *B. subtilis* spores from the effluent stream. As in the PSL beads test, the entire effluent from the POU system was passed through the membrane. The final volume of *B. subtilis* effluent sample was ~ 1 L; a representative 100-mL sample was analyzed following the method described elsewhere (Rice et al, 1994).

TABLE 1 Summary of PSL bead challenge results for filter 1 system*

Test Run	Total Number of Beads		Log Removal	Average Log Removal for All Runs
	In	Out		
1	1.49×10^9	1.68×10^6	2.95	3.14
2	1.50×10^9	1.00×10^6	3.17	
3	1.48×10^9	7.80×10^5	3.30	

PSL—polystyrene latex

*KX Matrikx®, KX Technologies, Orange, Conn.

TABLE 2 Summary of PSL bead challenge results for filter 2 system*

Test Run	Total Number of Beads		Log Removal	Average Log Removal for All Runs
	In	Out		
1	1.78×10^9	6.04×10^5	3.47	3.56
2	1.89×10^9	2.71×10^5	3.84	
3	1.57×10^9	1.57×10^5	3.37	

PSL—polystyrene latex

*Clorox-Brita, Clorox Co., Oakland, Calif.

TABLE 3 Summary of *E. coli* challenge results for filter 1 system*

Test Run	Number of ***E. coli*** per 100 mL		Log Removal	Average Log Removal for All Runs
	In	Out		
1	2.52×10^5	0	> 5.40	> 5.98
2	9.60×10^5	0	> 5.94	
3	4.02×10^6	0	> 6.60	

E. coli—Escherichia coli

*KX Matrikx®, KX Technologies, Orange, Conn.

RESULTS AND DISCUSSIONS

PSL bead challenges. Table 1 shows the results for PSL bead challenges for filter 1. The log removals of PSL beads in the three test runs varied between 2.95 and 3.30, with an average of 3.14.

Table 2 shows the results of the three test runs using PSL beads for filter 2. Log removals varied between 3.37 and 3.84, with an average of 3.56. The influent PSL bead concentrations for filter 2 were slightly higher than those for filter 1, whereas the effluent bead concentrations for filter 2 were lower than those for filter 1. Both test series

demonstrated excellent and comparable removal efficiencies with a slightly greater removal of beads occurring in filter 2.

***E. coli* challenges.** Table 3 shows the results of *E. coli* challenges for filter 1. The influent data shown were the average concentrations of the sampling during each test run. No organisms were detected in the effluent.

Table 4 shows the results of *E. coli* challenges for filter 2. The influent data shown were the average concentrations of the sampling during each test run. No organisms were detected in the effluent.

For both filters, no *E. coli* were detected in the effluent; each filter achieved > 5- to 6-log removal of the challenge organisms. The apparent quantitative differences were attributable to variations in the influent *E. coli* concentrations and not reflective of the log-removal capabilities of the filters.

***B. subtilis* challenges.** Table 5 shows the results of *B. subtilis* spore challenges for filter 1. The influent data shown were the average concentrations of the sampling for each test run. No organisms were detected in the effluent.

Table 6 shows the results of *B. subtilis* challenges for filter 2. No organisms were detected in the effluent. The influent data shown were the average concentrations of the sampling for each test run.

For both filters, no *B. subtilis* were detected in the effluent from either test series, and each filter removed > 5–6 logs of challenge organisms. The apparent quantitative differences were attributable to variations in the influent *B. subtilis* concentrations and not reflective of the log-removal capabilities of the filters.

TABLE 4 Summary of *E. coli* challenge for filter 2 system*

Test Run	Number of ***E. coli*** per 100 mL		Log Removal	Average Log Removal for All Runs
	In	Out		
1	2.40×10^6	0	> 6.37	> 6.50
2	3.18×10^6	0	> 6.50	
3	4.04×10^6	0	> 6.61	

E. coli—Escherichia coli

*Clorox-Brita, Clorox Co., Oakland, Calif.

TABLE 5 Summary of *B. subtilis* challenge results for filter 1 system*

Test Run	Number of ***B. subtilis*** per 100 mL		Log Removal	Average Log Removal for All Runs
	In	Out		
1	1.58×10^5	0	> 5.19	> 5.18
2	1.64×10^5	0	> 5.21	
3	1.49×10^5	0	> 5.15	

B. subtilis—Bacillus subtilis

*KX Matrikx®, KX Technologies, Orange, Conn.

