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Decontamination of *Bacillus* Spores from Drinking Water Infrastructure with Physical Removal (Pigging)

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Decontamination of *Bacillus* Spores from Drinking Water Infrastructure with Physical Removal (Pigging)

by

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The contractor role did not include establishing Agency policy.

Table of Contents

List of Figures

Abbreviations

BWS	bulk water sample
cfu	colony forming units
CT	chlorine concentration, C, in mg/L and contact time, T, in minutes
ft	foot
hr	hour
gpm	gallons per minute
HPC	heterotrophic plate count
INL	Idaho National Laboratory
LOD	limit of detection
M	meter
min	minute
MPN	most probable number
HSRP	Homeland Security Research Program
USC	Utility Services Co., Inc.
WSTB	Water Security Test Bed

Acknowledgements

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Executive Summary

The U.S. Environmental Protection Agency's (EPA) National Homeland Security Research Center partnered with the Idaho National Laboratory (INL) to build the Water Security Test Bed (WSTB) at the INL test site outside of Idaho Falls, Idaho. The WSTB was built using an 8-inch diameter cement-mortar lined drinking water pipe that had been previously taken out of service. The pipe was exhumed from the INL grounds and oriented in the shape of a small drinking water distribution system that is 450 feet (ft) long. The WSTB can support drinking water distribution system research on a variety of topics including biofilms, water quality, sensors, and homeland security related contaminants. Since the WSTB is constructed of real drinking water distribution system pipes, research can be conducted under conditions similar to those in a real drinking water system (USEPA, 2016).

This report summarizes the results of biological decontamination experiments performed at the WSTB focused on removing *Bacillus globigii* spores adhered to the inner surface of the 8-inch water pipe. *B. globigii* spores are a non-pathogenic surrogate for *B. anthracis*, which is the causative agent of anthrax. Decontamination was undertaken with a technique known as pigging, or physical scouring of the inner pipe surface, followed by disinfection with free chlorine. Two pigging techniques were evaluated in separate experiments. First, ice pigging technology was used via a proprietary truck mounted mobile technology developed by the Utility Services Co., Inc. (USC). Ice pigging works by pumping a slurry of ice and water down the pipe. The ice in the slurry was expected to scour the inner pipe surface. The second pigging technique used a KEG® chain cutter. In this technique, water was pumped from a combination (Vactor®) truck at high flow and pressure and through a nozzle with a chain attached to it. The water flow caused the chains to spin and scour the pipe interior. Both pigging techniques were used to decontamination the 450 ft long pipe, as well as individual sections of cement-mortar lined iron pipe, and unlined iron pipe with corrosion. The results of this study indicate that the chain cutter followed by chlorination was more effective at reducing *B. globigii* spores than ice pigging followed by chlorination.

The following is a summary of the results that came from the pigging experiments performed at the INL WSTB:

- No change in the number of spores adhered to the inner pipe surface was observed after ice-pigging was conducted in the 450 ft pipe. After pigging, chlorination of the water in the pipe at an initial concentration of 52 mg/L for 24 hours (55 mg/L after 24 hours) resulted in a 1.0-log inactivation of the spores adhered to the pipe inner surface.

- Pigging with chain cutting (2,300 psi, 70 gpm) resulted in a 3.3-log reduction of the number of spores adhered to the inner surface of the 450 ft pipe. After pigging, chlorination of the water in the pipe at an initial concentration of 82 mg/L for 24 hours (31 mg/L after 24 hours) resulted in an additional 1.0-log inactivation of the spores adhered to the pipe inner surface, for a total reduction of 4.3-log.

- Chain cutter pigging (1,200 psi, 25 gpm) of an individual cement-mortar lined iron pipe section resulted in a 1.5-log reduction of the number of spores adhered to the inner pipe

surface. After pigging, chlorination of the water in the pipe at an initial concentration of 201 mg/L for 24 hours (154 mg/L after 24 hours) resulted in an additional 2.3-log inactivation of the spores adhered to the pipe inner surface, for a total reduction of 3.8-log.

- Chain cutter pigging (1,200 psi, 25 gpm) of an individual corroded iron pipe section resulted in a 3.2-log reduction of the number of spores adhered to the inner pipe surface. Chlorination of the water in the pipe at an initial concentration of 201 mg/L for 24 hours (154 mg/L after 24 hours) resulted in an additional 0.6-log inactivation of the spores adhered to the pipe inner surface, for a total reduction of 3.8-log. However, it should be noted that the initial number of adhered spores were one log higher on the iron pipe compared to the cement mortar pipe, likely due to the presence of tuberculation in the iron pipe.

In summary, the best spore removal, a 4.3 log reduction, was achieved with the combination of chain cutter pigging and chlorine treatment. However, this combination of decontamination techniques was not enough to remove all detectable spores from the pipe surface. Should this situation occur in reality, a technique like pipe lining or infrastructure replacement may need to be implemented to ensure that human exposure to spores via drinking water does not occur.

1.0 Introduction

1.1 Background

The U.S. Environmental Protection Agency's (EPA) Homeland Security Research Program (HSRP) has partnered with Idaho National Laboratory (INL) to build the Water Security Test Bed (WSTB) at INL in Idaho Falls, Idaho. The centerpiece of the WSTB is an 8-inch diameter drinking water pipe that had been taken out of service. The pipe was exhumed from the INL grounds and oriented in the shape of a small drinking water distribution system. The WSTB has been fitted with service connections, fire hydrants, and removable coupons to collect samples from the pipe interiors (USEPA, 2016).

Experiments focused on decontamination of *Bacillus globigii* spores adhered to the inner surface of the 8-inch water pipe have been conducted at the WSTB in recent years. *B. globigii* spores are a non-pathogenic surrogate for *B. anthracis*, which is the causative agent of anthrax. In one experiment using the full-scale WSTB, chlorine dioxide was used to decontaminate adhered spores. Two-log removal of *B. globigii* spores was observed with chlorine dioxide decontamination, which was less effective than anticipated based on previous pilot-scale experiments (Szabo et al, 2017; USEPA, 2016). Chlorine dioxide is a powerful disinfectant, and it was not anticipated that other common drinking water disinfectants such as free chlorine or monochloramine would be more effective. Therefore, subsequent efforts at removing adhered *B. globigii* spores were focused on physical removal, or scouring of the inner pipe surface, hereafter referred to as pigging. Two technologies were selected: ice pigging and chain cutter pigging.

1.2 Project Objective

The objective of the project was to conduct decontamination experiments at the WSTB with two pigging technologies following intentional contamination of the WSTB with *B. globigii* spores. The effectiveness of the pigging technologies at removing *B. globigii* from the inner pipe surface was evaluated.

1.3 WSTB System Description

The primarily feature of the WSTB is an 8-inch (20 cm) diameter drinking water pipe oriented in the shape of a small drinking water distribution system. The WSTB contains ports for service connections and a 15-foot (5 m) removable coupon section designed to sample the pipe interior to examine the results from contamination/decontamination experiments on the pipe wall. Figure 1 schematically depicts the main features of the WSTB.

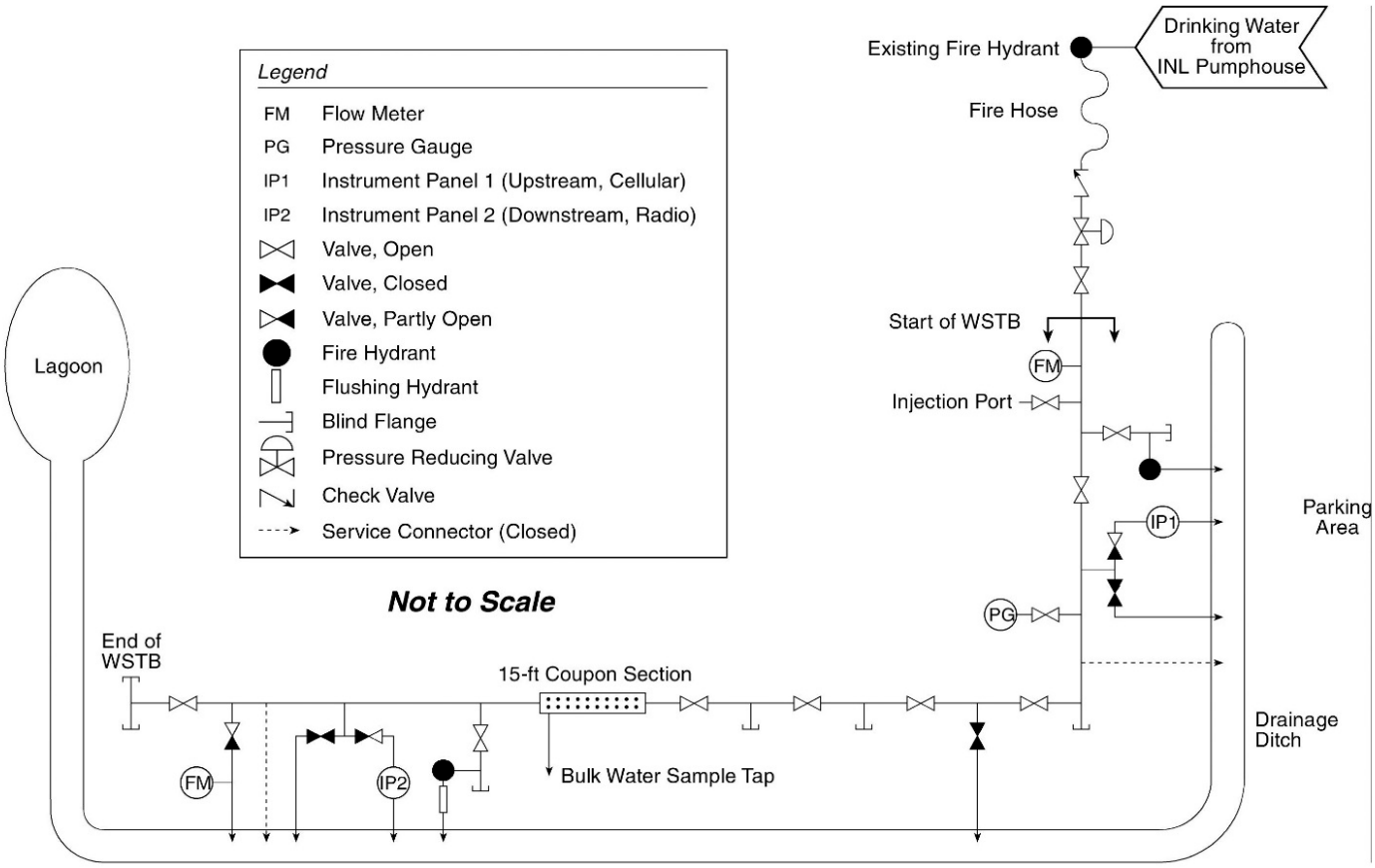

Figure 1: Schematic overview of Water Security Test Bed (WSTB).

