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technical BRIEF
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Decontamination of Drinking Water Infrastructure Contaminated with *Bacillus* Spores on Iron and Cement-mortar Surfaces

INTRODUCTION

This study examines the effectiveness of decontaminating corroded iron and cement-mortar coupons that have been contaminated with spores of *Bacillus atrophaeus* subsp. *globigii* (*B. globigii*), which is often used as a surrogate for pathogenic *B. anthracis* (anthrax) in disinfection studies.

Bacillus spores are persistent on common drinking water material surfaces like corroded iron, requiring physical or chemical methods to decontaminate the infrastructure. In the United States, free chlorine and monochloramine are the primary chemical disinfectants used by the drinking water industry to inactivate microorganisms.

Flushing is also a common, easily implemented practice in drinking water distribution systems, although large volumes of contaminated water needing treatment could be generated.

Identifying readily available alternative disinfectant formulations for infrastructure decontamination give water utilities options for responding to specific types of contamination events.

In addition to presenting data on flushing alone, which demonstrated the persistence of spores on water infrastructure in the absence of high levels of disinfectants, data on acidified nitrite, chlorine dioxide, free chlorine, monochloramine, ozone, peracetic acid, and followed by flushing are provided. [2]

DISINFECTANT USE IN U.S. WATER UTILITIES

In the most recent American Water Works Association survey of disinfectant use [1], 63% of U.S. water utilities reported using chlorine gas; 30% used chloramine; 8% used chlorine dioxide; and 9% reported using ozone. Some utilities used multiple forms of chlorine or combinations of chemical and physical disinfection practices.

In addition to investigating chlorine and chloramine, U.S. EPA's Homeland Security Research Program chose ozone and chlorine dioxide, used at water treatment plants for disinfection and taste and odor control. Because they are strong oxidants, their reactivity may limit their application to small areas in a distribution system.

Two other disinfectants, not reported in use by water utilities, were used to see if they were candidates for efficacious spore removal. Peracetic acid (PAA) is used in the food and beverage industry, as well as the medical device industry for cleaning equipment. It is gaining acceptance as an effective decontaminating agent for drinking water. Acidified nitrite, which can be formulated from common reagents, is less reactive on infrastructure than oxidant disinfectants and could potentially be effective against spores in water.

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INVESTIGATION [2]

Decontamination of two common drinking water infrastructure surfaces contaminated with *B. globigii* spores was evaluated using six disinfectants, plus flushing with water.

The study was conducted in a drinking water distribution system simulator (DSS). The DSS consisted of 23 m (approximately 75 ft) of 15 cm (approx. 6 in) diameter polyvinyl chloride (PVC) pipe connected in a rectangular shape to an in-line recirculation tank. Total DSS volume was 832 L (approximately 220 gal). A 3780 L (approximately 1000 gal) feed tank supplied tap water from the Greater Cincinnati Water Works (GCWW) to the DSS. For details, see [3].

Two types of coupons (6.5 cm^2) represented the infrastructure surfaces: corroded iron cut from a water main and cement-mortar.[1] The coupons were conditioned and allowed to form biofilm in the DSS for one month prior to contamination with spores.

For all coupons, unless adjusted, baseline conditions were: pH's ranging from 8.4 to 8.6 and free chlorine levels[2] during all disinfectant and flushing treatments ranging from 0.9 to 1.1 mg/L. Water temperature fluctuated between 25 ° and 30 °C. Water was kept stagnant for the disinfectant tests.[3] All disinfectant treatments were followed by flushing at 0.3 m/sec (1 ft/sec). Table 1 lists disinfectants and conditions.

Table 1. Disinfectants Tested: Concentrations, pH Levels, and Flushing (25 ° to 30 °C)

Disinfectant	1st Treatment Condition		2nd Treatment Condition		3rd Treatment Condition		Flushing (m/sec)
Acidified Nitrite	0.1 mol/L	adjusted to pH 2	0.1 mol/L	adjusted to pH 3			0.3 *
Chlorine Dioxide	5 mg/L	pH 8.4 – 8.6	25 mg/L	pH 8.4 – 8.6			0.3
Free Chlorine	5 mg/L	adjusted to pH 8	25 mg/L	adjusted to pH 7	25 mg/L	adjusted to pH 8	0.3
Monochloramine	25 mg/L	pH 8.4 – 8.6					0.3
Ozone	2.0 mg/L	pH 8.4 – 8.6					0.3 +
Peracetic acid	25 mg/L	pH 8.4 – 8.6					0.3

* 0.3 m/sec is 1 ft/sec + flow of 314 L/min during disinfectant testing

[1] Made using ANSI/AWWA method C104/A21.4-08. Standards for Cement-Mortar Lining for Ductile-Iron Pipe and Fittings. Effective September 23, 2008, AWWA. Denver: American Water Works Association.
[2] Free chlorine was also tested at two pH levels and two concentrations.
[3] During the ozone treatment (2 mg/L), decontamination occurred for 12 hours in the presence of flow at 314 L/min before flushing.