TABLE 6 Summary of *B. subtilis* challenge results for filter 2 system*

Test Run	Number of ***B. subtilis*** per 100 mL		Log Removal	Average Log Removal for All Runs
	In	Out		
1	6.25×10^5	0	> 5.77	> 5.97
2	1.14×10^6	0	> 5.96	
3	1.75×10^6	0	> 6.17	

B. subtilis—Bacillus subtilis

*Clorox-Brita, Clorox Co., Oakland, Calif.

TABLE 7 Summary of MS2 bacteriophage challenge results for filter 1 system*

Test Run	Number of MS2 Bacteriophage per 100 mL		Log Removal	Average Log Removal for All Runs
	In	Out		
1	1.28×106	0	> 6.11	> 5.95
2	1.13×106	0	> 6.01	
3	6.35×105	0	> 5.74	

*KX Matrikx®, KX Technologies, Orange, Conn.

One additional challenge was carried out for filter 2 using the postinjection membrane sampling technique for direct comparison with the data for PSL beads and *Cryptosporidium* challenges. For this challenge, the total number of cells in the influent was 5.25×10^8 and that in the effluent was 585, with an associated log removal of 5.95.

MS2 bacteriophage challenges. Table 7 shows the results of MS2 bacteriophage challenges for filter 1. The average removal was > 5.95 logs. The influent data shown were the average concentrations of the sampling for each test run. No organisms were detected in the effluent.

Table 8 shows the results of MS2 bacteriophage challenges with the filter 2 system. The average removal was > 6.34 logs.

For both filters, no MS2 bacteriophage were detected in the effluent from either standard test series, and both achieved > 5- to 6-log removal of the challenge organisms.

TABLE 8 Summary of MS2 bacteriophage challenge results for filter 2 system*

Test Run	Number of MS2 Bacteriophage per 100 mL		Log Removal	Average Log Removal for All Runs
	In	Out		
1	5.40×10^6	0	> 6.63	> 6.34
2	4.70×10^6	0	> 6.67	
3	5.84×10^5	0	> 5.72	

*Clorox-Brita, Clorox Co., Oakland, Calif.

TABLE 9 Summary of *Cryptosporidium* challenge results for filter 1 system*

Test Run	Total Number of Oocysts		Log Removal	Average Log Removal for All Runs
	In	Out		
1	7.83×10^8	0	> 8.89	> 8.84
2	7.92×10^8	0	> 8.90	
3	5.92×10^8	0	> 8.77	

*KX Matrikx®, KX Technologies, Orange, Conn.

TABLE 10 Summary of *Cryptosporidium* challenge results for filter 2 system*

Test Run	Total Number of Oocysts		Log Removal
	In	Out	
Run 1	8.5×10^8	0	> 8.93

*Clorox-Brita, Clorox Co., Oakland, Calif.

The influent challenge concentrations varied during the test series; thus the calculated log removal values differed, depending on the influent challenges in each case.

***Cryptosporidium* challenges.** Table 9 shows the results of *Cryptosporidium* challenges for the filter 1 systems tested. For all the test runs, the data indicated complete removal of *Cryptosporidium* by filter 1.

Table 10 shows the data obtained for the *Cryptosporidium* oocyst challenge of filter 2. Only one test on this filter was planned because of limited oocyst availability. In this test, the filter 2 system achieved complete removal of *Cryptosporidium*.

Evaluation of test filter performance for *E. coli* and MS2 bacteriophage. Both the filter 1 and filter 2 systems performed comparably; both filters demonstrated a high capacity to remove *E. coli* and the MS2 bacteriophage. Neither system allowed any detectable *E. coli* or MS2 bacteriophage to be present in the effluent. *E. coli* removals for filters 1 and 2 were greater than 5.98 and 6.50 logs, respectively. MS2 bacteriophage removals for filters 1 and 2 were greater than 5.95 and 6.34 logs, respectively. The influent challenges differed; thus, the calculated log removal values were limited by the influent challenges in each case. Because neither filter contained a disinfection system, the removal of the microorganisms occurred through physical removal processes associated with the specific design of each filter. Both filters performed favorably when compared with the reduction requirement of the USEPA Guide Standard and Protocol for Microbiological Water Purifiers (USEPA, 1987), which is 6 logs for bacteria; the filters were exceptional with respect to the 4-log requirement for viruses.

