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INNOVATIVE RESEARCH FOR A SUSTAINABLE FUTURE

Research Results to Enhance Management of *Bacillus anthracis* Contaminated Wash Water

Purpose

This technical brief provides decision makers with practical information that could be useful for managing and treating decontamination wash water generated during remediation activities following a *Bacillus anthracis* (*B. anthracis*, anthrax) contamination incident. Research results related to sampling and analysis methods for challenging water matrices and various treatment methods are summarized.

Introduction

Following a *B. anthracis* contamination incident, wash water will likely be generated during site remediation activities, potentially through direct use of liquids in decontamination methods, from equipment decontamination, or through washing personal protective equipment (PPE) onsite (Figure 1). For example, following the intentional mailing of letters containing *B. anthracis* spores in 2001, decontamination of personnel in PPE working to clean up the U.S. Capitol buildings generated approximately 14,000 gallons of wash water which required steam sterilization prior to disposal because of the potential presence of *B. anthracis* spores [1]. Of recent concern is an urban wide-area biothreat agent contamination event, which has the potential to generate much larger volumes of liquid waste from decontamination activities. This wash water will likely be collected and stored onsite, and because it poses difficult and unique challenges to the waste stream, it will potentially require onsite treatment prior to disposal.

Previous work has focused on improving detection and treatment of highly pathogenic microorganisms in drinking water distribution systems. These methods have generally involved concentrating pathogens from a larger volume of tap water to increase the probability of detection when target organisms are present at low levels. Traditional drinking water sampling and treatment methods may be inadequate when applied to decontamination wash water, which poses unique challenges. Decontamination wash water is generally more turbid than tap water and can contain high levels of particulate and

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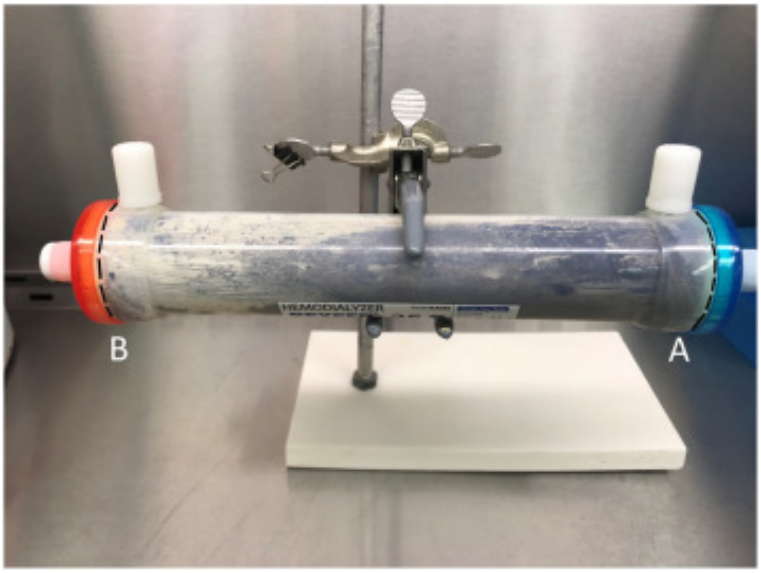
organic matter (Figure 2), causing a higher decontaminant demand [2,3]. These challenging matrices can also be problematic when employing traditional methods for concentrating pathogens in drinking water (i.e., particulate matter causes filter clogging). The U.S. Environmental Protection Agency (EPA) has been developing and testing sampling and treatment methods designed to overcome these operational challenges for bio-contaminated wash water.

Sampling and Analysis Methods

Ultrafiltration Concentration

Biothreat agents (e.g., *B. anthracis* spores) can be present in low concentrations in decontamination wash water. Collecting and concentrating particulates in larger water samples (40–100 liters) would increase the likelihood of agent detection when sampling to determine the best treatment and disposal options, but traditional ultrafiltration methods have proved problematic due to filter fouling issues arising from high levels of particulate matter [4].

Axial flow hollow fiber ultrafiltration (HFUF), in which the filtrate and retentate flow paths of single-use dialysis filters (Figure 3) are switched, has proved to be an effective concentration method for microorganisms in water with high levels of particulate matter [5]. The axial flow method has a much higher capacity for particulate matter since the space for particle accumulation is about 500 times larger compared to conventional HFUF. Operating with recirculation, axial flow HFUF of water with different particulate matter concentrations (0 to 150 mg solids/L) yielded a recovery of 35 to 53% of *B. globigii* spores (a nonpathogenic surrogate for *B. anthracis*). Recovery of MS2 (a surrogate for pathogenic viruses) was comparable (45% organism recovery at 150 mg solids/L). Operating without recirculation (dead end method) also proved effective for concentrating spores, even with a very high solids concentration of 750 mg/L. Although axial flow ultrafiltration has been shown to be an effective concentration method in turbid water matrices, one disadvantage is that microorganism recovery is more cumbersome because it involves additional steps not required as part of traditional HFUF (e.g., filter dissection is required for maximum biothreat agent recovery, Figure 3) [5].