Figure 2 shows the aerial view of the WSTB. The lower right corner shows the upstream and system inlet; the upper left corner shows the lagoon. The upstream fire hydrant was used for injecting the ice slurry and the downstream fire hydrant was used to retrieve the injected material.

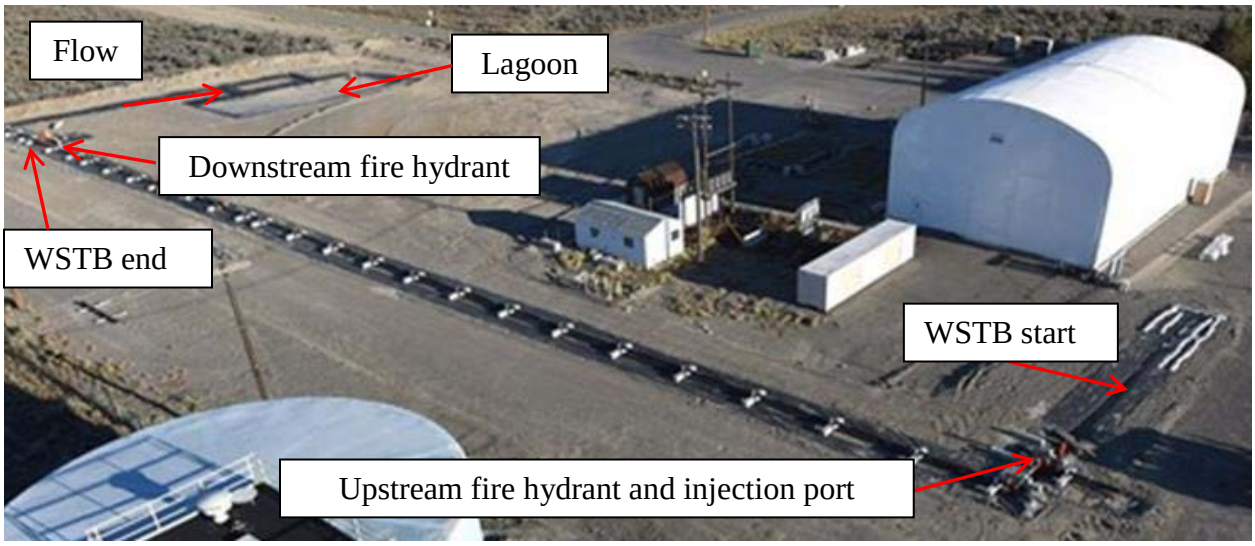

Figure 2: Aerial view of the Water Security Test Bed (WSTB).

Drinking water supplied to WSTB is chlorinated ground water that also supplied the surrounding INL facilities. The WSTB incorporates approximately 450 feet (137 m) of 8 inch (20 cm) diameter cement-lined pipe. The 8 inch (20 cm) pipe system is constructed directly over the lined drainage ditch for spill/ leak containment (as shown in Figure 2). The total volume of the WSTB was estimated to be around 1,150 gallons (4,353 L). The valve near the end of WSTB along with the flow meter (shown in Figure 3) was used to regulate and maintain flow.

Figure 3: Water Security Test Bed system flow regulator.

The effluent water and ice slurry from the WSTB system was discharged to a lagoon (Figure 4) which has a total water storage capacity of 28,000 gallons (105,980 L).

Figure 4: Water Security Test Bed discharge lagoon.

Bulk water samples (BWSs) and coupon samples were taken from the 15-foot (5 m) polyvinyl chloride (PVC) pipe-segment designed and fabricated to contain 10 sets of duplicate removable coupons (totaling 20 coupons). The coupons were made from cement-lined pipe used to construct the rest of the WSTB. The coupons were installed such that they were flush with the interior pipe surface. The coupons allow for the measurement of contaminant persistence on pipe material, and the determination of the effectiveness of decontamination. Figure 5 shows a portion of the 15-foot (5 m) PVC coupon section.

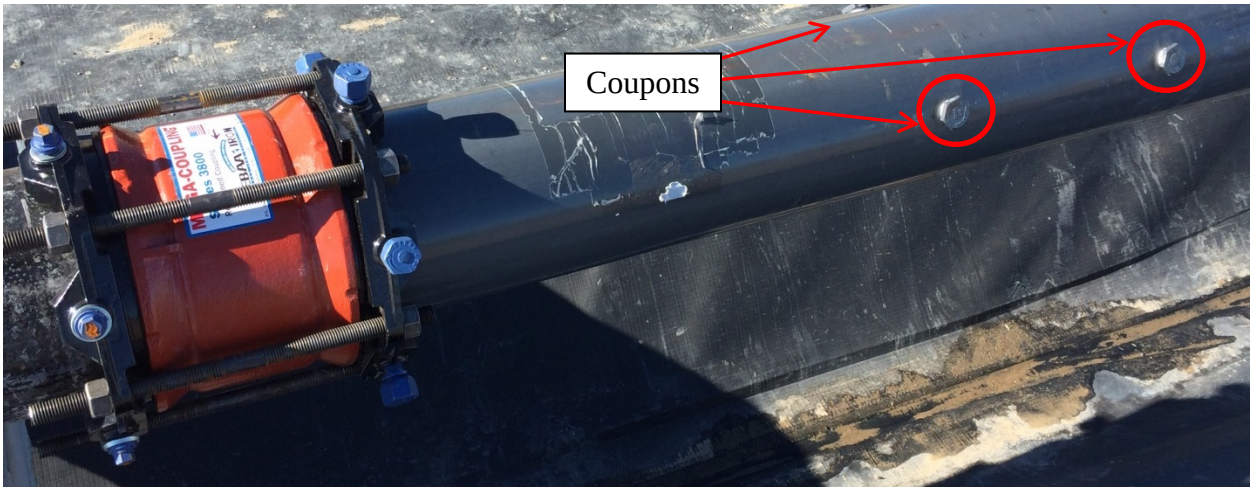

Figure 5: Removable 15-ft PVC coupon section.

The 20 small coupons (22/32 of an inch [1.8 cm] in diameter and 0.371 square inches [2.4 square centimeters] in area) were cut from the cement mortar-lined iron pipe obtained from INL and set into threaded plugs that were inserted into the PVC-coupon section of the pipe. Figure 6 shows a picture of the threaded coupon that was inserted into the pipe main. Furthermore, as seen in

Figure 7, sampling could also be performed by removing pipe caps and directly sampling the pipe interior.

Figure 6: Extracted pipe coupon.

Figure 7: 450-ft Water Security Test Bed pipe interior.

2.0 Description of Pigging Techniques and Experimental Design

Historically, the term "pigging," in the context of pipeline cleanup operations, refers to the practice of using mechanical devices known as "pigs" to perform cleanup activities. The original mechanical pigs used for cleaning pipes were made from straw wrapped in wire. This device would make a squealing noise when scraping the pipe walls and traveling through the pipe, which led to the process being called pigging. In general, pipeline cleaning operations using pigs are accomplished by launching the "pig" at an upstream location and pushing the pig down the pipe until it reaches a downstream receiving station for retrieval.

In industrial applications, soft "foam" pigs are most commonly used for pipe cleaning applications. The soft pigs are constructed using flexible open cell polyurethane foam materials (or other materials with similar properties) topped with select external wrapping that is suited for individual application. The soft pigs are slightly oversized and designed to form a "sliding seal" in the pipe. When pushed through the pipe, the soft pig is able to mechanically scrape and remove product buildup, foreign matter and loose sediment from the pipe walls.

In 2011, Utility Services Co. (USC) launched ice pigging services (USC was subsequently acquired by Suez North America and renamed Utility Services Group). Ice pigging combines the operational advantages of flushing with the cleaning impact of soft pigging. The ice pig is a semisolid ice slurry that is pumped like a liquid and flows through pipe bends and fittings without blockage. Ice pigging has a minimum impact on operations. The ice pig is simply pumped into and recovered from a hydrant at each end of the pipe section without excavation of pipe or modification to the hydrant. According to the vendor, the benefits of ice pigging (in comparison to other forms of pigging) to clean pipes include (USC, 2016):

- Requires only 1/2 the time of other mechanical techniques
- No requirement to disinfect post cleaning
- Less disruptive/expensive than foam pigging
- Combines operational advantages of flushing with cleaning impact of soft pigging
- Pig behaves like a liquid, flows through changes in diameter, through bends, and through butterfly valves without blockage

Physical scouring of the pipe interior can be performed using a variety of nozzles, or metal tips that are attached to a high pressure hose. These pigs are activated by pumping water at high pressure and flow through the nozzles. Some nozzles scour, or pig, the pipe using high pressure water jet (or spray) that comes out of the nozzle at an angle. Other pigs use metal hooks, screws or other metal protrusions to scour the pipe interior. The chain cutter described later in this report falls into the category of physical scouring nozzles. Water pumped at high flow and pressure through the nozzle causes the chains to spin and scour the pipe interior. Detailed descriptions of the ice pigging and chain cutter pigging are provided in the following sections.

2.1 Ice Pigging

Prior to coming to the site to perform decontamination activities, the USC team prepared the ice slurry using their mobile trailer mounted equipment at a parking lot in Idaho Falls, Idaho (approximately 50 miles from the test site). When the USC team arrived on-site, they transferred

the slurry to a tanker truck in preparation for the decontamination event (Figure 8).