Evaluation of the use of PSL beads and *B. subtilis* spores as surrogates for demonstrating *Cryptosporidium* removal. The two POU devices that were tested demonstrated significantly greater removal efficiency for *Cryptosporidium*, compared with removal efficiency for PSL beads or *B. subtilis* spores. This may be attributed to size as well as the different surface and solution properties, such as the pH and the ionic strength as well as other factors such as cationic charge. It has been suggested that the rigidity of the PSL beads (compared with oocysts) may lead to different attachment/detachment behavior as the filter influent particle load or composition changes (Emelko & Huck, 2004). This hypothesis, however, seems to be operating in the opposite direction for filtration retention, i.e., the more rigid PSL beads should be more subject to size exclusion than the flexible oocysts. Surface characteristics such as zeta potential, hydrophobicity, and filterability of microspheres are important in determining the potential as a surrogate for *Cryptosporidium* (Dai & Hozalski, 2003). Researchers have shown a negative zeta potential for *Cryptosporidium* (Dai & Hozalski, 2003; Brush et al, 1998; Drozd & Schwartzbrod, 1996; Ongerth & Pecoraro, 1996; Rice et al, 1996; Van der Mei et al, 1993) and PSL microspheres (Dai & Hozalski, 2003; Dewez et al, 1997). The electrophoretic mobilities of *C. parvum* oocysts and *B. subtilis* endospores are pH-dependent (Lytle et al, 2002); at the operating pH range (6.5–8) of this study, however, they were similar (see Table 11).

Several authors have concluded that PSL beads can be used as a conservative surrogate for removal of *Cryptosporidium* from water by treatment processes, depending on the water quality, operating condition, and filter composition. It has been stated that PSL microspheres maintain a relatively greater negative charge and are more hydrophobic (Dai & Hozalski, 2003) than oocysts. However, in the current study involving test systems with cationic charged surfaces, this may not have been borne

out because smaller *B. subtilis* spores were always retained in the test system much more effectively than were the PSL beads. Similarly sized oocysts were retained even more effectively than either PSL beads or *B. subtilis* spores. These tests showed the PSL beads to be so conservative as potential surrogates—more than five orders of magnitude less removal than the oocysts—as to be nonrepresentative of filtration performance for oocyst removal. This may be the result of poorer adhesion of the PSL beads to the filtration media surface, compared with *Cryptosporidium* oocysts and *B. subtilis* spores because of the surface smoothness and rigidity of the PSL beads. Electrical charge interactions appeared to be more significant than size exclusion in the performance of these systems; thus, the removal differences among the three test species and the PSL beads may be more reflective of charge neutralization or charge retention on the surface of the species. The attraction/repulsion and the corresponding removal of *Cryptosporidium* and microspheres were dictated by the composition and characteristics of the filter and filtration media (Dai & Hozalski, 2003), the pH and the ionic strength, and the cationic charge, among other factors (Lytle et al, 2002).

Both POU systems achieved complete removal of *B. subtilis* during the challenges. Similar to PSL microspheres, *B. subtilis* spores are hydrophobic in nature and possess negative zeta potential (Ahimou et al, 2001). Compared with *Cryptosporidium*, *B. subtilis* spores are significantly smaller in size. Thus, *B. subtilis* spores can be used as a conservative surrogate for *Cryptosporidium* if the surface characteristics of the filtration media dictate removal performance.

CONCLUSION

The two tested POU devices achieved similar and virtually complete removal of microbiological contaminants including *E. coli* (> ~ 6 logs), *B. subtilis* spores (> ~ 5–6 logs), MS2 bacteriophage (> ~ 6 logs), and *Cryptosporidium* (> 8.8–8.9 logs), whereas PSL beads were much less effectively removed than any of the microorganisms. The log removals of PSL beads by filters 1 and 2 were 3.14 and 3.56, respectively. It is difficult to explain the significantly reduced performance efficiency of the PSL beads compared with any of the actual microorganisms that were tested. Neither size exclusion nor apparent surface charges of the beads correlated with their relatively poor performance.

