Evaluation of an environmentally sustainable UV-assisted water treatment system for the removal of *Bacillus globigii* spores in water

R. G. Silva, J. Szabo, V. Namboodiri, E. R. Krishnan, J. Rodriguez and A. Zeigler

ABSTRACT

Development of greener water treatment technologies is important for the production of safe drinking water and water security applications, such as decontamination. Chlorine assisted disinfection is common and economical, but can generate disinfection byproducts (DBPs) that may be of health concern. DBPs are formed due to the reaction of chlorine with naturally occurring organic and inorganic substances in water. Currently, various innovative technologies are being developed as alternative approaches for preventing DBPs during water treatment. In this study, we evaluated the effectiveness of a novel combination of high efficiency flow filtration and UV disinfection treatment system for the removal of *Bacillus globigii* (*B. globigii*) spores in water. The filtration system consists of a charged membrane filter (CMF) that not only helps to remove suspended particles but also reduces the impact of other impurities including bio organisms. In order to get most performance details, the CMF was evaluated at clean, half-life, and end of life (EOL) conditions along with 100% UV transmittance (UVT). In addition, the effectiveness of the UV system was evaluated as a stand alone system at 100% and 70% EOL intensity. The study was conducted at the US EPA's Test and Evaluation (T&E) Facility in Cincinnati, OH, using *B. globigii,* a surrogate for *B. anthracis* spores. This non-chemical environmentally-friendly CMF/UV combination system and the stand alone UV unit showed greater than 6.0 log removal of *B. globigii* during the tests.

Key words | *B. globigii*, disinfection, filtration, green technology, membrane, UV

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INTRODUCTION

In both developing and industrialized nations, a growing number of contaminants are entering water supplies from human activities. Annually, millions of people die from diseases due to consumption of contaminated water (Hilborn *et al.* 2013). A leading cause of water-related human death is related to intestinal parasitic infections and diarrheal diseases caused by waterborne bacteria and enteric viruses (CDC 2012). In addition, the events of September 11, 2001, in the US have raised concerns about the potential for intentional contamination of water supply systems using biological agents, as have natural disasters. These public health and environmental concerns have driven efforts to treat water sources, ensuring that the pathogens are removed and water sources are safe.

Although chlorination continues to be the most utilized disinfection treatment, the production of carcinogenic disinfection by-products (DBPs) and safety concerns with transporting, storage, and handling of chlorine gas have led to the development of alternate disinfection technologies (Bull *et al.* 2011). In addition, disinfection or inactivation of

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some pathogenic biological contaminants through chlorination is ineffective at typical drinking water treatment disinfectant concentrations and contact times (Kotecha *et al.* 2013). As a result, stakeholders are increasingly looking into new technologies or combinations of technologies for efficient removal of chemical and biological contaminants from drinking water systems. Sequential disinfection systems, such as combined UV/chlorine or ozone/chlorine, are being used by many drinking water treatment utilities. UV and ozone combined systems are very effective for disinfection compared with free chlorine or monochloramine (WHO 2004; Sun *et al.* 2016). Free or combined chlorine disinfection systems can provide a residual in distribution systems with low levels of regulated DBPs (Shannon *et al.* 2008). However, changing disinfection technologies have raised new concerns because viruses, such as adenovirus, although effectively controlled by ozone, are resistant to both conventional UV dose and combined chlorine disinfection (WHO 2004). Moreover, ozone can form bromate ion, a carcinogen, if bromide ion is present in the feed water. Combined chlorine disinfection can form other unregulated DBPs, for example, haloacetonitriles and iodoacetic acid, that may be more toxic and carcinogenic than the DBPs associated with free chlorine (Krasner *et al.* 2006; Muellner *et al.* 2007; Dotson *et al.* 2012). The implementation of a combination system, such as filtration and UV disinfection, while overcoming the aforementioned adverse effects, is expected to become more wide spread because of an EPA water treatment rule and guidance released in 2007 (US EPA 2006a). Thus, there is a need for research to be conducted to identify robust new methods of treating water at lower cost with less energy while minimizing the use of chemicals for environmental protection.

In this study, a non-chemical based, zero backwashing, environmentally sustainable combined system including a charged membrane filter (CMF) cartridge (Waterline Technology, Mansfield, OH) and a high dose UV unit (Aqua Treatment Services, ATS, Mechanicsburg, PA) was evaluated for the treatment of Bacillus globigii (*B. globigii*) in water. *B. globigii,* a surrogate for *B. anthracis* spores, has been widely used as a biological warfare simulant for tracking water or air contamination because *B. globigii* generally mimics the physical properties of the real biological agent without causing adverse health effects.

