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## ORIGINAL ARTICLE

# Fumigation of a laboratory-scale HVAC system with hydrogen peroxide for decontamination following a biological contamination incident

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## Keywords

anthrax, *Bacillus anthracis*, biological agent, decontamination, fumigation, heating, ventilation and air conditioning, hydrogen peroxide.

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## Abstract

**Aims:** To evaluate hydrogen peroxide vapour (H<sub>2</sub>O<sub>2</sub>) for its ability to inactivate *Bacillus* spores within a laboratory-scale heating, ventilation and air-conditioning (HVAC) duct system.

**Methods and Results:** Experiments were conducted in a closed-loop duct system, constructed of either internally lined or unlined galvanized metal. Bacterial spores were aerosol-deposited onto 18-mm-diameter test material coupons and strategically placed at several locations within the duct environment. Various concentrations of H<sub>2</sub>O<sub>2</sub> and exposure times were evaluated to determine the sporicidal efficacy and minimum exposure needed for decontamination. For the unlined duct, high variability was observed in the recovery of spores between sample locations, likely due to complex, unpredictable flow patterns within the ducts. In comparison, the lined duct exhibited a significant desorption of the H<sub>2</sub>O<sub>2</sub> following the fumigant dwell period and thus resulted in complete decontamination at all sampling locations.

**Conclusions:** These findings suggest that decontamination of *Bacillus* spore-contaminated unlined HVAC ducts by hydrogen peroxide fumigation may require more stringent conditions (higher concentrations, longer dwell duration) than internally insulated ductwork.

**Significance and Impact of the Study:** These data may help emergency responders when developing remediation plans during building decontamination.

## Introduction

Deliberate dissemination of *Bacillus anthracis* spores through letters processed by the U.S. Postal Service in the fall of 2001 led to the contamination of numerous offices, buildings and residences across Florida, New Jersey, New York and the Washington DC area (Canter 2005; Canter *et al.* 2005; US Department of Justice 2010). Response to the contamination incidents and the resulting remediation required unprecedented cross-government efforts, utilizing several decontamination agents and tools to remediate the affected areas (Canter *et al.* 2005; Campbell *et al.* 2012).

Surface treatment methods utilizing liquid sporicides were used for small areas where minimal contamination (thought to be cross-contamination) occurred (Canter *et al.* 2005). Fumigation was used as the primary means to decontaminate areas where direct contamination was suspected. Fumigation-based approaches are advantageous for decontaminating the inside of buildings because they are easily dispersed, penetrate into difficult to access areas, decontaminate the interior volume of the space (not only surfaces) and are less labour intensive than many spray-based approaches (Campbell *et al.* 2012). Three different fumigant agents were used in the Amerithrax 2001

remediation—paraformaldehyde, gaseous chlorine dioxide and hydrogen peroxide vapour (H<sub>2</sub>O<sub>2</sub>) (Canter *et al.* 2005). Of the seven mail facilities and office buildings fumigated, H<sub>2</sub>O<sub>2</sub> was used in two (Canter *et al.* 2005). At that time, the optimal application conditions (concentration, contact time, temperature and relative humidity) for this fumigant were unknown.

The germicidal properties of hydrogen peroxide have been demonstrated previously (Yoshpe-Purer and Eylan 1968; Gasset *et al.* 1975; Wardle and Renninger 1975). More recently, however, several studies have shown that H<sub>2</sub>O<sub>2</sub> can be used as an environmentally friendly sterilant, as its decomposition products are oxygen and water. Although H<sub>2</sub>O<sub>2</sub> has a lower capacity to penetrate materials when compared to other fumigants (Beswick *et al.* 2011; Jia *et al.* 2013), it has wide material compatibility (Rogers *et al.* 2005) and is a safe alternative to more carcinogenic fumigants. Accordingly, H<sub>2</sub>O<sub>2</sub> has been used widely for biodecontamination of healthcare, pharmaceutical, animal and food industry facilities (Klapes and Vesley 1990; McDonnell and Russell 1999; Krause *et al.* 2001; McDonnell *et al.* 2002; Johnston *et al.* 2005). Additionally, decontamination efficacy has been demonstrated for H<sub>2</sub>O<sub>2</sub> against a wide range of micro-organisms, including vegetative bacteria (French *et al.* 2004; Kahnert *et al.* 2005; Rogers *et al.* 2008), viruses (Heckert *et al.* 1997), prions (Fichet *et al.* 2007) and more recently against bacterial spores (Johnston *et al.* 2005; Rogers *et al.* 2005, 2009).