The PSL bead tests and the *Cryptosporidium* tests were conducted using a membrane to filter the effluent to capture a significant fraction of the influent challenge organisms. The *B. subtilis* tests were conducted by collecting effluent grab samples (which allowed some organisms to be discharged with the effluent) and estimating by ratio the number of organisms in the effluent. To remove any possible bias stemming from the difference in sampling methodology for the different challenge contaminants, one *B. subtilis* test was conducted to incorporate postinjection membrane sampling (instead of collecting effluent grab samples). For this test, the log removal for *B. subtilis* for the single test conducted on filter 2 was 5.95 with an insignificant number of cells (585 cells in 400 L) observed in the effluent, showing no bias attributable to the different sampling methodologies.

The two point-of-use devices demonstrated significantly greater removal efficiency for *Cryptosporidium* than did polystyrene latex beads or *Bacillus subtilis* spores.

On the basis of the experimental results in the test systems (which incorporated frank surface charge interaction processes), PSL beads proved a very conservative surrogate for *Cryptosporidium* oocyst removal to the point of not being particularly sensitive to likely differences between POU units that would use those mechanisms. For

TABLE 11 Expected EPM and zeta potential of microorganisms/surrogates at the operating condition of this study (pH 6.5–8.0)

Microorganisms/Surrogates	Size—*μm*	EPM—*μm cm V^{-1} s^{-1}*	Zeta Potential—*mV*
Cryptosporidium parvum oocysts	4 to 6	–1.30 to –1.50 (Lytle et al, 2002)	–16.0 to –17.0 (Lytle et al, 2002)
			–1.50 to –12.5 (Dai & Hozalski, 2003)*
Bacillus subtilis† endospores	~ 1.2	–1.40 to –1.55 (Lytle et al, 2002)	–15.0 to –22.0 (Ahimou et al, 2001)‡
PSL beads	~ 3	NA	–7.4 to –50.2 (Dai & Hozalski, 2003)*

EPM—electrophoretic mobility, NA—not available, PSL—polystyrene latex

*Tests were conducted with different concentrations of calcium.
†ATCC 9372, American Type Culture Collection, Manassas, Va.
‡*Bacillus subtilis* spore ATCC 7058 (American Type Culture Collection, Manassas, Va.) was used for the experiments.

other noncharged devices, PSL beads may still be useful because the analytical methods are much simpler and subject to less system error, compared with *Cryptosporidium* analysis; nevertheless, the degree of conservatism in their performance should be considered.

The experimental results of the evaluated test systems demonstrate *Bacillus subtilis* spores to be a more effective conservative surrogate for *Cryptosporidium* oocyst removal.

The experimental results of the evaluated test systems demonstrated *B. subtilis* spores to be a more reasonable and effective conservative surrogate for *Cryptosporidium* oocyst removal. The spores are easy to separate and quantify. The potential use of the aerobic spores as a surrogate for oocysts appears to be a better and more realistic choice than PSL beads for separation systems that function predominantly by electrostatic charge interaction treatment mechanisms involving surface characteristics of the treatment media and the organisms. Therefore, if the treatment mechanism relies on surface charge to effect removal, *B. subtilis* spores constitute a better surrogate for *Cryptosporidium* oocysts for demonstration of performance. The analytical methods for *B. subtilis* are simpler than those for *Cryptosporidium* and PSL beads and are subject to less system error. In systems that operate by combinations of charge and size exclusion mechanisms, including coagulation and filtration, *B. subtilis* may be the surrogate of choice, as has been demonstrated in lime softening processes in operating water supplies (Cornwell et al, 2003). For the reasons cited here, it may be postulated that situations involving groundwater transport of oocysts in soils that contain substantial levels of cations (e.g., karstic limestone aquifers) might also be better simulated by *B. subtilis* spores than by PSL beads.

ACKNOWLEDGMENT

Any opinions expressed in this article are those of the authors and do not necessarily reflect the official posi-

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Date of submission: 01/03/08
Date of acceptance: 05/09/08

FOOTNOTES

[1]KX Matrikx®, KX Technologies, Orange, Conn.
[2]Clorox-Brita, Clorox Co., Oakland, Calif.
[3]GUR 2122®, Tycona, Florence, Ky.
[4]TWEEN® 20, Sigma-Aldrich, St. Louis, Mo.
[5]Raven Labs, Omaha, Neb.
[6]BioVir Laboratories, Benicia, Calif.
[7]ATCC 15222™, American Type Culture Collection, Manassas, Va.
[8]Fluoresbrite® Plain YG, Polysciences Inc., Warrington, Pa.

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