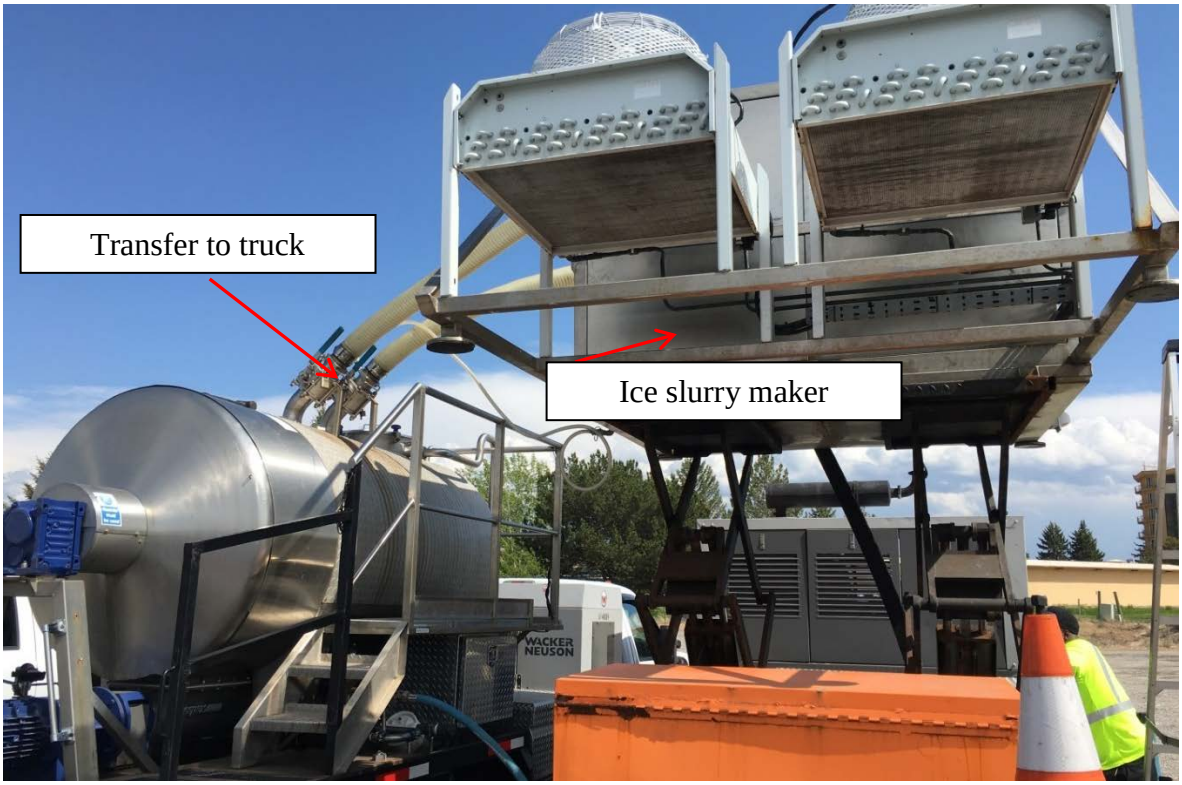

Figure 8: Ice slurry transfer to a mobile truck.

Before decontamination took place, the flow to main WSTB pipe was shut off, and the pipe depressurized. The ice pigging crew then connected hose to deliver slurry mix to upstream hydrant (Figure 9).

Figure 9: Ice slurry injection into the upstream fire hydrant.

The ice pigging decontamination event involved injecting one 600 gallon (2,271 L) slug of the blended ice slurry into the upstream fire hydrant, and letting it scour the inner pipe wall as it traveled down the pipe. The slurry was drained from the downstream fire hydrant (Figure 10) into the lagoon (Figure 11). Once the 600 gallons (2,271 L) of ice slurry was injected into the pipe, the upstream hydrant was closed and the downstream hydrant steam port was connected to a discharge hose which routed the ice slurry to the lagoon. Flow and pressure were re-established into the main water line, which pushed the ice slurry slug through the pipe and out through the downstream hydrant discharge. After the ice slurry had cleared the pipeline, the downstream hydrant was closed and the post pigging coupon samples were collected. This process took approximately one hour.

The ice pigging crew described the consistency of the ice slurry as being similar to a slushie beverage or frozen margarita. It was observed during the test that the ice slurry came out as a semi-solid slurry in the lagoon. However, it was difficult to determine how much of the ice slurry melted as it travelled down the pipe. It was possible that some melting took place since the sun was shining directly onto the black pipe exterior, which is above ground.

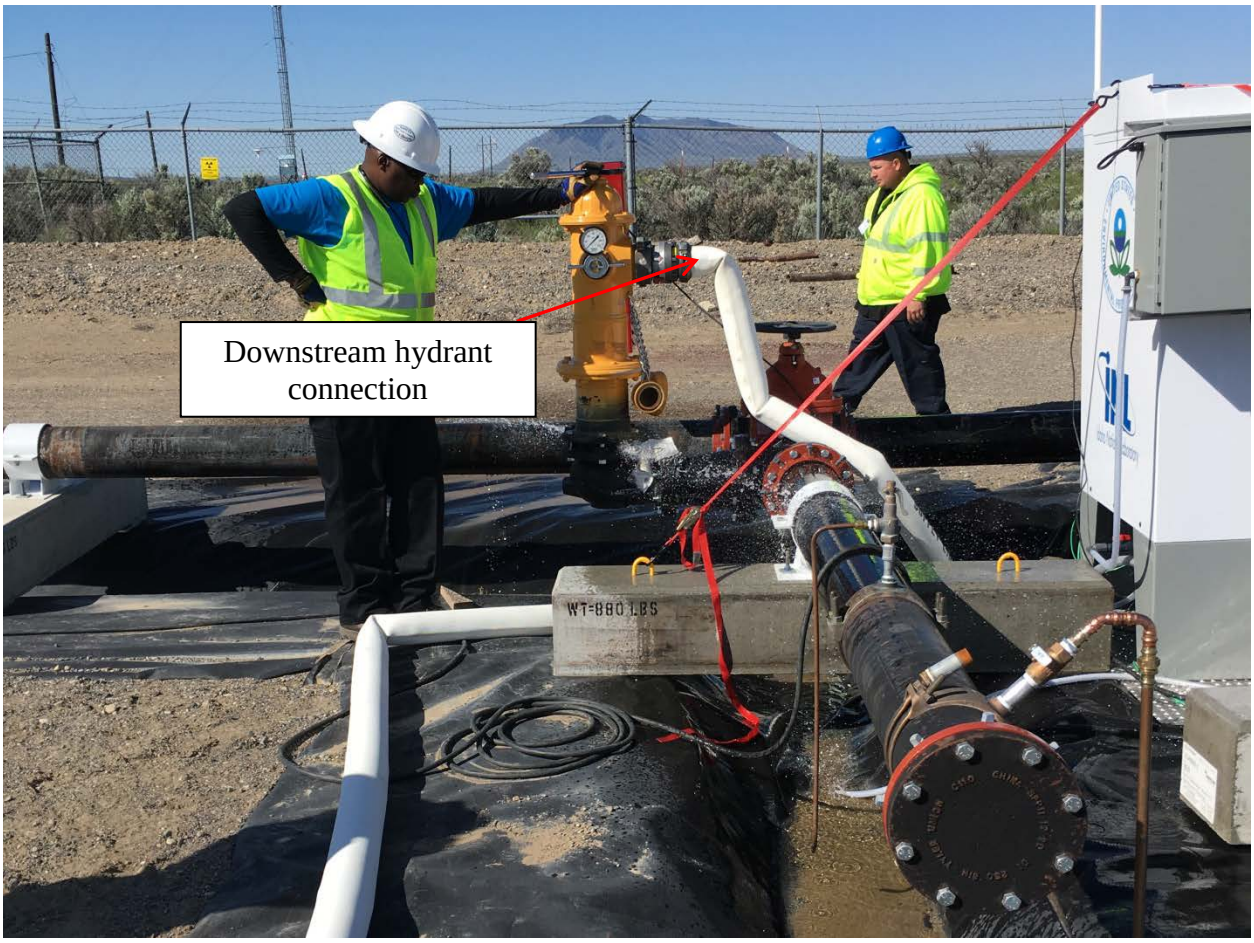

Figure 10: Ice slurry retrieval from the downstream fire hydrant.

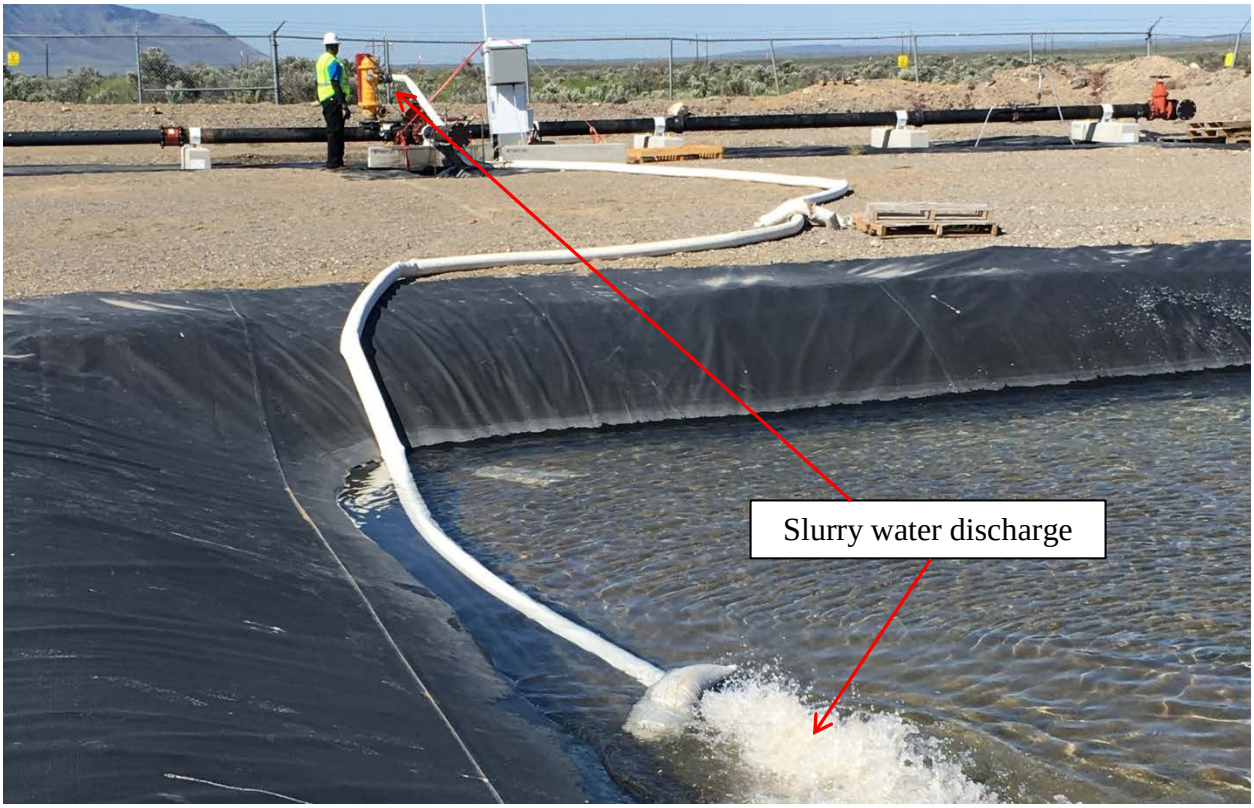

Figure 11: Slurry water downstream discharge.