METHODS AND MATERIALS

Description of the CMF-cartridge component

The one-end open CMF cartridge (RC-CMF-4524-20) consists of three layers of pleated materials with 1.85 square meters of surface area. The outer 5 μm glass fiber pre-filter layer helps to remove coarser particles. The interim and final electro-adsorptive sub-micron layers consist of Nano Alumina Fibers grafted to glass structures with a Zeta potential of approximately 51 millivolts, and are made to remove finer particles and microbial contaminants. To maximize contaminant removal efficacy, approximately 400 layers of 0.8 mm thick Nano Alumina structures are randomly dispersed to create a tortuous flow pattern in the sub-micron layers. The CMF stand alone component is rated for 1,034 kilopascal (kPA) operating pressure, 93°C water temperature, and terminal pressure differential of 483 kPa. When the CMF component is combined with the UV treatment component, the operating pressure and water temperature need to be reduced to 862 kPa and 46.5°C, respectively, to match the UV specifications. The design flow rate for this filter is 76 liters per minute (Lpm) with a maximum operational pressure differential (ΔP) of 241 kPa. The initial ΔP of the clean CMF is approximately 41 kPa. The CMF cartridge is easily replaceable, and the lifespan is generally dependent on the water quality parameters, particularly turbidity levels.

The outside/in flow, single open end CMF cartridge with an exclusive dual O-ring bottom seal design was mounted into a CMF-4524-20 stainless steel filter vessel, which contains the female mating component to provide the integral seal. Prior to use, each RC-CMF-4524-20 cartridge was tested using a reverse bubble point test by the manufacturer for non-destructive performance, as outlined in the EPA Membrane Filtration Guidance Manual (US EPA 2006b).

Description of the UV component

The ATS-186 K UV disinfection system operates at 254 nm wavelength in a closed vessel and uses four low pressure, high output amalgam lamps. The UV reactor vessel includes a set of UV lamps and baffles spaced along the vessel, which

Table 1 | UVT sensor and monitor percent readings for individual lamp setting

UV lamp number	Lamp reading (mW)	Probe temperature (°C)	UVT sensor (%)
1	7.816	23	73
2	1.235	23	11
3	2.149	23	20
4	1.212	23	11

generate a helical flow of the water flowing along the UV lamps to enhance exposure of microorganisms carried in the water to UV radiation. The rated minimum UV dose of the system is 186 mJ/cm^2 at 76 Lpm flow rate through the disinfection chamber at the end-of-life (EOL) 70% UV transmittance (UVT) settings. The recommended EOL of the unit is 12,000 hours. As required by the EPA UV Disinfection Guidance Manual (UVDGM), a pre-calibrated UV sensor is fitted into the system to register the intensity and real-time visual indication of output as a percentage (US EPA 2006c). The individual UV lamp output within the reactor is presented in Table 1 as required by the EPA UVDGM. The ATS-186 K UV system has been independently validated by 3rd party Carollo Engineers, PA, as per UVDGM to provide 4 log reduction of *B. pumilus*, a surrogate for Adenovirus.

Experimental approach

The CMF and UV components were installed to test the system in either a combined mode or stand alone mode (Figure 1). The CMF component was evaluated at clean, half-life, and EOL conditions along with the UV component at 100% UVT. The UV component was also evaluated as a stand alone system at clean (100%) and EOL (70%) conditions as required by the UVDGM (US EPA 2006c). The combined CMF/UV mode and stand alone mode test conditions are presented in Table 2. In each combined test condition, a new CMF cartridge was placed in the CMF-4524-20 stainless steel filter vessel, and the effluent from the CMF component was passed into the UV component as influent. As mentioned in the description of the CMF-cartridge component section, the CMF in clean condition has a ΔP of approximately 41 kPa. To create the CMF half-life (ΔP = 117 kPa) and EOL (ΔP = 241 kPa) conditions,

