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Research article

Inactivation of *Bacillus anthracis* spores to decontaminate subway railcar and related materials via the fogging of peracetic acid and hydrogen peroxide sporicidal liquids

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ABSTRACT

The inactivation of *Bacillus anthracis* spores on subway and used subway railcar materials was evaluated using fogged peracetic acid/hydrogen peroxide (PAA) and hydrogen peroxide (H_2O_2). A total of 21 separate decontamination tests were conducted using bacterial spores of both *B. anthracis* Ames (*B.a.*) and *Bacillus atrophaeus* (*B.g.*) inoculated onto several types of materials. Tests were conducted using commercial off-the-shelf fogging equipment filled with either PAA or H_2O_2 to fumigate a ~15 cubic meter chamber under uncontrolled ambient relative humidity and controlled temperature (10 or 20 °C) from 8 to 168 h. For the present study, no conditions were found that resulted in complete inactivation of either *B.a.* Ames or *B.g.* on all test materials. Approximately 41% and 38% of the decontamination efficacies for *B.a.* and *B.g.*, respectively, exhibited ≥6 $\log_{10}$ reduction (LR); efficacy depended greatly on the material. When testing at 10 °C, the mean LR was consistently lower for both *B.a.* and *B.g.* as compared to 20 °C. Based on the statistical comparison of the LR results, *B.g.* exhibited equivalent or greater resistance than *B.a.* for approximately 92% of the time across all 21 tests. The efficacy data suggest that *B.g.* may be a suitable surrogate for *B.a.* Ames when assessing the decontamination efficacy of fogged PAA or H_2O_2. Moreover, the results of this testing indicate that in the event of *B.a.* spore release into a subway system, the fogging of PAA or H_2O_2 represents a decontamination option for consideration.

1. Introduction

The deliberate release of *Bacillus anthracis* spores in the mail in 2001 led to the contamination of offices, buildings and residences across Washington DC, New York, New Jersey, and Florida. The resulting decontamination and remediation efforts were estimated to be approximately 320 million dollars (Canter et al., 2009, 2005). From the knowledge gained during cleanup and remediation of the 2001 bioterrorist attack, it has been suggested that the process include characterization, decontamination, and source reduction (Canter et al., 2009).

In the event of a wide area release of *B. anthracis* spores within a subway system, rapid remediation will be a challenge due to the vast and complex system of tunnels and platforms. Moreover, the abundance of porous structural materials (e.g., concrete) in a subway system may be problematic for surface treatment. Additionally, there are several factors that should be considered if cleanup and remediation of a subway system is needed following the intentional release of a biological agent. These include surface area and volume of the contaminated space, composition and porosity of contaminated surfaces, environmental conditions (temperature and relative humidity), type of decontaminant(s), and method of delivery (e.g., fumigant).

In the event of a biological contamination incident with *B. anthracis* spores or other agent, there are several considerations that must be made to implement the best strategy for large-scale remediation (Krauter et al., 2011; Rogers et al., 2008). For example, a volumetric decontamination approach (e.g., gases and vapors) is advantageous when decontaminating rooms or buildings that contain complex surfaces within a sealed area. However, this approach often requires large, expensive equipment requiring experienced and knowledgeable operators. For decontamination of smaller areas and surfaces, fogging of liquids and sporicides using commercial off-the-shelf (COTS) equipment is a strategy that could

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be implemented with less training and expertise than would be required for fumigation.

The inactivation of vegetative bacteria, viruses, and bacterial spores has been demonstrated using commercially-available fogging machines generating aerosolized droplets of hypochlorous acid (Clark et al., 2006; Park et al., 2007), quaternary ammonium compounds (Friedman et al., 1968), peroxymonosulfate (Dunowska et al., 2005), peracetic acid (Wood et al., 2013), and hydrogen peroxide/silver nitrate (Taneja et al., 2011). These fogging demonstrations have been mainly employed for disinfecting healthcare environments. Whereas the use of fogging as a sporicide against *B.a.* and surrogate spores has not been extensively investigated. One such study used a pilot-scale test chamber to evaluate the decontamination efficacy of fogging a peracetic acid (PAA) solution (Wood et al., 2013) against a *B. antharcis* surrogate spore. The present study builds on the previous work by evaluating additional experimental parameters that may impact efficacy. These include (but not limited to) the use of two types of foggers, fogging greater/varying amounts of sporicidal solution to seek improved efficacy, a wider range of materials, an additional sporicide (aqueous hydrogen peroxide), and a virulent microorganism.

In this investigation, the efficacy of fogging two sporicidal liquids, PAA and hydrogen peroxide (H_2O_2) solutions, to inactivate *B. anthracis* Ames and potential surrogate *B. atrophaeus* spores at different temperatures was evaluated on both used subway car materials and common building materials. Both microorganisms were included to compare their resistance to inactivation by the PAA and H_2O_2 fog. Similar resistance would allow the use of *B. atrophaeus* in lieu of *B. anthracis* in future decontamination studies using PAA and H_2O_2 fog. The results of this investigation provide decontamination stakeholders and decision-makers with data on the effectiveness of dispersing sporicidal liquids as a fog onto subway materials as a function of the spore contaminant, material type, temperature, equipment type, and sporicidal liquid. Moreover, implementation of fogging could provide an easier and inexpensive approach to decontaminate an area in the event of a wide-area *B. anthracis* release.