2.2 Chain Cutter Pigging

The nozzle shown in Figure 12 is the KEG® mini chain cutter (KEG Technologies, Inc., Spartanburg, SC) was used for scouring the internal surface of the pipe. INL contracted Big Sky Industrial (Big Sky) to perform the chain cutting service for the Water Security Test Bed. This chain cutter is capable of being inserted into pipes from 4 to 8 inches in diameter, and was designed to remove obstructions and blockages in pipes such as roots and tuberculation (like iron corrosion). This type of chain cutter is primarily used to clear wastewater piping clogged with persistent tree roots, however the field operation of the device was the same for drinking water pipe decontamination. When water flows into the rear end of the chain cutter (at the left of Figure 12), the front end (right end of Figure 12) rotates, and the chains scour the pipe wall. Water flows out in a high pressure jet through openings in the middle of the nozzle. This high pressure jet flows toward the rear end of the nozzle and helps propel the nozzle forward. The lengths of chain can be adjusted by adding or subtracting links. The chain length was set to allow the chains to just touch the interior pipe walls.

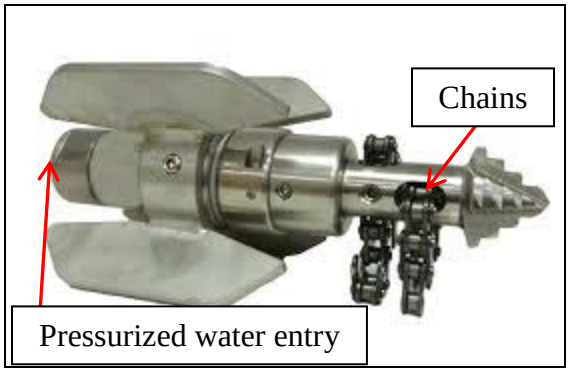

Figure 12: KEG chain cutter nozzle.

In order to achieve the appropriate amount of chain rotation and scouring action, water must be supplied from a combination truck, which is shown in Figure 13. Big Sky provided the Vactor® truck and the operator for this chain cutting experiment. In the wastewater industry, combination trucks are commonly referred to as "Vactor trucks" after one of the manufacturers of these vehicles. Combination trucks have a dual function. The hose on the front can vacuum solids out of a sewer and pump them into the tank on the back. The truck can also store approximately 1,300 gallons (4,921 L) of potable water, and pump it through a high pressure hose at up to 2500 psi (170 atm) and 80 gpm (302 L/min). Water at a flow of 70 gpm (265 L/min) and 2300 psi (157 atm) pressure were used to operate the chain cutter inside of the 450 foot (ft) section of the WSTB pipe. Figure 14 shows the chain cutter installed at the end of the high pressure hose.

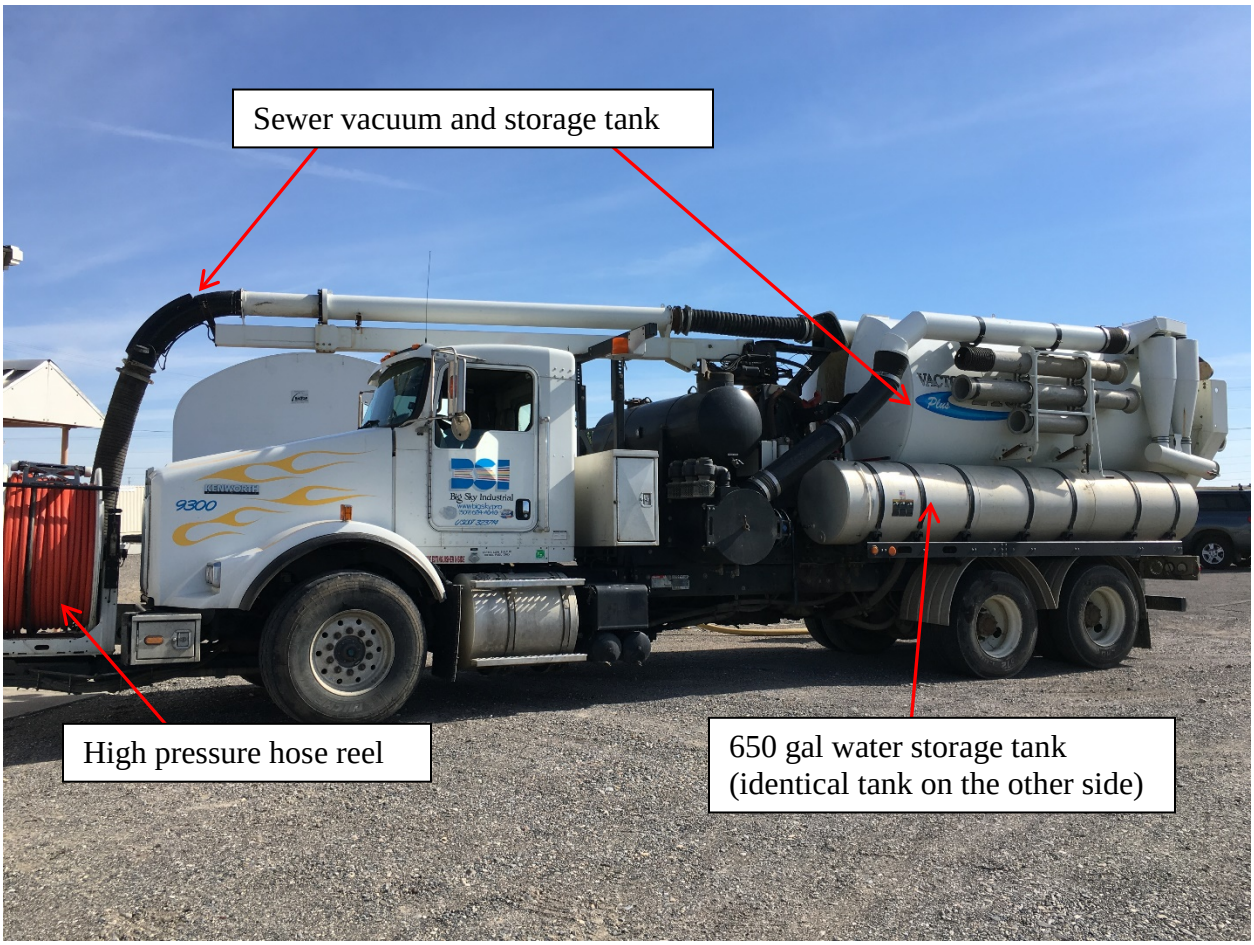

Figure 13: Combination (Vactor) truck.

Figure 14: Chain cutter nozzle attached to the combination truck high pressure hose.

Images showing the process of pigging the 450 ft WSTB pipe are shown in Figure 15 and Figure 16. After the combination truck was filled with water and the chain cutter was installed on the hose, the 450 ft WSTB pipe was depressurized. A cap on the end of the 450 ft pipe was removed and the pipe was drained. The chain cutter was then inserted into the end of the dry, depressurized pipe (Figure 15). When the high pressure water pump on the combination truck was turned on, the water began flowing through the nozzle, and the chain cutter began spinning. High pressure water exited through an opening in the rear of the chain cutter nozzle, and this pressurized water flow propelled the chain cutter down the pipe (notice the water spray exiting in the pipe in Figure 15).

Figure 16 shows the chain cutter at the other opposite end of the 450 ft pipe. Once the chain cutter had traversed the length of the pipe, the hose reel on the combination truck pulled the chain cutter back down the pipe. The chain cutter was then retrieved and removed from the Vactor truck hose reel. After chain cutter pigging, a water flushing "bullet" attachment was attached to the hose reel and reinserted into the 8 inch main to flush out any materials chipped off the interior pipe surfaces during the chain cutting procedure.

It should be noted that the chain lengths were considerably eroded or shortened (approx. 1/8 inch) during the pigging operations due to contacting the pipe walls. Sparks were very evident from the physical contact with the pipe walls as the chain cutter progressed down the pipe length. Care should be taken to reapply chain lengths to the assembly as needed in order to maintain the desired amount of pipe wall contact/abrasion.

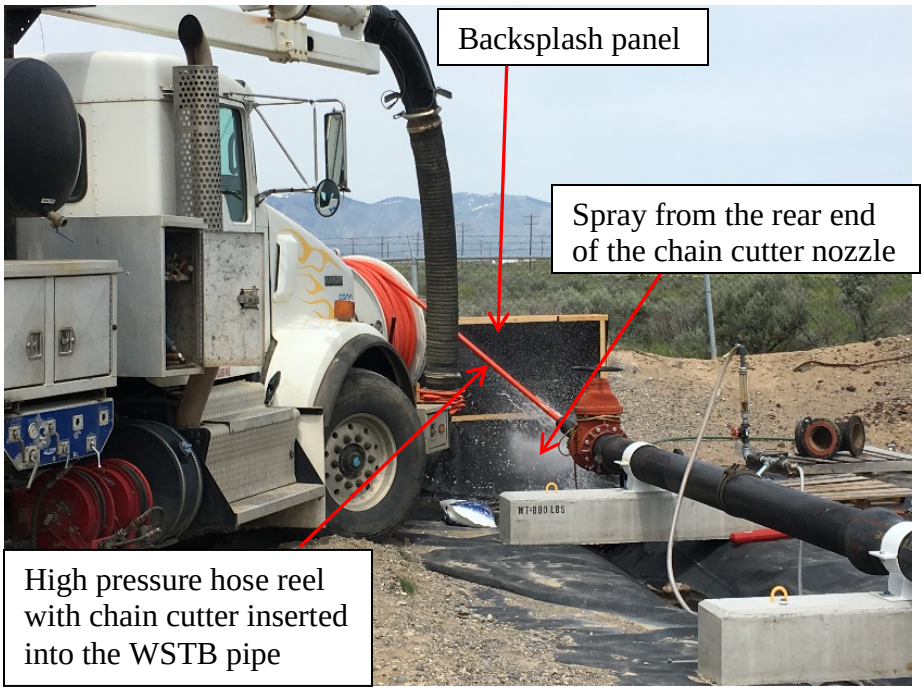

Figure 15: Chain cutter nozzle inserted into the 450 ft Water Security Test Bed (WSTB) pipe.