Analytical Detection Methods

Real-time polymerase chain reaction (PCR), rapid viability-polymerase chain reaction (RV-PCR), and culture methods have all been identified as suitable methods for detection of *B. anthracis* spores in decontamination wash water samples. When spore

concentrations are low, ultrafiltration prior to analysis will concentrate the biothreat agent and therefore may increase the probability of detecting target organisms. Amendments to traditional methods may be necessary to minimize the likelihood of false negatives with dirty water matrices (e.g., additional washes to remove PCR inhibitors, or extending incubation times) and to avoid clogging the filter during filter plating due to high suspended particle loads [6].

Wash Water Treatment Methods

Chlorine

The challenges of disposing of bio-contaminated wash water following cleanup of the *B. anthracis*-contaminated U.S. Capitol buildings led the development of the U.S. National Response Team (U.S. NRT) quick reference guide for the on-site treatment of PPE wash water containing *B. anthracis* spores [7]. The method described in this guide calls for a slightly acidic 10% bleach solution (1-part chlorine bleach and 1-part vinegar to 8 parts wash water, by volume) and a treatment time of 1-2 hours. The addition of vinegar, a dilute acid, decreases the pH of the resulting solution to ~7, which makes the chlorine species much more germicidal. Although this treatment is efficacious (> 5 $\log_{10}$ or 99.999% inactivation in < 1 minute), it also requires a relatively large volume of bleach (a hazardous material) and has the potential to form chlorine gas, a toxic gas, if too much acid (or the wrong type of acid) is added. At a pH of 4, chlorine gas levels begin to increase exponentially with decreasing pH. Therefore, it is better suited for treating relatively small volumes (< 30 gallons) of wash water.

Figure 1: Wash water used in bench-scale inactivation testing [10].

EPA has developed and tested chlorine inactivation methods that are safer for treating larger volumes of wash water. Approximately 5% (v/v) bleach (with no vinegar addition) was sufficient for > 7 $\log_{10}$ inactivation of *B. globigii* spores in simulated diluted wash water after a 10 minute exposure at room temperature, and inactivation occurred more rapidly when a detergent with buffering agents (i.e., 1% Alconox®) was added [8]. Chlorine inactivation efficacy generally decreases with increasing pH and decreasing temperatures, so contact times should be adjusted accordingly. Based on the results from an EPA study [9] in which a wide variety of wash waters were tested, it is estimated that a 6 $\log_{10}$ inactivation of viable *B. anthracis* spores in wash water can be achieved with contact time of 100 minutes and 400 minutes for wash water temperatures of ~20°C and ~4°C, respectively, when a 5 % bleach solution is used. This assumes a safety factor of 2 and is based on results from the wash waters that were most difficult to treat. Adjusting the pH of the wash water (following bleach addition) to ensure that it is below pH 9 (rather than below 7) can still decrease the contact time necessary for inactivation. A phosphate buffer

is an effective way to lower pH without introducing the hazard of chlorine gas formation, which could result from adding acid in excess of the U.S. NRT published guidelines [9].

Because wash water generated at an actual cleanup of *B. anthracis* spores will likely have unique characteristics, EPA has developed a procedure for testing the efficacy of chlorine bleach inactivation of *Bacillus* spores in actual wash water generated during site decontamination [10]. In this bench-scale procedure, which should be conducted in a biosafety level 3 laboratory (because virulent spores could be present in the wash water), a known amount of *B. globigii* is added to a known volume of the site-specific wash water (Figure 4). *B. globigii* concentrations are measured before and after chlorine bleach addition to the wash water (with initial, intermediate, and final sampling points), allowing measurement of 99.9999% (or 6 $\log_{10}$ removal) inactivation of the *B. globigii* spores. The method does not rely on measuring treatment of *B. anthracis* spores since levels in the wash water would likely be too low for detection, and, because *B. globigii* is more resistant to chlorine than *B. anthracis* [11], 6 $\log_{10}$ inactivation of *B. globigii* implies an even greater inactivation of *B. anthracis* spores. Results from this bench-scale testing can be used to estimate conservative contact times for full-scale wash water treatment (assuming chlorine concentrations do not decrease substantially) [10].