RESULTS

In the current study, flushing was tested at 0.5 m/sec (1.7 ft/sec) without disinfectants. This reduced adhered spores by 0.5 $\log_{10}$ from iron and 2.0 $\log_{10}$ from cement-mortar.

More of the disinfectant treatments on contaminated cement-mortar coupons reduced spores to undetectable levels than on iron. However, after 6 hours of treatment, chlorine dioxide (25 mg/L) did reduce spores to undetectable levels on iron coupons.

Acidified nitrite (pH 2, 0.1 mol/L) was the only tested disinfectant that performed better on iron coupons than on cement-mortar. There were undetectable spore levels on the iron surfaces during the flushing phase (at 0.3 m/sec (0.1 ft/sec)) that followed the disinfection treatment.

Overall, chlorine dioxide was the best performing disinfectant on both surfaces with >3.0 $\log_{10}$ removal from cement-mortar at 5 and 25 mg/L at 2 hours of treatment. For acidified nitrite and peracetic acid, there were no test conditions under which spores were reduced to undetectable levels on cement-mortar coupons.

Table 2 summarizes the effective disinfectant concentrations and conditions.

Table 2. Disinfectants Were Observed to Reduce the Number of Spores to Undetectable Levels on Cement-mortar Coupons (25° to 30°C) under these Times and Conditions

Conditions	Disinfectants				
	Chlorine Dioxide		Free Chlorine (pH 7)	Monochloramine	Ozone
Concentration (mg/L)	**5**	**25**	**25**	**25**	**2**
Time (hours)	**2**		**18**	**18**	**1**

Tables 3 (cement-mortar) and 4 (iron) present all the experimental results for disinfectant and flushing treatments.

Table 3. Surface Concentrations of Surviving *Bacillus globigii* Spores (cfu/cm^2) with Decontaminant Effectiveness (log reduction) in Parenthesis for Various Decontamination and Flushing Durations on Cement-mortar Coupons (6.5 cm$^{2)}$ (25° to 30°C)