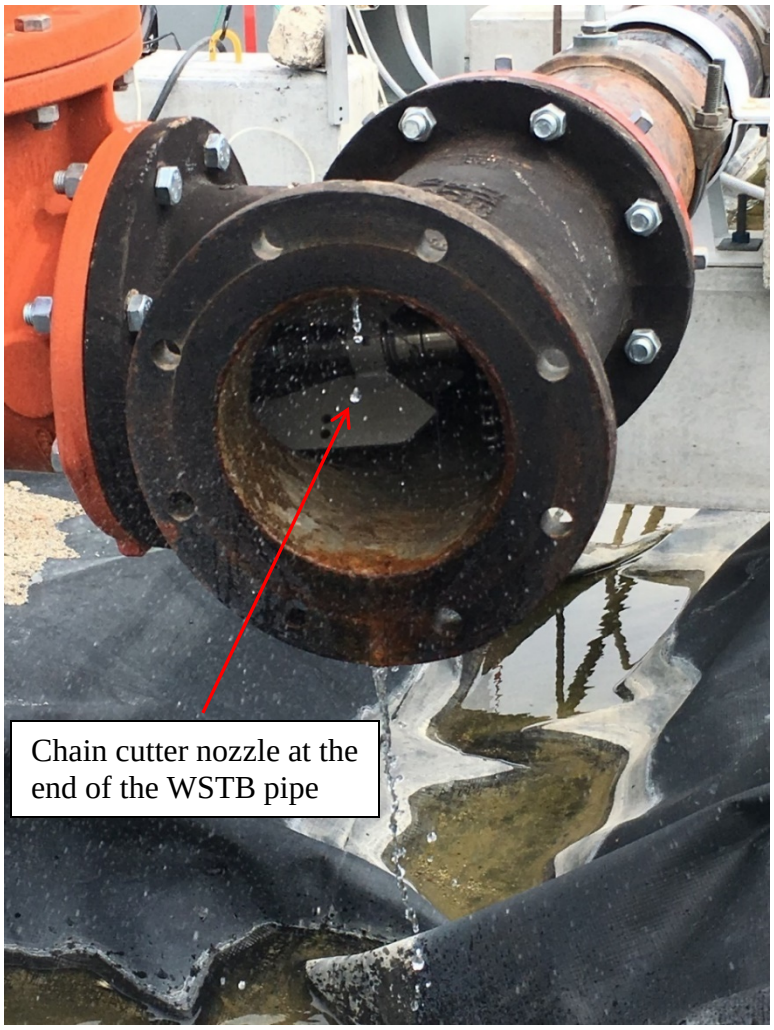

Figure 16: Chain cutter nozzle after traversing the 450 ft Water Security Test Bed (WSTB) pipe.

In addition to the 450 ft pipe, individual sections of pipe were pigged with the chain cutter. The individual pipe setup is shown in Figure 17, and the pigging process is shown in Figure 18. Pigging of the individual pipe sections took place in the same manner as the 450 ft pipe, with the exception that the water pressure and flow were reduced to 25 gpm and 1200 psi. Two types of pipe were pigged. One was the same cement-mortar lined iron pipe used in the 450 ft WSTB pipe. The other was iron pipe with heavy corrosion on the interior. This pipe was obtained from the District of Columbia Water and Sewer Authority (DC Water). All pipe surface samples taken from the individual pipes were direct scrapings of the inner surface. Figure 19 shows the scouring of the cement-mortar pipe after pigging, with some of the cement-mortar coating removed.

Figure 17: Individual pipe sections next to the Water Security Test Bed lagoon.

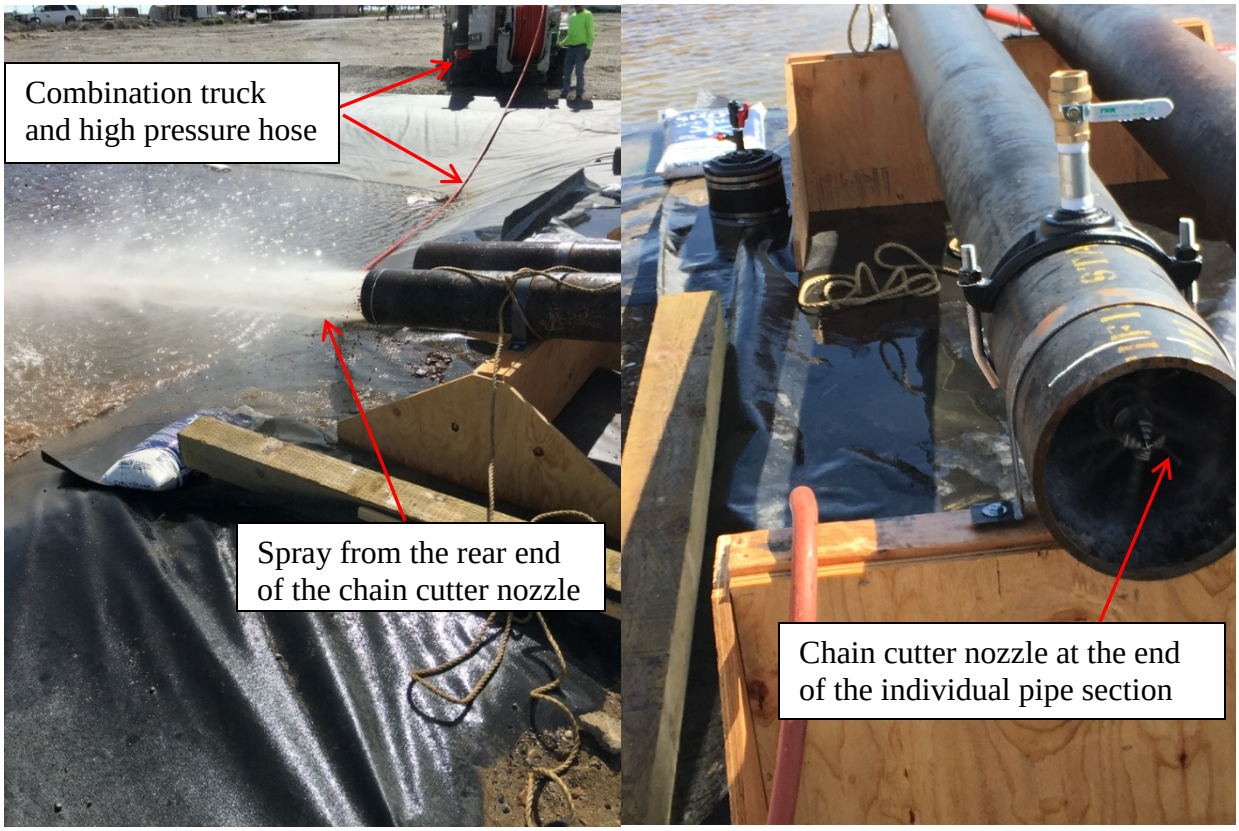

Figure 18: Chain cutter nozzle operating in the individual pipe sections.

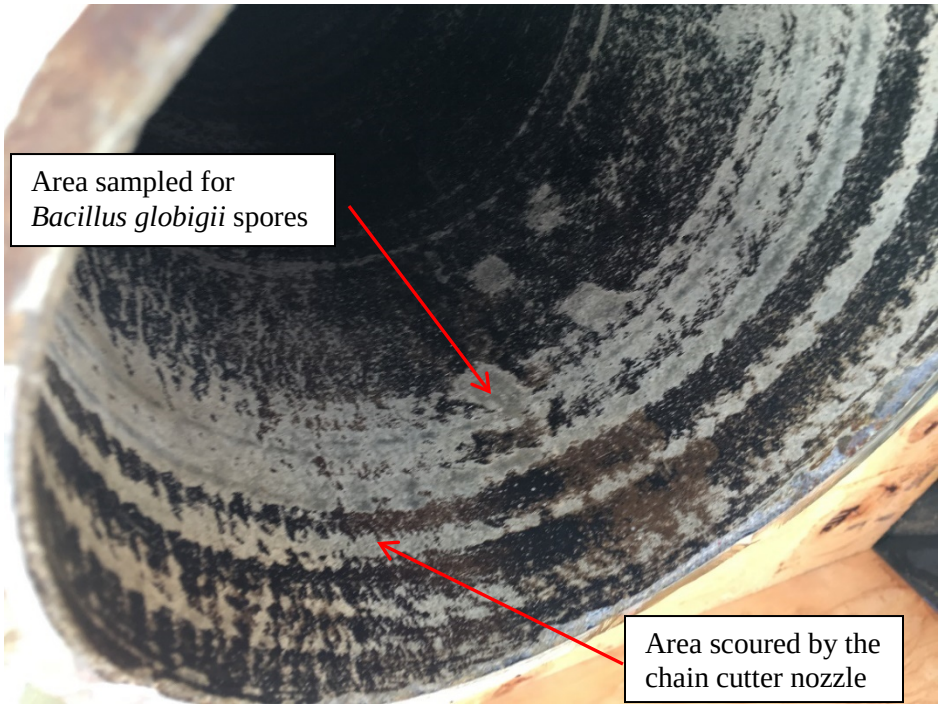

Figure 19: Inside of the cement-mortar lined pipe section after pigging with the chain cutter.

2.3 Contamination and Decontamination Experiments

Contamination and decontamination took place as follows:

Step 1 - Pipe conditioning (cultivation of biofilm)
Step 2 - Contamination (addition of *B. globigii* spores to WSTB)
Step 3 - Decontamination (ice pigging/flushing)
Step 4 - Post-Decontamination Flushing, Reconditioning and Monitoring

Step 1 - Pipe Conditioning (Cultivation of Biofilm)
To effectively study the adsorption of contaminants such as *B. globigii* on pipe walls, it was essential to ensure that there was a viable biofilm. The biofilm could influence adsorption of the contaminant on the pipe wall. Similar to the past studies at WSTB, natural cultivation of biofilm was chosen as the cultivation procedure for testing of the WSTB. This was accomplished by passing INL tap water through the WSTB continuously over approximately 4 weeks before both ice pigging and chain pigging experiments. After initial flushing to remove any debris, the normal operating flow rate was set at 2.5 gallons per minute (gpm) with a total discharge of 25,200 gallons per week to the lagoon which allowed for weekly trucking and disposal of the accumulated discharge.

For ice pigging and chain cutter pigging in the 450 ft WSTB pipe, samples of water from the WSTB pipe and samples from the pipe interior were taken to determine background levels of heterotrophic plate count (HPC) concentration and *B. globigii*. This sampling occurred prior to contamination with *B. globigii*. After background samples were taken, the main pipe flowrate was turned up from 2.5 to 15 gpm. For the individual pipes sections pigged with the chain cutter, sampling for HPC and *B. globigii* took place in the same manner as the 450 ft pipe. However, these pipes were not exposed to flow for 4 weeks before contamination.