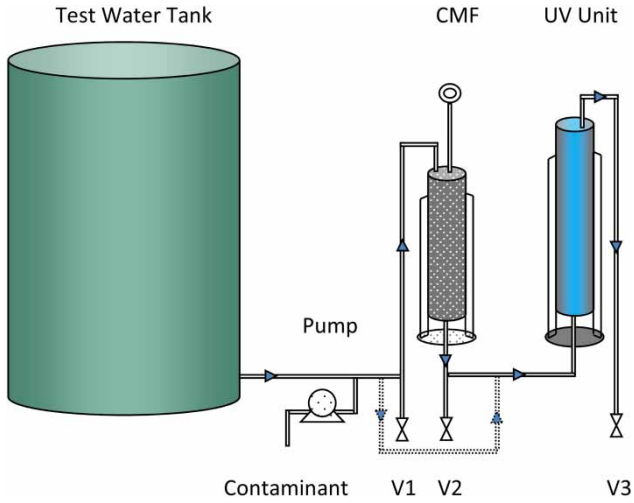

Figure 1 | Schematic of experimental set-up. V1, CMF influent sampling valve; V2, CMF effluent/UV influent valve; V3, UV effluent valve.

Arizona Road Dust (ARD)-blended water (approximately 6 NTU) was passed through the CMF according to the National Sanitation Foundation/American National Standards Institute (NSF/ANSI) Protocol 53 (NSF 2007) while monitoring ΔP using an online Monarch Instruments Data Chart 2000 paperless recorder. Based on the turbidity level, ARD-blended water was passed through the CMF cartridge for about 45 and 90 minutes to achieve half-life (50% block) and EOL (100% block), respectively, prior to the use of the respective cartridge for each challenge test. Approximately 96% (by volume) of ARD particles were below 5 μm, of which 40% (by volume) of the particles were greater than 2.5 μm. The ARD was obtained from Powder Technologies, Inc. (Burnsville, MN). Once the respective ΔP of the CMF was achieved, the turbid water test run was terminated, and the water tank was rinsed several times to remove residual ARD and refilled with activated carbon-

Table 2 | Different test configurations for the UV-assisted treatment system

Test conditions	Description
CMF + UV	Δp ~ 41 kPa (clean CMF) + 100% UVT
CMF + UV	50% block (Half-life) or at Δp = 117 kPa + 100% UVT
CMF + UV	100% block (EOL) at Δp = 241 kPa + 100% UVT
UV only	100% (clean) UVT
UV only	70% (EOL) UVT

filtered test water. To evaluate the UV component as a stand alone system, the influent was introduced while bypassing the CMF component of the combined system. The EOL (70% UVT) of the UV component was artificially created by mixing Bio-hume™ (Agri life, Hyderabad, India), a humic acid derived from leonardite shales, into the activated carbon-filtered test water to lower influent water UVT. A ratio of 25 g of humic acid to 1,000 L of activated carbon-filtered water was required to obtain test water.

The removal efficacy of *B. globigii* was used as an indicator to evaluate the combined CMF/UV treatment system as well as the UV stand alone component. A stock of *B. globigii* cells was grown in generic spore medium (8 g nutrient broth, 40 mg $MnSO_4$, and 100 mg $CaCl_2$ in 1 L deionized water) (Coroller *et al.* 2001; Szabo *et al.* 2007), for 5 days at 35 °C in a shaking (145 rpm) incubator, and the concentration was determined by the direct spread plate standard method. A sub-sample of the purified spores was heat-shocked and analyzed to determine the exact spore concentration. The *B. globigii* injection suspension was prepared by mixing 300 mL of stock (10^9/mL) in 700 mL of 0.01% Tween® 20 (Sigma-Aldrich, St Louis, Missouri) to achieve a target concentration (10^{10}/100 mL) and analyzed to verify the correct dose (Table 3). The percent *B. globigii* recovery of the injected stock solution was within the acceptable range of 85–115%. *B. globigii* suspension was injected at 40 mL/min for 25 minutes using a peristaltic pump into the water loop, in which test water was running at a flow rate of 76 Lpm to achieve a target influent *B. globigii* concentration of 10^6–10^7/100 mL. Test water was either clean activated carbon-filtered water or Bio-Hume blended activated carbon-filtered water, based on the experimental conditions. Both influent and effluent water samples were collected for CMF and UV stand alone components at 10 and 20 minutes to evaluate the system efficacy. When the combined CMF and UV system was tested, the CMF effluent sample was considered as the UV influent sample based on the system setup. In each test, a duplicate influent sample was collected at 20 minutes to verify accuracy. A small sample of injection suspension was also collected to verify the accuracy of the calculated value. Samples were analyzed to determine *B. globigii* concentration using the same method used to analyze stock solutions as mentioned above. Effluent *B. globigii* log concentrations were subtracted from influent *B. globigii* log concentrations to obtain log removal value (LRV), as an indication of the system efficacy.