While laboratory testing of H<sub>2</sub>O<sub>2</sub> has shown efficacy against aqueous suspensions of virulent *Bacillus* spores on both a small (Rogers *et al.* 2005) and large scale (Rogers *et al.* 2009), little is known about whether differences exist in the inactivation of spores on surfaces where they were deposited as an aerosol, resembling the dry powder aggregate that formed over surfaces in the 2001 anthrax incidents. Thus, the primary objective of this study was to investigate the decontamination efficacy of H<sub>2</sub>O<sub>2</sub> in the deactivation of aerosol-deposited *Bacillus subtilis* spores inside a laboratory-scale heating, ventilation and air-conditioning (HVAC) duct system. HVAC duct systems are integral components of most commercial and public buildings, as they circulate air and maintain acceptable indoor air quality and temperature control. As such, HVAC duct systems are a potential hot spot for contamination following an incident and therefore can potentially increase the risk of exposure to contaminants if not properly decontaminated following a biological attacks (Hitchcock *et al.* 2006). In the current study, both unlined and internally lined galvanized HVAC duct systems were included in the evaluations of H<sub>2</sub>O<sub>2</sub> decontamination effectiveness.

## Materials and methods

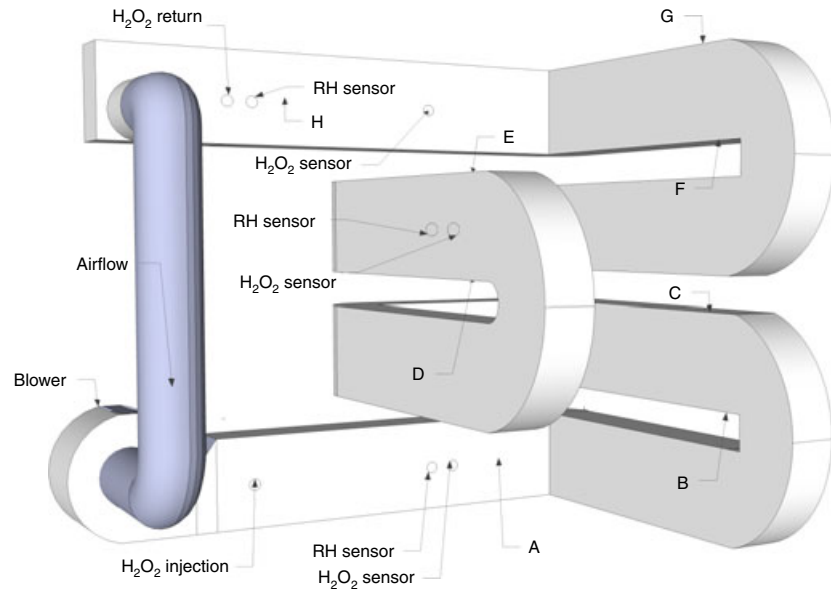
### Laboratory-scale HVAC duct system

To account for the two common types of HVAC duct environments in commercial and residential structures, a closed-loop duct was constructed in both unlined and lined forms. Both duct systems were constructed of 18-gauge galvanized steel (P/N 01170, Eastcoast Metal Distributors; Durham, NC) and included a series of straight sections and elbows to represent the complexities of actual HVAC duct systems. More specifically, the ducts consisted of ten 16" high by 8" wide straight duct sections, one 10" round duct section, 3 round 180° elbows, 6 square ell 90° turns and 1 radial ell (Fig. 1). The equivalent length of the ducts based on friction losses to the fittings was 539 ft, and the actual overall duct length was 72 ft. For tests with lined ductwork, Knauf Sonic XP 1.5# 1" fibreglass acoustic duct liner (Knauf Insulation; Shelbyville, IN) was used to cover all interior surfaces of the duct. An electric blower (Model 7C651, modified with 1/2 horsepower variable frequency inverted duty motor; Dayton Electric Manufacturing Co.; Niles, IL) was used to recirculate air (and fumigant) within the ductwork at two speeds (blower operated at 15 Hz or 60 Hz) during testing with the unlined duct. For the lined duct, the slower speed (blower operated at 15 Hz) was used for all tests. Eight sample ports were located at various points along the length of the duct to allow coupon holders (5 coupons per test holder; Fig. 2) to be inserted flush onto the inside of the duct. Differential pressure rates and air velocities were measured at the various sampling ports using an AirData™ Multimeter ADM-860 electronic micromanometer (Shortridge Instruments, Inc.; Scottsdale, AZ).

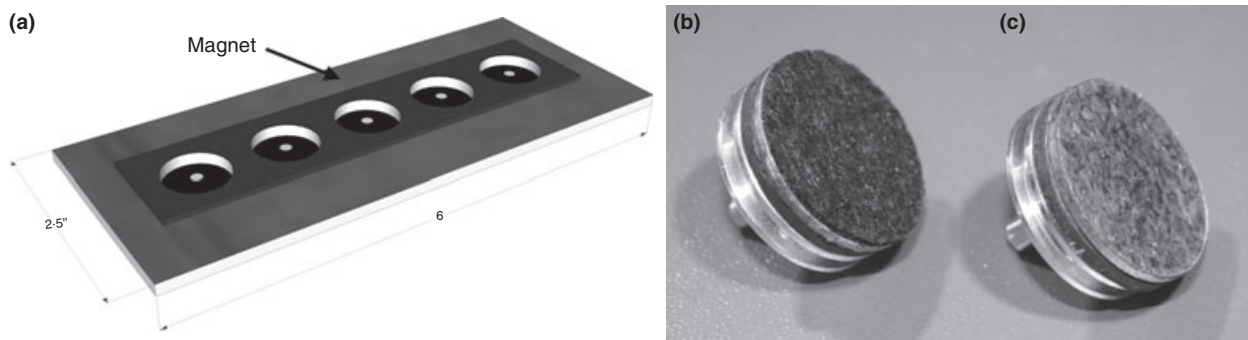
### Hydrogen peroxide fumigation and test conditions