Step 2 - Contamination (Addition of *B. globigii* Spores to WSTB)
During the contamination, the WSTB was operated at 15 gpm with a minimum contact time of approximately 1 hour (to accommodate for travel time). Injection duration was also estimated to be 1 hour so that there was a contact of one hour after the bolus of *B. globigii* suspension reaches the coupon section of the pipe. Immediately prior to contamination of the 450 ft pipe, the *B. globigii* stock was mixed with water to obtain 40 liters of the mixture with an expected in-pipe mixed concentration of 1×10^6 colony forming units (cfu)/mL (at 15 gpm main flowrate). *Bacillus globigii* injection was then started at 650 ml/min rate. The injection was complete after one hour. After the injection was complete, flow in the pipe was reduced to 5 gpm, which was maintained until the morning on the following day. Pipe surface and bulk water samples were taken during injection, and then the morning of the day following contamination (immediately before pigging).

For the individual pipe sections, caps were installed on each end of the pipe. The caps had two influent ports so that tap water and *B. globigii* spores could be injected simultaneously. The mixing action of simultaneous filling ensured that the spores were mixed evenly throughout the bulk water phase in the pipe. Like the 450 ft pipe, enough spores were injected to achieve 1×10^6 cfu/mL in the bulk water phase. After the pipe sections were filled, the spores were allowed to contact the pipe surfaces for 1 hr. The spore suspension was then drained, and the pipes were

filled with tap water until chain cutter pigging. Background samples were taken before contamination, immediately after contamination, and then the morning of the day following contamination (immediately before pigging).

Step 3 - Decontamination (Pigging)
Ice pigging or chain cutter pigging was performed as described in sections 2.1 and 2.2, respectively. As mentioned in Step 2, samples of bulk water and the scraped samples of the pipe inner surface were taken immediately before and after pigging

Step 4 - Post-ice pigging disinfection and return to service.
Following the completion of the ice or chain pigging, the water pressure was restored to 40 psi and water flow was reset to 15 gpm. One gallon of commercially available bleach (8.25% sodium hypochlorite) was diluted with water 4:1 and the resulting mixture was injected into the WSTB at 200 ml/min. Once the diluted bleach was injected, pipe flow as stopped and the bleach was allowed to sit stagnant in the pipe for 24 hours. The in-pipe concentration was expected to be between 70 to 80 mg/L once the bleach mixed throughout the pipe bulk water phase. After 24 hours of contact, flow was restored to 15 gpm and the bleach was flushed from the pipe. Both coupon and bulk water samples were collected after the bleach was flushed. A similar procedure was followed for the individual sections of pipe, except that 100 to 150 ml, depending on pipe volume, of bleach was added to each pipe as it was being refilled with tap water after pigging. Chlorination is a common method of pipe disinfection in the drinking water industry, which is why it was chosen for disinfection in this study. However, due to variations in pipe demand and volume of bleach added, chlorine concentrations differed from the target of 70 to 80 mg/L in some experiments. These results are described in the section 3.0.

2.4 Experimental Methods

Preparation and transport of *B. globigii* spores
Bacillus globigii spores were produced by mixing an inoculum of *B. globigii* spores with generic sporulation media and incubating with gentle shaking at 35 °C for 7 days. The *B. globigii* suspensions were heat-shocked and enumerated using the spread plate method with tryptic soy agar and membrane filtration (plating is described later in this section). Approximately 40 liters of prepared stock was shipped in separate 1 liter containers inside coolers (preserved at 4 ± 2 °C) to the site.

Extraction of biofilm and spores from coupon and pipe surfaces for microbial analyses

Pipe surface samples were taken either from coupons removed from the WSTB coupon section or scraped directly from the 450 ft pipe wall. Coupon/surface samples were collected from the WSTB carefully without touching the sampled surface that was exposed to WSTB water. The biofilm and spores were scraped from the surface using a disposable sterile surgical scalpel. For coupons, the entire coupon surface was scraped. For the pipe sections, an o-ring with the same diameter as the coupon surfaces was placed on the pipe wall, and the area inside the o-ring was scraped. This ensured that the area scraped was the same for the coupons and pipe wall. The extracted material was collected in a sterile sample bottle with a sodium thiosulfate tablet (for dechlorination of the water) and 100 mL of pre-filled carbon-filtered water. The extracted sample was transferred to a cooler at 4 ±2 °C. The samples were shipped cooled overnight to the

EPA laboratory and analyzed upon receipt.

Enumeration of *B. globigii* and Heterotrophic Plate Count

Upon receipt in the lab, samples containing *B. globigii* spores were heat-shocked at 80˚ C for 10 minutes and analyzed using the Standard Methods spread plate method 9215 (APHA, 2005). Tryptic soy agar plates were used for *B. globigii* spores. *B. globigii* plates were incubated at 35˚ to 37˚C for 24 hours. Heterotrophic plate count samples were analyzed using the IDEXX SimPlate® method (Westbrook, ME) according to Standard Methods 9215E (APHA, 2005). Plates were inculcated at 35˚ ± 0.5˚ C for 45 to 72 hours. When needed, samples were serially diluted (*B. globigii* and HPC) or membrane filtered (*B. globigii*).

Bulk water samples

The BWS for *B. globigii* were collected using the grab sampling technique in 100 mL sterile sample bottles with a sodium thiosulfate tablet. The BWS sampling port in the WSTB coupon section was opened and the water was drained for 15 seconds prior to collection of 100 ml of water from the WSTB.

Free Chlorine

During decontamination experiments, 100 ml grab samples were collected from the water sampling port in the WSTB coupon section. The water was drained for 15 seconds prior to collection of 100 ml of water from the WSTB. Samples were collected in a clean glass laboratory beaker and analyzed for free chlorine using a portable Hach® colorimeter Hach, Loveland, CO). The sample was immediately processed for free chlorine using the Hach Method 10102 using N,N-diethyl-p-phenylenediamine (DPD) at the WSTB site. Samples were diluted in distilled water as needed.

3.0 Experimental Results

3.1 Ice Pigging

The background HPC concentration on the inner pipe surface was analyzed via two coupon samples. These samples were removed after one month of water flow through the WSTB pipe, but before contamination with spores and decontamination with pigging. HPC values from the two coupons were 4.0 x 10^5 most probable number (MPN)/cm^2 and 2 x 10^5 MPN/cm^2, respectively. These results indicate that viable biofilm was present on the pipe walls at WSTB prior to the initiation of the tests. It should be noted that all coupon samples for HPC and *B. globigii* spores were removed from the WSTB coupon section.

Figure 20 graphically summarizes the data obtained from the ice pigging tests. The *B. globigii* spore values obtained from the pipe wall coupons have been converted to colony forming units per square centimeter (CFU/cm^2). For all bars in Figure 20, the "n" value shown in the legend represents the number of coupons samples taken during that phase of the experiment. The bar represents the average of those coupons, and the error bars represent standard deviation. The limit of detection (LOD) in Figure 20 was calculated as follows: The scrapings from the sampled coupon surface went into 100 ml of sterile buffer. Then 22.2 ml of the buffer suspension containing the coupons scrapings were membrane filtered in duplicate three sample volumes (0.1mL, 1mL, and 10mL). If one spore was contained in the filtered 22.2 ml, that scales up to 4.5 per 100 ml. When that value was normalized by the coupon area 2.4 cm^2, this yields a value of 1.9 CFU/cm^2.

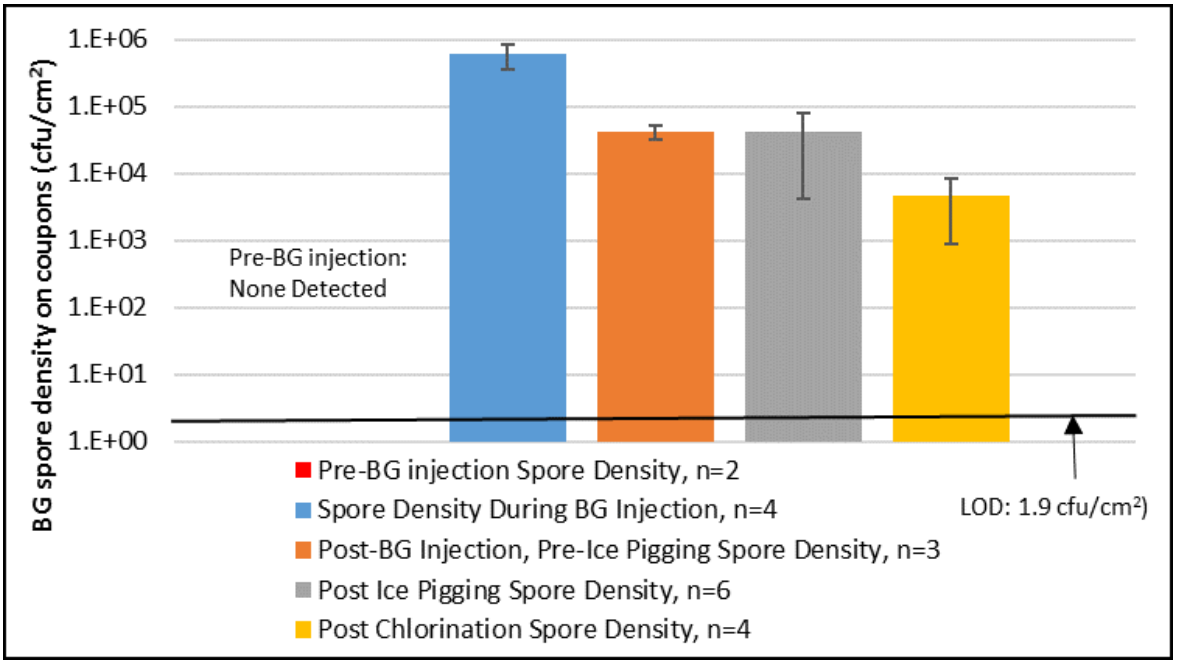

Figure 20: Decontamination of *Bacillus globigii* (BG) from the 450 ft Water Security Test Bed pipe with ice pigging.

The missing first bar (pre-BG injection spore density) is not visible on the graph because no spores were detected above the LOD in the background samples. These coupons had been in

contact with the water for 4 weeks before the background samples were taken. A text label has been added to reflect that no viable spores were detected in white space where the bar would appear if any background *B. globigii* were present. The second bar (spore density during BG injection) represents the number of viable spores detected on the coupons during the spore injection. The bulk water phase had 1.2 x10^6 CFU/mL during this period of sample collection. The third bar (Post-BG injection) represents the number of viable spores attached to the coupons after the *B. globigii* contaminant slug passed out of the pipe with clean water behind it. These coupon samples were taken the day after contamination, and immediately before ice pigging. BWS taken during this time showed that 5.0 x 10^1 CFU/ml were present in the bulk water of pipe. The fourth bar (Post ice pigging BG spore density) represents the number of viable *B. globigii* spores attached to the coupons after ice pigging. The data suggest that there is no difference between the pre and post ice pigging pipe wall spore densities. Furthermore, after water was restored at 40 psi to the pipe, 7.3 x 10^1 cfu/ml viable spores were detected in the bulk water phase. Therefore, it can be concluded that the ice pigging decontamination procedure is not an effective way to remove spore from the water under the tested conditions.