Aliquots of test water samples were analyzed using the Hach (Hach Company, Loveland, CO) Method 8021 to determine free chlorine concentration. Metal concentrations (Fe and Mn) were measured using a Perkin Elmer (Waltham, MA, USA) 2300 DV inductive coupled plasma-optical emission spectrometer (ICP-OES). The total hardness was measured using EDTA titrimetric method. Test water sample pH and turbidity were determined using a temperature compensated pH probe and turbidimeter with scattered-light detectors, respectively. Quantitative determination of test water color (water color index) was carried out by colorimetric analysis based on platinum salt and cobalt chloride reference solution mixture. Total dissolved solid concentrations were determined using the glass-fiber filtration technique. Each parameter was determined using duplicate test water samples under each test condition.

Collimated beam test

The effect of UV light on *B. globigii* viability was separately determined using a collimated beam test apparatus that utilized a low pressure UV lamp. This method involves exposing a known concentration of stock to a known UV intensity from the collimated beam apparatus over a specified time interval, as described in the UVDGM (US EPA

Table 3 | Verification of influent suspension *B. globigii* concentrations

Activity	Clean CMF + 100% UV	50% block CMF + 100% UV	100% block CMF + 100% UV	100% UV	70% UV
Expected stock *B. globigii* log conc./100 mL	10.39	10.25	10.65	10.65	10.55
Measured stock *B. globigii* log conc./100 mL	9.95	9.92	10.25	9.07	9.07
Percent recovery	96	97	96	85	86

2006c). A range of UV doses can be generated by varying the exposure time. The respective UV dose delivered for *B. globigii* inactivation at each time was calculated on the basis of UV intensity, the exposure time, and other apparatus-related constant factors. After each specified time of exposure, each sample was analyzed using the direct spread plate standard method to determine the viability, expressed as log inactivation, of the *B. globigii* stock. The function of UV dose in relation to *B. globigii* log inactivation was then established under controlled collimated beam test conditions (Figure 2). One collimated beam test was conducted to determine the viability of *B. globigii* independently when exposed to UV light using stock solution during the full-scale reactor experiment.

RESULTS AND DISCUSSION

The UV sensor monitored each lamp positioned at various angles throughout the UV reactor. The reading of Lamp 1 was the highest, as it was positioned at the closest distance from the UV sensor. The positions of Lamp 2 and Lamp 4 were almost identical, and thus the readings for these two lamps were identical. The reading of Lamp 3 was in the middle range. The UV irradiation dose range monitored was 100% ± 2% for all of the tests except the 70% UV test (Table 1).

Water quality parameters used for the study are presented in Table 4 for all five tests. Of the measured parameters, free chlorine, pH, total dissolved solid (TDS), hardness, and turbidity values were consistent among the five tests. The data revealed that iron and color increased slightly in the test water for the CMF and UV-blocked test conditions. Although the test water tank was flushed twice with clean activated carbon-filtered water after using ARD-blended water to block the CMF cartridge, some residual ARD could have resulted in these higher concentrations (Table 4). The slightly higher iron and color values for the 70% UV condition could be a result of Bio-hume, which was used to block the UV system. Free chlorine (Cl_2) levels were negligible across all the tests, and neither iron nor manganese was found in the test water. The pH values were marginally basic, and hardness values varied from 130 to 160 mg/L. In general, water quality parameters of the test water were similar to those recommended in the NSF/ANSI 53 Protocol (NSF/ANSI 2007).

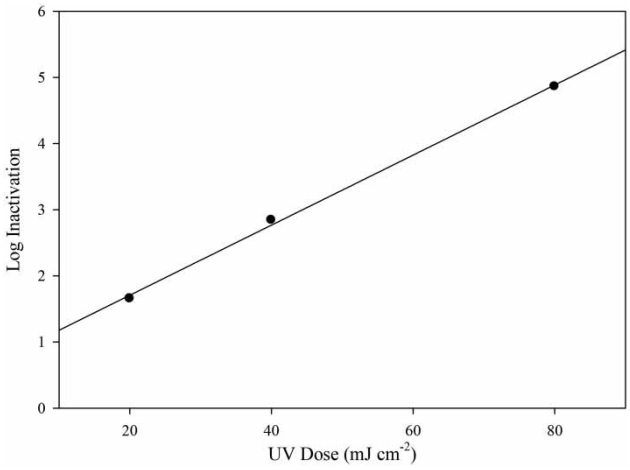

Figure 2 | UV Dose Response of *B. globigii* based on bench-scale Collimated beam test.