Experimental Phase	Time (hr)	Disinfectant with water pH (when adjusted) and concentration									
		Free Chlorine			Chlorine Dioxide ‡		Monochlor-amine ‡	Acidified Nitrite		Ozone *	PAA ‡
		pH 8, 5 mg/L	pH 8, 25 mg/L	pH 7, 25 mg/L	5 mg/L	25 mg/L	25 mg/L	pH 2, 0.1 mol/L,	pH 3, 0.1 mol/L	2 mg/L	25 mg/L
Spore Injection	0 (initial)	8.5×10^4 (0.0)	4.1×10^4 (0.0)	7.9×10^4 (0.0)	5.5×10^4 (0.0)	5.2×10^4 (0.0)	4.4×10^4 (0.0)	1.9×10^4 (0.0)	1.2×10^5 (0.0)	8.0×10^4 (0.0)	2.6×10^4 (1.7)
Decontamination with Disinfectant	1	7.0×10^3 (1.1)	4.6×10^3 (1.0)	7.5×10^2 (2.0)	5.0×10^2 (2.0)	2.5×10^2 (2.3)	3.5×10^4 (0.1)	2.8×10^4 (0.0)	1.1×10^5 (0.1)	0.0 (>3.2)	7.3×10^3 (0.6)
	2	1.3×10^4 (0.8)	2.0×10^3 (1.3)	1.5×10^3 (1.7)	0.0 (>3.0)	0.0 (>3.0)	2.0×10^4 (0.3)	2.5×10^4 (0.0)	6.1×10^4 (0.3)	0.0 (>3.2)	4.8×10^3 (0.7)
	3	7.8×10^3 (1.0)	2.5×10^3 (1.2)	2.5×10^2 (2.5)	0.0 (>3.0)	0.0 (>3.0)	1.2×10^4 (0.6)	1.8×10^4 (0.0)	4.0×10^4 (0.5)	0.0 (>3.2)	3.3×10^3 (0.9)
	4	7.0×10^3 (1.1)	3.3×10^3 (1.1)	1.5×10^3 (1.7)	0.0 (>3.0)	0.0 (>3.0)	1.1×10^4 (0.6)	1.2×10^4 (0.2)	5.5×10^4 (0.3)	0.0 (>3.2)	3.3×10^3 (0.9)
	6	6.8×10^3 (1.1)	1.5×10^3 (1.4)	3.3×10^3 (1.4)	0.0 (>3.0)	0.0 (>3.0)	2.3×10^3 (1.3)	1.5×10^4 (0.1)	4.6×10^4 (0.4)	0.0 (>3.2)	1.5×10^3 (1.2)
	18	2.0×10^3 (1.6)	5.0×10^2 (1.9)	0.0 (>3.2)	0.0 (>3.0)	0.0 (>3.0)	0.0 (>2.9)	2.0×10^3 (1.0)	4.6×10^4 (0.4)	0.0 (>3.2) +	3.5×10^3 (0.9)
	22	1.5×10^3 (1.8)	2.5×10^2 (2.2)	0.0 (>3.2)	0.0 (>3.0)	0.0 (>3.0)	0.0 (>2.9)	4.3×10^3 (0.6)	7.9×10^4 (0.2)	No Data	2.8×10^3 (1.0)
Flushing at 0.3 m/sec (1 ft/sec)	24	1.4×10^3 (1.8)	5.0×10^2 (1.9)	3.8×10^2 (2.3)	0.0 (>3.0)	0.0 (>3.0)	5.0×10^1 (2.9)	0.0 (>2.6)	6.0×10^4 (0.3)	0.0 (>3.2)	1.9×10^3 (1.1)
	26	2.5×10^3 (1.5)	0.0 (>2.9)	0.0 (>3.2)	0.0 (>3.0)	0.0 (>3.0)	0.0 (>2.9)	5.0×10^2 (1.6)	3.2×10^4 (0.6)	0.0 (>3.2)	5×10^2 (1.7)
	44	5.0×10^2 (2.2)	0.0 (>2.9)	0.0 (>3.2)	0.0 (>3.0)	0.0 (>3.0)	0.0 (>2.9)	1.5×10^3 (1.1)	5.4×10^3 (1.4)	0.0 (>3.2)	5×10^2 (1.7)

‡ pH ranging from 8.4 to 8.6; free chlorine ranging from 0.9 to 1.1 mg/L. * Flow 314 L/min before flushing. + Data is from 12 hr from start of treatment.

Table 4. Surface Concentrations of Surviving *Bacillus globigii* Spores (cfu/cm2) with Decontaminant Effectiveness (log reduction) in Parenthesis for Various Decontamination and Flushing Durations on Corroded Iron Coupons (6.5 cm^2) (25° to 30°C)