Acidified Nitrite

Disinfecting large volumes of wash water onsite with traditional drinking water treatment methods or with other published guidelines (e.g., U.S. NRT [7]) could necessitate transporting large quantities of chemicals (e.g., bleach) or placement and installation of large machinery (e.g., ozone or chlorine dioxide generators). Nitrate salts are relatively inexpensive, available in large quantities, and do not require neutralization with a reducing agent before discharge to the municipal sewer (although transporting large quantities of salts may provide alternative challenges, and pH adjustment may be necessary prior to sewer discharge). EPA [12] tested the efficacy of acidified nitrite for spore disinfection with varying pH, temperature, nitrite concentration, and buffer types (Butterfield's or phosphate buffered saline, PBS). Spore inactivation occurred more rapidly at lower pH and was slower in waters at colder temperatures or with a higher ionic strength. At optimal inactivation conditions (room temperature, pH 2 and low ionic strength), acidified nitrite is an adequate substitute for chlorine dioxide or free chlorine, and it is more effective than monochloramine at both optimal and suboptimal conditions. However, at low temperatures and sub-optimal pH conditions, free chlorine is more effective [12].

Water Treatment Units

The EPA HSRP has developed the Water Security Test Bed (WSTB) in conjunction with Idaho National Laboratory for testing decontamination technologies in previously-serviceable drinking water pipes. Water flowing through the WSTB exits into a lagoon, which contains sediment and algae (Figure 5). Experiments conducted in 2015 tested the ability of four different mobile treatment systems (e.g., EPA's Advanced Oxidation Process [AOP] UV-Ozone trailer unit, Figure 6) to treat biologically-contaminated (with *B.*

Figure 2: EPA's Water Security Test Bed lagoon. Haward Saline C™ 6.0 Chlorination System setup on table (left) with effluent entering lagoon during treatment [2].

globigii) "dirty" water, which has an increased disinfectant demand resulting from the dirt and algae. Treatment volumes ranged from 1,250 to 5,000 gallons, with experiments running from 5.5 hours to one day. All treatment units achieved at least a 4 $\log_{10}$ removal (99.99%) of *B. globigii* spores in the lagoon water over the course of the experiments. See Table 1 for a performance summary of the different mobile water treatment devices tested. All of the tested treatment devices can be scaled up (or multiple units could be put into place) to treat larger volumes of water [2].

Table 1. Mobile Water Treatment Device Performance Summary (adapted from U.S. EPA, 2016 [2])

Water Treatment Technology Tested	Capital Cost	Average Log Reduction (*B. globigii*)	Volume Treated (gallons)	Flow (gallons per minute)	Performance Summary
EPA Advanced Oxidative Process Trailer (UV and Ozone)	$40,000	4.0	2,000	5	Immediate disinfection, log reduction was unstable during this study due to experimental challenges.
Solstreme (UV)	$15,000	3.5 to 4.0	2,000	5	Stable, immediate disinfection, easy to transport and set up.
Water Step (Chlorinator)	$8,000	7.0	1,250	not applicable	6-log reduction in 300 min, lowest total treated volume.
Hayward (Chlorinator)	$4,000	4.3	5,000	40	4-log reduction in 1,350 min, under most difficult disinfection conditions.

Additional Challenges and Concerns

Figure 3: EPA Advanced Oxidative Process (AOP) mobile ozone/UV treatment system [2].

Additional considerations may be necessary during decontamination wash water treatment and disposal, depending on the inactivation method employed. For example, disinfectants may react with constituents in the water matrix other than the contaminant of concern, and/or disinfectant concentrations could degrade as a result of environmental exposure (e.g., sunlight). Monitoring disinfectant levels throughout the treatment process may be necessary to ensure that target concentrations are maintained for the duration of the contact time. Moreover, reactions between decontaminants (e.g., chlorine) and organic or inorganic substances in the wash water matrices can form disinfection byproducts that may create a secondary health concern [13]. Air quality monitoring (for spores and other contaminants) can be used to determine if pathogenic bioaerosols are generated during wash water treatment [14].

Following biothreat agent inactivation in decontamination wash water, additional water treatment may be necessary prior to disposal (e.g., before discharging to the publicly-owned water treatment facilities or natural waterways). For instance, the disinfectant may need to be neutralized with a quenching agent (e.g., sodium thiosulfate following disinfection with bleach), or the pH of the treated wash water may need to be adjusted. If present, gloves and other PPE debris may also need to be removed from wash water and disposed of properly. Communication with local water and sewer authorities is necessary to determine acceptability plans before releasing any treated water or runoff into sewers or natural waterways [15].

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Disclaimer

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