Experimental results revealed that the target influent *B. globigii* concentration of 10^6–10^7/100 mL was approximated in all test conditions (Table 5). Based on the influent *B. globigii* application rate, the CMF component showed 2.5 to 3.2 $\log_{10}$ removal of *B. globigii* for the tested CMF conditions. Results revealed that the impact of CMF on *B. globigii* $\log_{10}$ removal after 10 minutes was insignificant. The remainder of the *B. globigii* was inactivated by the UV component of the combined system. In the stand alone mode, the UV component showed more than 6.0 $\log_{10}$ reduction of *B. globigii* even at the 70% block condition at similar *B. globigii* application rates of 10^6–10^7/100 mL. Based on *B. globigii* influent application rates, the UV component of the combined system reduced more than two-fold of the *B. globigii* contaminant when compared to the CMF. Even though combining UV treatment with the CMF system was shown to enhance reduction of *B. globigii*, UV treatment alone may prove effective for *B. globigii* removal in the current study (Table 5). Although UV stand alone mode resulted in similar $\log_{10}$ reduction, it is important to note that UV systems are commonly combined with an appropriate pre-treatment mechanism to ensure desirable turbidity levels and influent water quality. The CMF system was documented to reduce turbidity of water prior to entering the UV system. In general, the data suggests that the CMF/UV combined system can be used to remove *B. globigii,* a surrogate *of B. anthracis,* or similar bacterium

Table 4 | Water quality parameters measured in the test water during the *Bacillus globigii* tests

Test conditions	Free chlorine (mg L^{-1})	pH	Iron (mg L^{-1})	Manganese (mg L^{-1})	Total dissolved solids (mg L^{-1})	Hardness (mg L^{-1})	Color (Pt-co unit)	Turbidity (NTU)
CMF clean + 100% UV	<MDL	7.99 (±0.1)	<MDL	<MDL	NA	138 (±2.8)	8 (±0.0)	0.61 (±0.0)
CMF 50% block + 100% UV	0.04 (±0.1)	8.05 (±0.1)	27.5 (±14.7)	<MDL	314 (±8.2)	140 (±0.0)	16 (±0.7)	0.68 (±0.1)
CMF 100% block + 100% UV	0.03 (±0.0)	8.17 (±0.0)	152 (±5.0)	1.7 (±0.6)	300 (±4.8)	160 (±0.0)	13 (±2.1)	0.65 (±0.0)
100% UV	<MDL	8.31 (±0.0	<MDL	<MDL	286 (±8.2)	130 (±28.3)	10 (±0.1)	0.33 (±0.0)
70% UV	0.16 (±0.0)	7.82 (±0.0)	2.1 (±0.1)	<MDL	278 (±5.1)	145 (±7.1)	17 (±0.7)	0.60 (±0.1)

<MDL = below method detection limit. MDLs for free chlorine = 0.02 mg L^{-1}, Fe = 0.017 mg L^{-1}, Mn = 0.002 mg L^{-1}, TDS = 2 mg L^{-1}, Hardness = 1 mg L^{-1}, Color = 2 Pt-Co unit, and Turbidity = 0.1 NTU.
NA = Not analyzed.
Data in parentheses are ± standard deviation, $n = 2$.

effectively. Results also revealed that operation of either the CMF/UV combined system or UV stand alone mode resulted in complete removal of *B. globigii* regardless of the treatment condition.

Rose & Rice (2014) cited that *B. globigii* is a commonly used surrogate for *B. anthracis*, and *B. globigii* is more resistant to free available chlorine than the virulent *B anthracis* if pH is maintained between 6.2 and 8.6 in buffered water. Even though the mode of inactivation was different, an equivalent resistance of *B. globigii* in the current study with test water in the pH range of 7.8–8.3 was possible.