H<sub>2</sub>O<sub>2</sub> was generated using a STERIS VHP® 1000ED generator (VHP; Steris Life Sciences; Mentor, OH) loaded with a 35% hydrogen peroxide Vaprox® cartridge. H<sub>2</sub>O<sub>2</sub> was injected at the duct blower outlet using the VHP fumigation cycle (~24 total hours), which was programmed to include 3 operational phases: conditioning, decontamination and aeration.

During the conditioning phase, the STERIS VHP® 1000ED pulled 17 actual cubic feet per minute (acfm) of air from the duct and pushed it through a desiccator and a high-efficiency particulate air (HEPA) filter. H<sub>2</sub>O<sub>2</sub> was then injected at a variable rate (1–12 g min<sup>-1</sup>) into the dry, filtered air, and the air was returned to the duct. In the decontamination phase, a target concentration (50 or



**Figure 1** Heating, ventilation and air-conditioning duct system design. Schematic of laboratory-scale HVAC duct system. Shown are locations of  $\text{H}_2\text{O}_2$  injection and return ports,  $\text{H}_2\text{O}_2$  and RH sensors and spore sampling locations indicated by letters A–H.



**Figure 2** Test coupon holder and test coupons. Image of the test coupon holder (a) which held four test coupons and one negative coupon at each sample location. Magnetic seals were used to ensure alignment of the coupon holders with their corresponding holes in the duct. Photograph of representative lined (b) and unlined (c) coupons. Coupons were 18 mm in diameter and affixed to SEM stubs.

250 parts per million by volume (ppmv) of  $\text{H}_2\text{O}_2$  in air was maintained automatically by the STERIS VHP<sup>®</sup> 1000ED using a variable  $\text{H}_2\text{O}_2$  injection rate. Once the target concentration was achieved, this condition was maintained for either 90 or 240 min (decontamination phase). The aeration phase was used to reduce and ultimately remove the hydrogen peroxide vapour from the duct. It consisted of two stages, one controlled by the STERIS VHP<sup>®</sup> 1000ED and one by a portable data acquisition system (IOtech Personal Daq/56, Measurement Computing Corp.; Norton, MA) controlled by LabVIEW (National Instruments Corp.; Austin, TX). For the STERIS aeration stage,  $\text{H}_2\text{O}_2$  injection ceased and the recirculation flow of dry HEPA-filtered air through a platinum metal group catalytic converter at 17 acfm continued for 4 h to reduce the  $\text{H}_2\text{O}_2$  concentration within the duct. After the 4 h, a pressure relief blower was used to remove air from the duct and pass it through

activated carbon before its release to the environment. Laboratory air was used to replace air removed from the duct. To monitor the concentration of  $\text{H}_2\text{O}_2$  in the duct during testing, three  $\text{H}_2\text{O}_2$  electrochemical sensors (model B12-34-6-1000-1, Analytical Technology Corp.; Collegeville, PA) were installed at three locations along the length of the duct (Fig. 1). The sensor placed closest to the  $\text{H}_2\text{O}_2$  injection site (near sample location A) controlled an automatic feedback loop for the  $\text{H}_2\text{O}_2$ ; once the sensor indicated that the target  $\text{H}_2\text{O}_2$  concentration had been reached, the injection solenoid valves closed and were switched to bypass. This sensor was also used for the determination of the concentration time (CT; ppmv\*hours).

Tests were performed at 250 ppmv for 4 hrs (for a total CT of 1000 ppmv\*hours) in the unlined HVAC duct. For the lined duct, tests were performed at either 1.5 or 4 h and exposed to either 50 ppmv or 250 ppmv,

respectively. Lined Tests 1, 2 and 3 were expected to achieve 1000, 375 and 75 ppmv\*hours, respectively. Due to a significant H<sub>2</sub>O<sub>2</sub> desorption from the lining during the aeration phase, the lined duct was actually exposed to a total of 1763, 1400 and 278 ppmv\*hours, respectively, over these tests. RH within the duct was monitored, but not controlled. During fumigation, minor increases in RH were observed, yet RH values varied less than 10% throughout the entire duct. Such increases were likely due to H<sub>2</sub>O<sub>2</sub> degradation into water and oxygen as it passed through the duct. A summary of the test conditions are presented in Table 1.