Experimental Phase	Time (hr)	Disinfectant with water pH (when adjusted) and concentration									
		Free Chlorine			Chlorine Dioxide ‡		Monochlor-amine ‡	Acidified Nitrite		Ozone *	PAA ‡
		pH 8, 5 mg/L	pH 8, 25 mg/L	pH 7, 25 mg/L	5 mg/L	25 mg/L	25 mg/L	pH 2, 0.1 mol/L	pH 3, 0.1 mol/L	2 mg/L	25 mg/L
Spore Injection	**0 (initial)**	2.2×10^{5} (0.0)	2.5×10^{5} (0.0)	1.7×10^{5} (0.0)	2.2×10^{5} (0.0)	2.0×10^{5} (0.0)	1.9×10^{5} (0.0)	2.2×10^{5} (0.0)	7.1×10^{5} (0.0)	2.0×10^{5} (0.0)	1.5×10^{5} (0.0)
Decontamination with Disinfectant	**1**	1.8×10^{5} (0.1)	4.2×10^{4} (0.8)	1.5×10^{4} (1.1)	6.3×10^{4} (0.5)	2.5×10^{3} (1.9)	2.0×10^{5} (0.0)	7.0×10^{3} (1.5)	9.7×10^{4} (0.9)	2.5×10^{4} (0.9)	8.6×10^{4} (0.2)
	2	1.5×10^{5} (0.2)	3.8×10^{4} (0.8)	1.5×10^{4} (1.1)	5.6×10^{4} (0.6)	1.5×10^{4} (1.1)	2.0×10^{5} (0.0)	4.9×10^{3} (1.6)	1.5×10^{5} (0.7)	1.5×10^{4} (1.1)	7.0×10^{4} (0.3)
	3	1.3×10^{5} (0.2)	4.2×10^{4} (0.8)	1.7×10^{4} (1.0)	4.9×10^{4} (0.7)	2.8×10^{3} (1.9)	1.0×10^{5} (0.3)	4.0×10^{3} (1.7)	9.9×10^{4} (0.9)	2.0×10^{4} (1.0)	8.5×10^{4} (0.2)
	4	2.3×10^{5} (0.0)	4.1×10^{4} (0.8)	2.0×10^{4} (0.9)	3.5×10^{4} (0.8)	4.5×10^{3} (1.6)	1.1×10^{5} (0.2)	2.4×10^{3} (2.0)	1.8×10^{5} (0.6)	1.9×10^{4} (1.0)	4.7×10^{4} (0.5)
	6	1.4×10^{5} (0.2)	2.8×10^{4} (1.0)	1.7×10^{4} (1.0)	2.7×10^{4} (0.9)	0.0 (>3.6)	9.8×10^{4} (0.3)	5.2×10^{3} (1.6)	1.1×10^{5} (0.8)	1.2×10^{4} (1.2)	4.5×10^{4} (0.5)
	18	1.6×10^{5} (0.1)	2.5×10^{4} (1.0)	1.8×10^{4} (1.0)	2.5×10^{4} (0.9)	0.0 (>3.6)	2.5×10^{4} (0.9)	5.0×10^{2} (2.6)	2.7×10^{4} (1.4)	1.4×10^{4} (1.2)+	2.3×10^{4} (0.8)
	22	1.3×10^{5} (0.2)	1.6×10^{4} (1.2)	1.2×10^{4} (1.2)	2.7×10^{4} (0.9)	0.0 (>3.6)	1.7×10^{4} (1.0)	1.0×10^{3} (2.3)	1.5×10^{5} (0.7)	No Data	1.9×10^{4} (0.9)
Flushing at 0.3 m/sec (1 ft/sec)	**24**	9.8×10^{4} (0.3)	1.4×10^{4} (1.3)	9.8×10^{3} (1.2)	2.0×10^{4} (1.0)	2.5×10^{2} (2.9)	1.9×10^{4} (1.0)	0.0 (>3.6)	1.6×10^{4} (1.6)	4.5×10^{3} (1.6)	1.6×10^{4} (0.9)
	26	7.3×10^{4} (0.5)	1.2×10^{4} (1.3)	1.3×10^{4} (1.1)	1.6×10^{4} (1.1)	2.5×10^{2} (2.9)	1.7×10^{4} (1.1)	2.5×10^{2} (2.9)	1.9×10^{4} (1.6)	1.8×10^{3} (2.1)	2.1×10^{4} (0.8)
	44	8.1×10^{4} (0.4)	7.3×10^{3} (1.5)	7.3×10^{3} (1.4)	1.5×10^{4} (1.2)	5.0×10^{2} (2.6)	1.3×10^{4} (1.2)	0.0 (>3.6)	6.6×10^{3} (2.0)	2.0×10^{3} (2.0)	1.6×10^{4} (1.0)

‡ pH ranging from 8.4 to 8.6; free chlorine ranging from 0.9 to 1.1 mg/L. * Flow 314 L/min before flushing. + Data is from 12 hr from start of treatment.

The study concludes: “These data will help individuals such as incident commanders and drinking water utility personnel make informed decisions about how to decontaminate a drinking water distribution system after a biological contamination incident. Ultimately, decontamination performance along with the cost and effort of disseminating disinfectants over a sufficient area of the distribution system will dictate their use.”

REFERENCES

[1] AWWA Disinfection Systems Committee. 2008. Committee Report: Disinfection Survey, Part 1 – Recent changes, current practices, and water quality. *Journal AWWA,* 100(10):76-90.

[2] Szabo, J. G., Meiners, G., Heckman, L., Rice, E. W, and Hall, J. 2015. Decontamination of *Bacillus* spores adhered to iron and cement-mortar drinking water infrastructure in a model system using disinfectants.

[3] U.S. EPA. 2008. Pilot-scale Tests and Systems Evaluation for the Containment, Treatment, and Decontamination of Selected Materials from T&E Building Pipe Loop Equipment. Washington, D.C.: U.S. Environmental Protection Agency. EPA/600/R08/016.

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