The last bar in Figure 20 (Post chlorination spore density) represents the number of viable spores recovered from the coupons after chlorination. As mentioned previously in the report, one gallon of free chlorine (Clorox bleach) was pumped into the WSTB pipe after ice pigging so that it spread out evenly through the pipe volume. The initial free chlorine concentration was 52 mg/L. After 24 hours of contact, the free chlorine concentration was 55 mg/L. These values yield a bulk phase CT (chlorine concentration, C, in mg/L and contact time, T, in minutes) of 77,040 mg-min/L. After chlorination, the number of viable spores recovered from the pipe surface decreased by 1 log. No viable spores were detected in the bulk water phase after the bleach had been flushed from the pipe and tap water restored.

3.2 Chain Cutter Pigging

The background HPC concentration on inner pipe surface was analyzed via two coupon samples. These samples were removed after one month of water flow through the WSTB pipe, but before contamination with spores and decontamination with pigging. HPC values from the two coupons were 1.8 x 10^3 MPN/cm^2 and 3.1 x 10^3 MPN/cm^2, respectively. These results indicate that viable biofilm was present on the pipe walls at WSTB prior to the initiation of the tests. It should be noted that coupon samples for HPC were removed from the WSTB coupon section.

Figure 21 graphically summarizes the data obtained from the chain cutter pigging tests. The *B. globigii* spore values obtained from the pipe wall coupons have been converted to colony forming units per square centimeter (CFU/cm^2). For all bars in Figure 21, the "n" value shown in the legend represents the number of coupons samples taken during that phase of the experiment. The bar represents the average of those coupons, and the error bars represent standard deviation. The LOD in Figure 21 was calculated as follows: The scrapings from the sampled coupon surface went into 100 ml of sterile buffer. Then 22.2 ml of the buffer suspension containing the coupons scrapings was membrane filtered in duplicate three sample volumes (0.1mL, 1mL, and 10mL). If one spore was contained in the filtered 22.2 ml, that scales up to 4.5 per 100 ml. When that value was normalized by the coupon area 2.4 cm^2, you get a value of 1.9 CFU/cm^2. In Figure 21, note that the pre-BG injection spore density and the spore density during BG injection were taken from the WSTB coupon section. This was done because the pipe was full and

pressurized during these samples. During the next three sampling phases, the pipe was open and depressurized, so pipe surface scrapings were taken directly from the pipe wall.

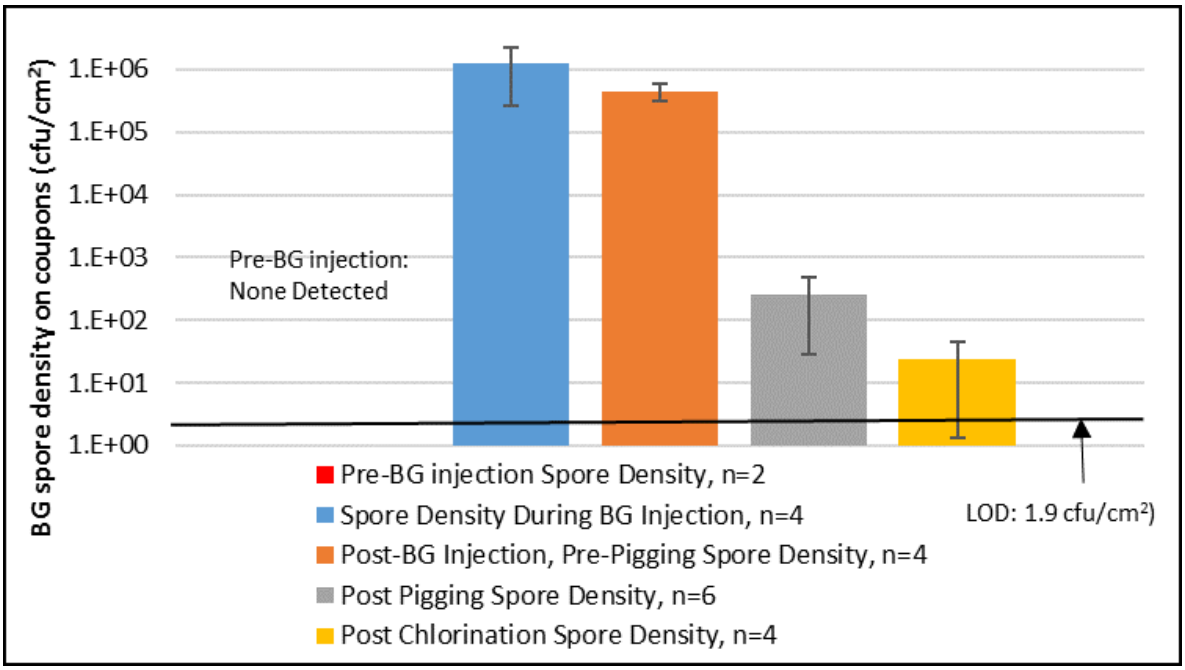

Figure 21: Decontamination of *Bacillus globigii* (BG) from the 450 ft Water Security Test Bed pipe with chain cutter pigging.

The missing first bar (pre-BG injection spore density) is not visible on the graph because no viable spores were detected above the LOD in the background samples. These coupons had been in contact with the water for 4 weeks before the background samples were taken. A text label has been added to reflect that no spores were detected in white space where the bar would appear if any background *B. globigii* were present. The second bar (spore density during BG injection) represents the number of viable spores detected on the coupons during the spore injection. The bulk water phase had 1.9 $x10^6$ CFU/mL during this period of sample collection. The third bar (Post-BG injection…) represents the number of viable spores recovered from the coupons after the *B. globigii* contaminant slug passed out of the pipe with clean water behind it. These coupon samples were taken the day after contamination, and immediately before chain cutter pigging. BWS taken during this time showed that 4.3 x 10^1 CFU/ml were present in the bulk water of pipe. The fourth bar (Post ice pigging BG spore density) represents the number of viable *B. globigii* spores recovered from the coupons after chain cutter pigging. The data shows a 3.3-log decrease in the number of spores after chain cutter pigging. This suggests that scouring the pipe surface with the chain cutter removed enough of the cement-mortar matrix and biofilm to impact the number of adhered spores. After water was restored at 40 psi to the pipe, 1.3 x 10^1 cfu/ml viable spores were detected in the bulk water phase.

The last bar in Figure 21 (Post chlorination spore density) represents the number of viable spores recovered from the coupons after chlorination. As mentioned previously in the report, one gallon of free chlorine (Clorox bleach) was pumped into the WSTB pipe after chain cutter pigging so that it spread out evenly through the pipe volume. The initial free chlorine concentration was 82 mg/L. After 17 hours of contact, the free chlorine concentration was 31 mg/L. These values yield

a bulk phase CT of 109,650 mg-min/L. After chlorination, the number of viable spores recovered from the pipe surface decreased by 1.0 log. No viable spores were detected in the bulk water phase after the bleach had been flushed from the pipe and tap water restored. Between chain cutter pigging and chlorination, viable spores adhered to the pipe wall decreased by 4.3 log.

In addition to the 450 ft WSTB pipe, individual cement-mortar lined iron and corroded iron pipe sections were also contaminated and pigged. The results from the cement-mortar lined pipe are shown in Figure 22 and the corroded iron pipe in Figure 23. The bars in each figure correspond with the same experimental phases as those used in Figure 20 and Figure 21. Like Figure 20 and Figure 21, the "n" value shown in the legend represents the number of coupons samples taken during that phase of the experiment. The bar represents the average of those coupons, and the error bars represent standard deviation. All samples taken in Figure 22 and Figure 23 were scraping from the pipe wall of the individual pipe sections.

Figure 22 shows that the number of viable spores adhered to the inner surface of the cement-mortar pipe was between 10^5 and 10^6 cfu/cm^2. The same range of initially adhered spores was observed in contamination and pigging experiments performed in the 450 ft WSTB pipe. Chain cutter pigging resulted in a 1.5 log reduction of adhered spores. Chlorination was conducted in the same manner as the experiments in the 450 ft pipe, with household bleach added to the pipe section and a contact time of 17 hours. The initial free chlorine concentration was 201 mg/L. After 17 hours of contact, the free chlorine concentration was 154 mg/L. These values yield a bulk phase CT of 228,990 mg-min/L. After chlorination, inactivation of the spores adhered to the pipe inner surface decreased by 2.3 log. No spores were detected in the bulk water phase after chlorination. Between chain cutter pigging and chlorination, spores adhered to the pipe wall decreased by 3.8 log.

Results from the individual pipe differs from the pigging that took place in the 450 ft WSTB pipe, which also contains cement-mortar lined iron pipe. Specifically, the reduction of spores adhered to the pipe wall due to chain cutter pigging was 3.3 log in the 450 ft pipe, but only 1.5 log in the individual pipe section. This was likely because the chain cutter nozzle was not operated at full strength in the individual pipe section. In the 450 ft pipe, the chain cutter nozzle was operated with 2300 psi and 70 gpm. In the individual pipe section, 1200 psi and 25 gpm was used, and this pressure and flow resulted in less scouring action compared to the 450 ft pipe experiment. The combination truck operator used the reduced flow and pressure in the individual pipe section for safety reasons. The individual pipe section was open at both ends, and there was concern that operation at the maximum pressure and flow would push the chain cutter out of the pipe and cause damage to the chain cutter or the surrounding area.

Chlorination in the individual pipe section inactivated the spores adhered to the pipe inner surface by 2.3 log, with an initial chorine value of 201 mg/L and a CT of 228,990 mg-min/L. In the 450 ft pipe, 1.0 log inactivation was observed with an initial concentration of 82 mg/L and a CT of 109,650 mg-min/L. The increased spore inactivation in the individual pipe section was likely due to the fact that a higher chlorine concentration was used. Ideally, the same chlorine concentration would have been applied to both the 450 ft pipe and the individual pipe section. However, achieving equal concentrations in a field setting was difficult due to the different pipe

setups and varying disinfectant demand between individual pipe sections. However, it is notable that if the chain cutter was used at a reduced pressure or flow, or if it does not contact a portion of the pipe efficiently, application of a higher level of free chlorine can make up for the reduced log reduction resulting from pigging alone.