### Bacterial spore preparation

Spores of *B. subtilis* (ATCC 19659; Manassas, VA) were used in combination with silicon dioxide particles as a surrogate for the biological agent, *B. anthracis*. Spore preparations were obtained from the U.S. Army Edgewood Chemical Biological Center (ECBC) and have been described previously (Carrera *et al.* 2005).

### Coupon preparation and inoculation

Eighteen-millimetre-diameter circular test coupons were prepared from the galvanized steel (18-gauge) and fibreglass duct liner used in the creation of the duct system (Fig. 2). To create the liner test coupon, a 1-mm-thick section of the liner was affixed atop a galvanized coupon using double-adhesive tape (P/N 16073-2, Ted Pella, Inc.; Redding, CA). Both steel and liner coupons were fastened to 11-mm-diameter aluminium stubs (P/N 16119, Ted Pella, Inc.) using an adhesive-backed magnet (P/N 5775K8,

McMaster-Carr; Atlanta, GA). Galvanized coupons were sterilized prior to use by subjecting them to a 1-h gravity autoclave cycle at 121°C and 103 kPa. Liner coupons were sterilized by exposure to ethylene oxide (Anderson EOGas AN333 Sterilization System; Haw River, NC).

HVAC coupons were placed on a sterile stub (18-mm-diameter SEM pin stub; Ted Pella, Inc.) prior to inoculation. Coupons were inoculated with *B. subtilis* spores from metered dose inhalers (MDIs) using a previously described method (Lee *et al.* 2011). Briefly, the MDI was loaded into a stainless steel actuator, which was used to provide a dose containing  $\sim 1 \times 10^8$  aerosolized spores per actuation. Following inoculation, the coupons were aseptically transferred to new sterile stubs. To verify that coupon inoculums were consistent within an experiment, at least 8 positive control coupons (not fumigated) were also inoculated, four both at the beginning and end of the coupon inoculation sequence, and for some experiments, four were also inoculated during the middle as well. These positive control coupons were inoculated, sampled and analysed in the same manner as the test coupons. For the eight sample locations, 32 test coupons, eight negative controls (not inoculated with spores, but exposed to H<sub>2</sub>O<sub>2</sub>) and 8–12 positive controls were used. A set of five coupons (4 test and 1 negative) were collocated on a test coupon holder (Fig. 2) and inserted at each sampling location immediately before the start of each test.

### Sample extraction and spore recovery

Sampling of coupon surfaces was conducted the day following fumigation. Test coupons were aseptically

**Table 1** Summary of test parameters and fumigation conditions

Test no.	Concentration (ppmv)	Exposure time (min)	CT (ppmv*hours) Target; Actual	Lined
1	250	240	1000; NA*	No
2	250	240	1000; 1250	No
3	250	240	1000; 1100	No
4	250	90	375; 482	No
5	250	90	375; 486	No
6	250	240	1000; 1250	No
1	250	240	1000; 1763	Yes
1p†	Residual	1440	NA; 631	Yes
2	250	90	375; 1400	Yes
2p†	Residual	1440	NA; 569	Yes
3	50	90	75; 278	Yes
3p†	Residual	1440	NA; 233	Yes

NA—no target concentration was administered for 'post-test' samples

\*For unlined Test 1, there was a data acquisition failure, leading to unknown fumigation conditions.

†The 'p' at the end of the test ID indicates that the test was conducted during desorption following the test (i.e. post-test) with the similar test ID, where no new H<sub>2</sub>O<sub>2</sub> was injected.

transferred to 50-ml conical tubes, and 10 ml of phosphate-buffered saline containing 0.05% Tween<sup>®</sup>20 (PBST; P/N P3563, Sigma Aldrich; St. Louis, MO) was added. Sample tubes were sonicated for 10 min using a Branson 8510 Ultrasonic Cleaner (Branson Ultrasonics; Danbury, CT) at 44 kHz and 250 watts immediately followed by two continuous minutes of vortexing to further dislodge any viable spores from the coupon surfaces. Following extraction, sequential 10-fold serial dilutions were prepared in PBST and subsequently spread-plated in triplicate onto tryptic soy agar (TSA) plates. Plates were incubated at  $35 \pm 2^\circ\text{C}$  for 18–24 h, and colony forming units (CFU) were enumerated by visual inspection of the plates. When fewer than 30 CFU were detected on the plates, the remainder of the extract was analysed by filtration through  $0.2\text{-}\mu\text{m}$  filters (P/N 130-4020, Nalgene; Rochester, NY) and placed onto TSA plates followed by incubation at  $35 \pm 2^\circ\text{C}$  for 18–24 h.