2. Materials and methods

2.1. Test organisms

Bacillus anthracis Ames spores (referred hereafter as *B.a.*) were prepared by fermentation as previously described (Rogers et al., 2005). Lyophilized *B. atrophaeus* (ATCC 9372; formerly *B. subtilis* var. niger and *B. globigii*) spores (referred hereafter as *B.g.*) were prepared in sterile phosphate-buffered saline containing 0.1% Triton X-100 surfactant (PBST). Both preparations were diluted to approximately 1×10^9 colony-forming units (CFU) mL^{-1} in sterile-filtered water, and stored at 2–8 °C.

2.2. Test materials and inoculation

Test materials were prepared from a retired subway railcar and included used railcar carpet, Mylar® coating (used on glass windows), aluminum seat back, rubber flooring, seat upholstery, fiberglass interior siding, new railcar air filter, and a used railcar air filter. Other common building materials (unpainted concrete and new industrial carpet) were also used for decontamination testing. In addition, both new and used grease (from a railcar) were used as a coupon when applied to a glass slide. Materials (Fig. 1) were cut into equally sized coupons (pieces), and then were sterilized. All coupons were sterilized via autoclave (121 °C, 60 min), with the exception of the carpet coupons, which were sterilized by electron beam (E-beam) irradiation (~200 kGy; E-Beam Services, Inc., Lebanon, Ohio).

Coupons of railcar carpet, aluminum, upholstery, rubber flooring, Mylar® coating, fiberglass, new cabin air filter, and used cabin air filter coupons were 1.9 cm × 3.8 cm. Glass, new industrial carpet, and unpainted concrete coupons were 1.9 cm × 7.6 cm. New or used grease (1 mL) was applied to glass coupons. The grease test materials were prepared by first applying 1 mL of grease using a 3 mL syringe at one end of the glass material. The grease was then spread across the test material using a sterile colony spreader, creating a thin film, and then the target organism was applied in an identical manner as other test materials, designated as spores on top (SOT). For the "coupon" where the spores were mixed, or encapsulated, into the clean grease, after the spore inoculum was dried, a sterile glass rod was used to mix the dried spores into the grease using a circular motion across the glass. All coupons were inoculated inside a Class II biological safety cabinet (BSC) with ten droplets, 100 μl total volume, of the stock suspension, yielding an inoculum of approximately 1×10^8 CFU per coupon. All coupons were transferred to a Class III BSC and left undisturbed overnight to dry under ambient conditions, approximately 22 °C and 40% relative humidity (RH).

2.3. Decontaminants and application procedures

The sporicidal liquids used for decontamination testing included a ready-to-use solution of PAA (Minncare® Cold Sterilant; 22% H_2O_2/4.5% PAA; Minntech Corporation, Minneapolis, MN) and H_2O_2. Three concentrations of H_2O_2 in aqueous solution (8%, 22%, and 35%; Bioquell HPV-AQ; Bioquell, Horsham, PA) were tested (Table 1). The 8 and 22% solutions were prepared fresh on each day of testing while the 35% stock solution was used as received.

Two commercially-available fogging technologies were utilized for dissemination of the PAA and H_2O_2. The Sani-Tizer 3001-1 (Curtis Dyna-Fog Ltd., Jackson, GA) was equipped with a one-gallon tank, three spray nozzles, and a rotary knob for control of liquid flow rates. All testing conducted used the low flow setting as indicated on the rotary knob and resulted in flow rates ranging from 63 to 187 mL min^{-1}. The Minncare mini Dry Fog System (Mar Cor Purification, Plymouth, MN), was equipped with one spray nozzle, 500 mL liquid reservoir, and an in-line regulator to maintain pressure at the nozzle. The device required a controlled pressure of 75 pounds per square inch (psi) change to metric as well as minimum flow rate of 56 L min^{-1}. Pressure was measured using a Dwyer DPG-205-NIST (Dwyer, Michigan City, IN). Flow rate was measured using an Aalborg GFM47 flow meter (Aalborg Instruments and Controls, Orangeburg, NY). Data from these devices was recorded every minute during operation using a UX120-006M HOBO data logger.

Decontamination testing was conducted inside the Aerosol Research and Component Assessment (ARCA) test chamber (Rogers et al., 2009), with the supply and exhaust system closed to create a sealed, static chamber. On the day following inoculation, coupons intended for decontamination (including blanks) were transferred into the ARCA and placed in one of five designated positions (three horizontal, one vertical, and one inverted position). That is, one replicate spore-inoculated coupon of each material was placed at each of the five locations in the chamber. One position was located off the main chamber. This position was selected to challenge the ability of the decontaminant fog through a more complex area, as the duct was off set from the main ARCA test chamber.

The fogging technology selected for each test was placed in the center of the ARCA chamber with the nozzles positioned upwards. A measured amount of sporicidal liquid was placed into the liquid reservoir and each technology operated until all the liquid had been disseminated. After the specified contact time, the exhaust and