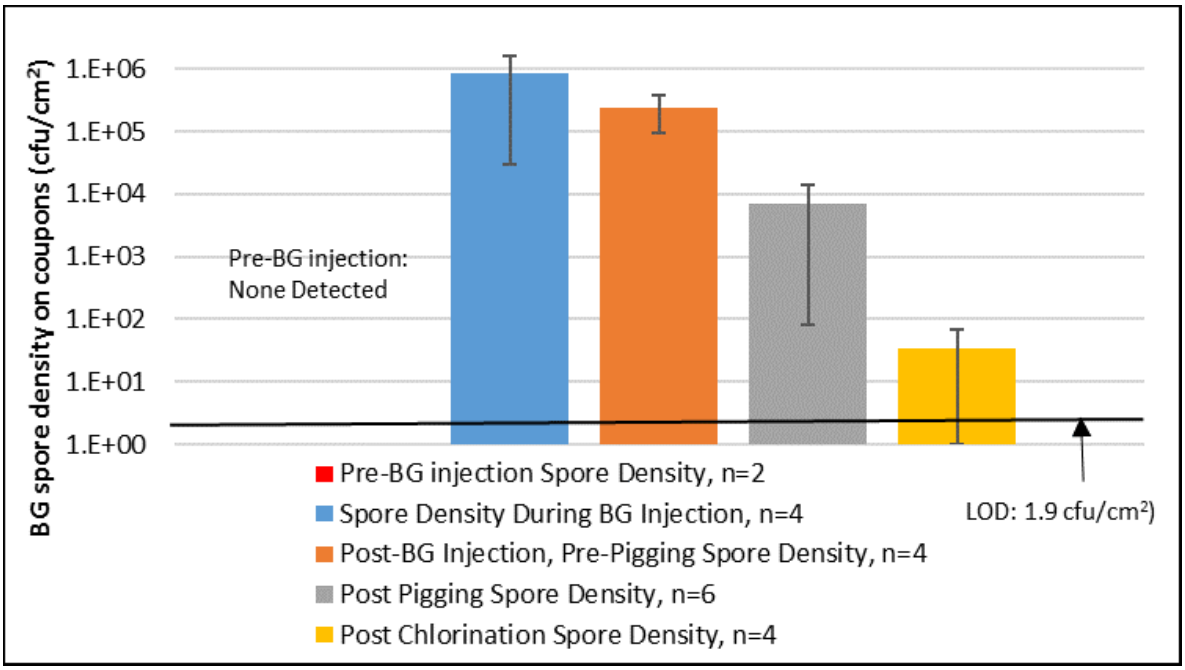

Figure 22: Decontamination of *Bacillus globigii* (BG) from an individual cement-mortar lined iron pipe with chain cutter pigging.

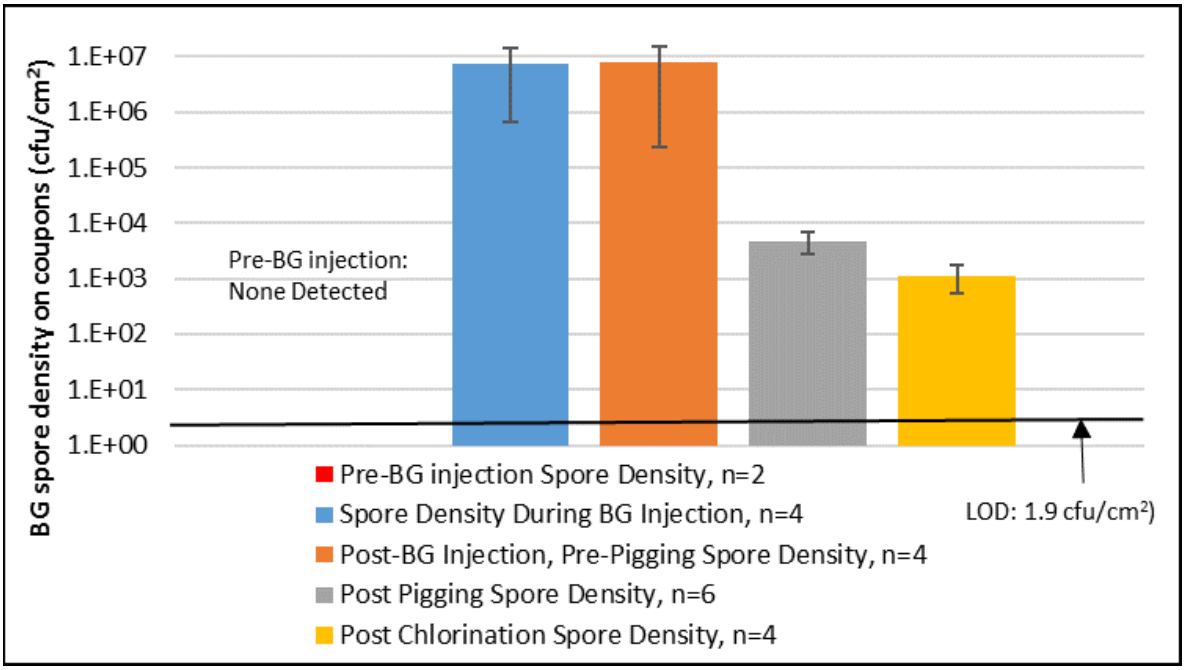

Figure 23: Decontamination of *Bacillus globigii* (BG) from an individual corroded iron pipe with chain cutter pigging.

Figure 23 shows that the number of spores adhered to the inner surface of corroded iron was between 10^6 and 10^7 cfu/cm^2. This range of initially adhered spores was higher than what was observed in contamination and pigging experiments performed in the 450 ft WSTB pipe and the individual cement-mortar lined iron pipe. The increase in initially adhered spores was likely due to the tuberculation (iron corrosion) that protruded from the inner surface of the iron pipe. The rough tuberculation has many ridges and crevasses where spores and particles can adhere.

Chain cutter pigging resulted in a 3.2 log reduction of adhered spores. Chlorination was conducted in the same manner as the experiments in the 450 ft pipe, with household bleach added to the pipe section and a contact time of 17 hours. The initial free chlorine concentration was 140 mg/L. After 17 hours of contact, the free chlorine concentration was 25 mg/L. These values yield a bulk phase CT of 201,450 mg-min/L. Chlorination resulted in a 0.6 log inactivation of the spores adhered to the pipe inner surface. No spores were detected in the bulk water phase after chlorination. Between chain cutter pigging and chlorination, spores adhered to the pipe wall decreased by 3.8 log.

No comparable data exists for pigging of corroded iron pipe contaminated with *Bacillus* spores. However, like the individual cement-mortar pipe section, the chain cutter nozzle was operated at a reduced pressure and flow of 1200 psi and 25 gpm. In the individual corroded iron pipe, a 3.2 log reduction of adhered spores was observed compared to a 1.5 log reduction in the individual cement-mortar lined pipe. Chain cutter pigging of the iron pipe section removed the tuberculation protruding from the pipe interior, so it is possible that additional pressure and flow may not have resulted in more removal. Chlorination of the individual corroded iron pipe only resulted in a 0.6 log inactivation of the spores adhered to the pipe inner surface, with an initial chorine value of 140 mg/L and a CT of 201,450 mg-min/L. The chlorination level was comparable to in the individual cement-mortar lined pipe, but the log reduction was less due to the increased disinfectant demand of the iron pipe.

4.0 Conclusions

Removing adhered *Bacillus* spores from drinking water infrastructure can be challenging. This study examined the ability of pigging, or physical scouring of the inside of pipes, followed by chlorination, to remove adhered *Bacillus* spores. A summary of results are as follows:

- No change in the number of spores adhered to the inner pipe surface was observed after ice-pigging was conducted in the 450 ft pipe. After pigging, chlorination of the water in the pipe at an initial concentration of 52 mg/L for 24 hours (55 mg/L after 24 hours) resulted in a 1.0-log inactivation of the spores adhered to the pipe inner surface. Elevated temperature may have impacted the efficacy of the ice pigging since the pipes were above ground.

- Pigging with chain cutting (2,300 psi, 70 gpm) resulted in a 3.3-log reduction of the number of spores adhered to the inner surface of the 450 ft pipe. After pigging, chlorination of the water in the pipe at an initial concentration of 82 mg/L for 24 hours (31 mg/L after 24 hours) resulted in an additional 1.0-log inactivation of the spores adhered to the pipe inner surface, for a total reduction of 4.3-log.

- Chain cutter pigging (1,200 psi, 25 gpm) of an individual cement-mortar lined iron pipe section resulted in a 1.5-log reduction of the number of spores adhered to the inner pipe surface. After pigging, chlorination of the water in the pipe at an initial concentration of 201 mg/L for 24 hours (154 mg/L after 24 hours) resulted in an additional 2.3-log inactivation of the spores adhered to the pipe inner surface, for a total reduction of 3.8-log.

- Chain cutter pigging (1,200 psi, 25 gpm) of an individual corroded iron pipe section resulted in a 3.2-log reduction of the number of spores adhered to the inner pipe surface. Chlorination of the water in the pipe at an initial concentration of 201 mg/L for 24 hours (154 mg/L after 24 hours) resulted in an additional 0.6-log inactivation of the spores adhered to the pipe inner surface, for a total reduction of 3.8-log. However, it should be noted that the initial number of adhered spores were one log higher on the iron pipe compared to the cement mortar pipe, likely due to the presence of tuberculation in the iron pipe.

These results suggest that physical scouring of pipe interior with a chain cutter nozzle is an effective way to achieve a 3.3 log reduction of spores when operated at maximum flow and pressure. Chlorination at 82 mg/L for 17 hours immediately following the pigging resulted in additional log reduction, for a total of a 4.3 log reduction of spores. In the experiments described in this study, the 4.3 log reduction was not enough to remove all detectable spores from the pipe surface. Should this situation occur in reality, a technique like pipe lining or replacement may need to be implemented to ensure that human exposure to spores via drinking water does not occur. However, pigging and chlorination can significantly reduce the number of spores adhered to drinking water infrastructure, which may make further remedial actions easier. It should also be considered that the consequence of pigging is the generation of contaminated waste and wash water, which must be treated or disposed of properly.

It should also be noted that the skill and experience of the operators applying either pigging technology are important. There is an art to “working the pigs” effectively through the pipeline, especially for the chain cutter. The speed of the chain cutter is controlled by the operator and certain sections of pipe may require several passes or rework as the pig progresses through the pipe. Similarly, for ice pigging, the pipe conditions and operator experience dictate whether several smaller slugs of ice or one large slug of ice is needed to effectively scour the pipe.

5.0 References

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