Spore recovery was determined by comparing the number of recovered spores from positive control coupons and test coupons and was expressed as 'log<sub>10</sub> reduction'. Replicates ( $n = 32$ ) were used to determine the log<sub>10</sub> reduction (LR) values for each test according to Eqn (1):

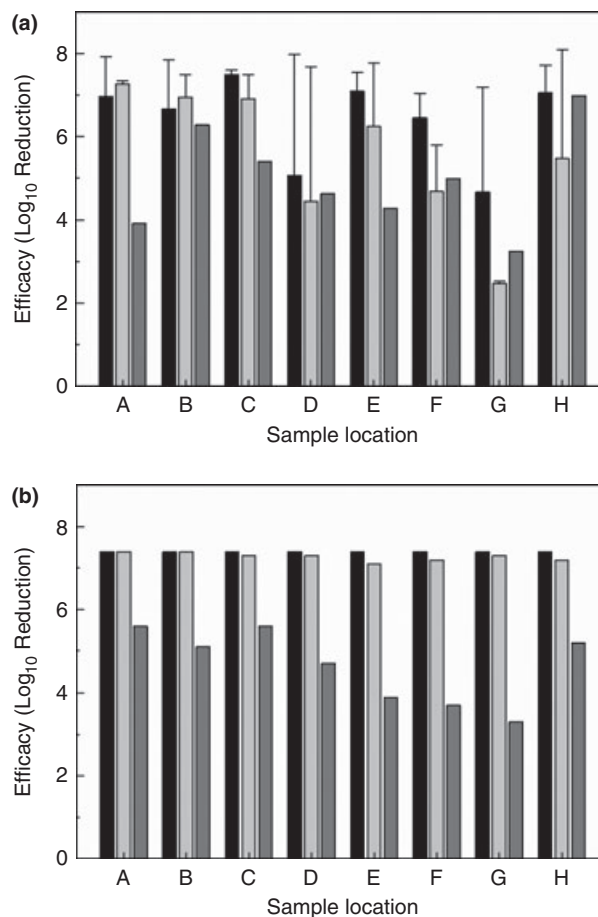
$$\text{Efficacy} = \overline{(\log_{10} \text{CFUc})} - \overline{(\log_{10} \text{CFUt})} \quad (1)$$

where CFUc is the number of CFU recovered from the positive control coupons and CFUt is the number of CFU recovered from the test samples. The mean log<sub>10</sub> recovery values from the 32 replicate test samples were subtracted from the mean log<sub>10</sub> recovery values from the 8–12 positive control coupons.

## Results

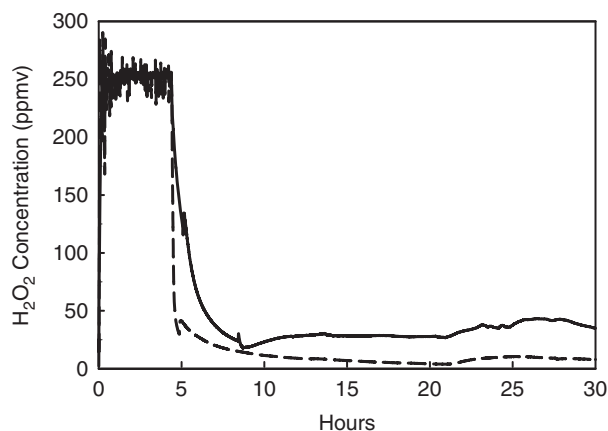
### Unlined duct

The first set of fumigation tests were conducted using a blower speed of 15 Hz and 250 ppmv H<sub>2</sub>O<sub>2</sub> for 4 h as the decontamination phase. Upon exposure of the coupons to the fumigant, the average number of surviving spores (as CFU) recovered per coupon was calculated for each sample point in each test. Across the tests, there was high variability in the postdecontamination recoveries; that is, the achieved efficacy at any particular location varied between the tests. Exposures of shorter duration (90 min vs 240 min) did not result in significant differences in achieved efficacy, suggesting a nonlinear kill curve. When the blower speed was increased roughly 4 times to 60 Hz (250 ppmv for 4 h), increasing the flow velocity within the duct, efficacy was reduced (Fig. 3a). The complex duct design resulted in regions of high flow and areas of lower flow. Average H<sub>2</sub>O<sub>2</sub> concentration across sensors measured at locations A, D/E and H



**Figure 3** Efficacy (log<sub>10</sub> reduction) by sample location within the (a) unlined and (b) lined ducts. Efficacy data are reported as mean log<sub>10</sub> reduction from four replicate coupons per location. Sample locations A through H are shown in Fig. 1. (A) Unlined duct—all samples were exposed to 250 ppmv of VHP. Black bars represent the mean  $\pm$  SD for Tests 1–3 performed with 15-Hz blower speed for 240 min ( $n = 3$ ), light grey bars represent the mean  $\pm$  SD for Tests 4–5 performed with 15 Hz for 90 min ( $n = 2$ ), and dark grey bars represent Test 6 performed at 60 Hz for 240 min ( $n = 1$ ). (Note: sample location B for the 15-Hz, 240-min test is composed of two replicates, instead of three, as the samples were mistakenly absent from the duct during the 3rd exposure). (b) Lined duct (exposure coupons only)—black bars represent Test 1 exposed to 250 ppmv of VHP for 240 min, light grey bars represent Test 2 exposed to 250 ppmv of VHP for 90 min, and dark grey bars represent Test 3 exposed to 50 ppmv of VHP for 90 min.