Previous studies have shown that UV doses of 40–45 mJ/cm^2 inactivated 1 to 2 $\log_{10}$ of *B. anthracis* spores (depending upon strain) in non-turbid water, and combining ozone treatment with UV treatment was shown to enhance reduction of *B. subtilis* spores by 33% (Jung *et al.* 2008) and may prove effective for *B. anthracis* spores, as well. Other researchers reported that inactivation can vary with the growth media or physiological conditions of the cells when sporulation occurs (Rose & O'Connell 2009). Mamane-Gravetz & Linden (2005) also noted that the dose-response curve tailed off at a UV dose greater than 60 mJ/cm^2 when *B. subtilis* spores were challenged with UV light. Based on hydrophobicity and particle size testing, they found that the tailing off was due to aggregates of spores providing protection for spores within the aggregate from UV light. Spores that are more hydrophobic

Table 5 | *Bacillus globigii* (*B. globigii*) measured concentrations and system LRVs

		***Bacillus globigii* concentration per 100 mL**			**LRV**		
Test conditions	**Test samples**	**CMF influent**	**CMF effluent/ UV influent**	**UV effluent**	**CMF**	**UV**	**Total**
CMF clean + 100% UV	*B. globigii* conc. at 10 min	3.90E + 06	4.00E + 03	0.00E + 00	2.99	3.60	6.59
	B. globigii conc. at 20 min	4.30E + 06	1.10E + 04	0.00E + 00	2.59	4.04	6.63
CMF 50% block + 100% UV	*B. globigii* conc. at 10 min	6.20E + 06	3.50E + 03	0.00E + 00	3.25	3.54	6.79
	B. globigii conc. at 20 min	4.80E + 06	3.00E + 03	0.00E + 00	3.20	3.48	6.68
CMF 100% block + 100% UV	*B. globigii* conc. at 10 min	5.80E + 06	2.00E + 05	0.00E + 00	1.46	5.30	6.76
	B. globigii conc. at 20 min	1.20E + 07	3.00E + 04	0.00E + 00	2.60	4.48	7.08
100% UV	*B. globigii* conc. at 10 min		2.30E + 06	0.00E + 00		6.36	6.36
	B. globigii conc. at 20 min		4.10E + 06	0.00E + 00		6.61	6.61
70% UV	*B. globigii* conc. at 10 min		4.10E + 06	NR		NR	NR
	B. globigii conc. at 20 min		4.60E + 06	0.00E + 00		6.66	6.66

Relative standard deviation is less than 5%.
NR = not reported.
0 values used for log calculations and indicate below the detection.

demonstrate more aggregation, and their UV dose-response curves are more likely to tail off at the higher UV dose applications (Mamane-Gravetz & Linden 2005). In another study, Nicholson & Galeano (2003) found no difference in UV inactivation between *B. anthracis* Sterne spores and two *B. subtilis* spore strains. In the current study, only 25–30 mJ/cm^2 UV dose was required for 1 $\log_{10}$ removal of *B. globigii*. The low dose requirement in the current study could be a result of high UV exposure of microorganisms due to the unique internal water flow baffling design of the ATS-186 K UV system. The aforementioned system features and *B. globigii* suspension characteristics may have resulted in rapid reduction of test organisms.

Inactivation of *B. globigii* based on bench-scale collimated beam test

The results from the collimated beam test revealed a linear relationship between the log inactivation and UV dose. The maximum *B. globigii* concentration used for the collimated beam test was 10^5/ mL, and the dose response of *Bacillus globigii* showed that a UV dose of 80 mJ/cm^2 was required to inactivate the maximum concentration, 5 $\log_{10}$, of *B. globigii* (Figure 2). Based on the collimated beam test results, a 16 mJ/cm^2 UV dose per log inactivation can be approximated.

CONCLUSIONS

The LRVs of *B. globigii* achieved by the clean, half-life and EOL CMF were similar regardless of blockage and pore path reduction at half-life and EOL. Since average length and width of *B. globigii* ranged from 0.9–1.9 μm and 0.6–0.8 μm, respectively, electro-adsorptive sites of the CMF with about 3–5 μm pore size appeared to be the driving force for *B. globigii* contaminant removal mechanism in the CMF.

The overall challenge tests showed that the performance of the CMF/UV combined system and the stand alone UV component can achieve greater than 6.0 $\log_{10}$ removal of *B. globigii*. Tests with other types of microbial contaminants are planned to evaluate the UV-assisted treatment system for broader applications.

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DISCLAIMER

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