suggests that there was some degradation of H<sub>2</sub>O<sub>2</sub> in the duct, as levels dropped from about 248 ppm to 219 ppm across the sampling locations within the duct. These local differences, such as the high flow conditions observed at sample location E and low recirculation flow at location D, appear to have influenced decontamination efficacy (Fig. 3a, data not shown). For reasons unknown, samples from locations D and G repeatedly demonstrated the



**Figure 4** H<sub>2</sub>O<sub>2</sub> concentration during exposure and desorption phases for the unlined and lined ducts. Values are representative of a control sensor trace during all phases of exposure for an unlined and lined duct test performed at 250 ppmv for 4 h. The solid line indicates values obtained from the lined duct, and the dotted line represents data from the unlined duct.

lowest efficacies of all sample locations. During the aeration phase, H<sub>2</sub>O<sub>2</sub> concentration rapidly declined to levels close to 0 ppmv within the unlined duct (Fig. 4).

#### Lined duct

Although there was no measureable material absorbance in the unlined duct and the fumigant concentration declined rapidly, the lined duct, in comparison, exhibited a significant desorption during the aeration period (Fig. 4). As a result, the trailing concentration during the aeration phase (about 30 ppmv) contributed to a significant portion (about 75%) of the total H<sub>2</sub>O<sub>2</sub> exposure in the lined duct. Thus, two fumigation durations (1.5 and 4 h) and two exposure concentrations (50 and 250 ppmv) were used to determine the sporicidal effectiveness of H<sub>2</sub>O<sub>2</sub> under these varied conditions. Each test was divided into two distinct exposure periods, the exposure (consisting of the conditioning, decontamination and

aeration phases) and desorption (post aeration phase). Two separate sets of samples were collected during these tests, one set that was exposed to the H<sub>2</sub>O<sub>2</sub> during only the exposure period and a subsequent set that was exposed only during the H<sub>2</sub>O<sub>2</sub> desorption period. For both fumigation tests performed with 250 ppmv of H<sub>2</sub>O<sub>2</sub>, high decontamination efficacy ( $\geq 7.3 \log_{10}$  reduction) was achieved during the initial exposure/aeration phase (Table 2, Fig. 3b) and during the desorption phase (Table 2), with recoveries of less than 10 CFU for all samples in each test. Although different exposure times were used in the tests with 250 ppmv H<sub>2</sub>O<sub>2</sub> (1.5 or 4 h), due to desorption during the aeration phase, the exposure in terms of ppmv\*hours was similar (1400 vs 1760 ppmv\*hours). To evaluate a lower concentration for fumigation, Test 3 used 50 ppmv instead of 250 ppmv H<sub>2</sub>O<sub>2</sub>. Conditions were only moderately effective, however, resulting in a 4.6  $\log_{10}$  reduction during the exposure phase (Fig. 3a). For the coupons exposed during desorption (Test 3), no measurable  $\log_{10}$  reduction was observed (Table 2).

#### Discussion

Following a large-scale biological attack, numerous buildings and indoor areas may be contaminated. Fumigation would be among the strategies likely used to remediate such areas. Although numerous fumigants have demonstrated utility at decontaminating surface types commonly found in open spaces of buildings (walls, floors, etc.) (Rogers *et al.* 2009; Rastogi *et al.* 2010), few data have been reported in the literature for volumetric decontamination of HVAC interiors. One such study using a room-scale air duct investigated the behaviour of hydrogen peroxide vapour within galvanized steel to better understand the factors affecting its use for decontamination (Verge *et al.* 2008). Collection of these data is important as HVAC duct systems are areas that are likely to both concentrate and/or indirectly re-aerosolize contaminants (Verge *et al.* 2008). This study therefore focused on

**Table 2** Mean decontamination efficacy ( $\log_{10}$  reduction) during exposure and desorption phases in an internally lined HVAC duct system

Test	H <sub>2</sub> O <sub>2</sub> concentration (ppmv)	Exposure duration (min)	Total exposure (ppmv*hours)	Efficacy (mean LR)
1	250	240	1763	$\geq 7.4$
1p	Residual*	1440	631	$\geq 7.3$
2	250	90	1400	$\geq 7.3$
2p	Residual*	1440	569	$\geq 7.4$
3	50	90	278	4.7
3p	Residual*	1440	233	0

\*No H<sub>2</sub>O<sub>2</sub> was injected during these tests; exposure values indicate residual H<sub>2</sub>O<sub>2</sub> concentration (approximately 30 ppm)\*time during the postexposure time period. Data were collected from sample location A for Tests 1p and 4p and from locations A and H for Test 2p, respectively.

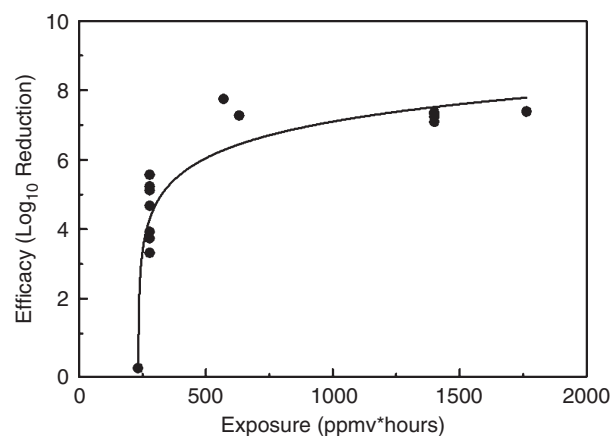


hydrogen peroxide vapour and its effect on the inactivation of spores inside the confined space of an HVAC duct. HVAC duct systems are complex, integral systems found within all building structures, and accordingly, determining effective application conditions for decontamination of *Bacillus* spores is of particular interest to emergency responders and remediation experts.

For the current study, an HVAC duct system was designed and constructed with a variety of elbows and square ell 90° turns, resulting in complex flow patterns expected within real-world HVAC duct systems. The system was designed as a closed loop in order to contain biologicals and more importantly hydrogen peroxide gas within the laboratory environment. In an actual building decontamination, the HVAC duct system may be utilized to distribute H<sub>2</sub>O<sub>2</sub> gas. Further, generated gasses may be recirculated within the building, using existing HVAC ducts, to achieve adequate mixing within the structure. This scenario is similar to the closed-loop system evaluated in our tests. Tests were conducted with ducts containing either no internal liner or an internal acoustic liner. Both lined and unlined ducts are common in commercial, industrial and large residential buildings; unlined ducts are most prevalent in small, detached residential structures.

For the unlined metal duct, the efficacy of fumigation with H<sub>2</sub>O<sub>2</sub> varied spatially within the duct. The complex design of the duct resulted in small variations in localized RH or spikes in H<sub>2</sub>O<sub>2</sub> concentration across experiments and ultimately resulted in significant differences in achieved efficacy at a particular sample location. For a single fumigation condition, the average log<sub>10</sub> reduction per location ranged from 2.95 log<sub>10</sub> reduction to full decontamination ( $\geq 7.57$  log<sub>10</sub> reduction, no viable spores recovered). Flow variation, eddying and flow reversal also may have contributed to the spatial variability in H<sub>2</sub>O<sub>2</sub> concentration and hence efficacy within the duct. Bulk airflow within the duct was estimated at both blower speeds (15 Hz and 60 Hz) from  $\Delta P$  measurements collected by traversing a location upstream of the blower (sample location H) and a location immediately downstream of the blower outlet (sample location A) with a pitot tube (ADM-860 airdata multimeter and airfoil probe, Shortridge Instruments, Inc.; Scottsdale, AZ). For both the lined and unlined ducts, airflow was estimated to be ~270 CFM and ~1150 CFM at 15 Hz and 60 Hz, respectively. The flow was considered turbulent for all test conditions, as Reynolds values (dimensionless number used to predict steady-state air flow or fluid flow patterns) were estimated to be  $\sim 1.5 \times 10^5$  and  $\sim 6.6 \times 10^5$  during operation at 15 Hz and 60 Hz, respectively. At a given blower speed, neither the bulk airflow rate nor the Reynolds values were significantly different between lined and unlined ducts.

The presence of an internal liner resulted in two major differences in the duct environment, when compared to the unlined duct. First, in terms of airflow, there were potentially fewer leaks in the lined duct as the presence of the liner covered gaps in the duct joints. And more importantly, the liner appeared to adsorb and desorb (commonly referred to as off-gassing) the H<sub>2</sub>O<sub>2</sub> over a period of 48 h, leading to much longer aeration times and overall exposure than the unlined duct (~24 h). Thus, in addition to testing the same fumigant conditions as the unlined duct, one additional test was completed to determine the sporicidal effectiveness of the fumigant at low concentration exposure (50 ppmv). Results show that exposures of low levels of H<sub>2</sub>O<sub>2</sub> over long periods of times can be effective at inactivating spores in lined ducts ( $>500$  ppmv\*hours, Fig. 5). The desorption appeared to contribute significantly to H<sub>2</sub>O<sub>2</sub> levels within the duct following the initial fumigation and resulted in higher and more repeatable efficacies than observed in unlined ductwork. For lined Tests 1 and 2, a smaller range of efficacies were observed per sample location (6.7 –  $\geq 7.3$  log<sub>10</sub> reduction), suggesting that the H<sub>2</sub>O<sub>2</sub> concentration within the lined duct was less variable than that within the unlined duct. Given the very high efficacy observed for the coupons following H<sub>2</sub>O<sub>2</sub> exposure, the results show that lined duct was more easily decontaminated than unlined duct at the same target fumigation conditions (Table 3). Additionally, using  $>500$  ppmv\*hours provided very effective decontamination of the lined duct (Test 2p, Fig. 5). An extended duration of H<sub>2</sub>O<sub>2</sub> desorption, however, may increase the amount of time needed before responders or occupants can re-enter the building.



**Figure 5** Efficacy (log<sub>10</sub> reduction) as a function of CT in the lined duct. Log<sub>10</sub> reduction and concentration (ppmv\*hours) plotted for each sample location in the three lined duct tests (n = 27), including both exposure and desorption phases: Test 1 = 250 ppmv, 240 min; Test 2 = 250 ppmv, 90 min; Test 3 = 50 ppmv, 90 min. The logarithmic curve fit of the data is also shown ( $P \leq 0.0001$ ,  $r^2 = 0.8735$ ).

**Table 3** Comparison of results from unlined and lined duct tests. For each set of conditions (250 ppmv H<sub>2</sub>O<sub>2</sub> for 4 h, or 250 ppmv H<sub>2</sub>O<sub>2</sub> for 90 min), decontamination efficacy (LR) from the lined test was compared by *t*-test to efficacy of unlined tests. Exposure (ppmv\*hours) is reported as the cumulative CT over 24 h

Parameter	250 ppmv H <sub>2</sub> O <sub>2</sub> for 4 h				250 ppmv H <sub>2</sub> O <sub>2</sub> for 90 min		
	Lined Test 1	Unlined Test 1	Unlined Test 2	Unlined Test 3	Lined Test 2	Unlined Test 4	Unlined Test 5
H <sub>2</sub> O <sub>2</sub> ppmv*hours	1763	NA	1250	1100	1400	482	486
LR	7.4	7.3	6.3	5.5	7.2	4.7	6.4
P-value	–	0.88	$8.3 \times 10^{-4}$	$2.7 \times 10^{-4}$	–	$4.9 \times 10^{-8}$	0.013

The efficacy of fumigants is influenced by several factors, including environmental conditions, surface material characteristics and spore load (Meszaros *et al.* 2005; Rogers *et al.* 2005; Rastogi *et al.* 2009; Richter *et al.* 2009). Generally, nonporous materials are more easily decontaminated than are porous materials (Henschel 2005; Rogers *et al.* 2005; Rastogi *et al.* 2010; Jia *et al.* 2013). For example, one previous study demonstrated the ability of H<sub>2</sub>O<sub>2</sub> (CT = 500 ppmv\*hours) to achieve complete kill (no viable spores recovered) on galvanized metal (US Environmental Protection Agency 2011). Interestingly, our results demonstrate that decontamination efficacy was higher for lined (porous) duct surfaces than for unlined (nonporous) duct surfaces. One potential explanation of these results is that H<sub>2</sub>O<sub>2</sub> exposure in the unlined duct (at the coupon surface) may have been lower than achieved in the lined duct, as galvanized metal is known to accelerate H<sub>2</sub>O<sub>2</sub> decomposition (Verge *et al.* 2008). In addition, adsorption and subsequent desorption of H<sub>2</sub>O<sub>2</sub> by the duct liner increased the duration of the exposure to H<sub>2</sub>O<sub>2</sub> and thereby may have increased the efficacy of the treatment. Moreover, Rastogi *et al.*, noted a significant decrease in the effectiveness of H<sub>2</sub>O<sub>2</sub> (CT = 870 ppmv\*hours) when the spore concentration on the coupon was increased from  $1 \times 10^6$  or  $1 \times 10^7$  to  $1 \times 10^8$  (Rastogi *et al.* 2009). Results obtained by the current study were consistent with those of Rastogi *et al.*, as a similar inoculum ( $1 \times 10^8$  spores per coupon) on unlined galvanized metal was not easily decontaminated. The extended exposure achieved in the lined duct was apparently able to overcome the challenge of the higher spore load. Collectively, the high spore inoculum and the differences in exposure duration may explain the disparity in decontamination efficacy between the lined and unlined ducts.

In summary, these findings suggest that fumigation with H<sub>2</sub>O<sub>2</sub> shows promise for decontaminating internally insulated ductwork. Although there appeared to be variable remediation in the unlined duct, the results suggest that using H<sub>2</sub>O<sub>2</sub> for longer exposure times can be used as an effective decontaminant within lined duct systems due to desorption. These findings suggest that total H<sub>2</sub>O<sub>2</sub> exposure time is more critical than the mere product of

concentration and time. Lastly, future efforts should consider determining the efficacy of H<sub>2</sub>O<sub>2</sub> in metal ducts under low flow conditions. Such conditions may reduce the complexities of flow regimes within the duct, reduce the spatial variability in efficacy and therefore result in a more comprehensive decontamination.

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### Conflict of interest

No conflict of interest declared.

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