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United States
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Ozone Gas Decontamination of Materials Contaminated with *Bacillus anthracis* Spores

TECHNOLOGY EVALUATION REPORT

Office of Research and Development
National Homeland Security Research Center

EPA/600/R-11/142
November 2011

Technology Evaluation Report

Ozone Gas Decontamination of Materials Contaminated with *Bacillus anthracis* Spores

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

Disclaimer

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development's National Homeland Security Research Center, funded, directed and managed this work through Contract Number EP-C-10-001 with Battelle Memorial Institute. This report has been peer and administratively reviewed and has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use of a specific product.

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Foreword

Following the events of September 11, 2001, addressing the critical needs related to homeland security became a clear requirement with respect to EPA's mission to protect human health and the environment. Presidential Directives further emphasized EPA as the primary federal agency responsible for the country's water supplies and for decontamination following a chemical, biological, and/or radiological (CBR) attack. To support EPA's mission to assist in and lead response and recovery activities associated with CBR incidents of national significance, the National Homeland Security Research Center (NHSRC) was established to conduct research and deliver products that improve the capability of the Agency and other federal, state and local agencies to carry out their homeland security responsibilities.

One goal of NHSRC's research is to provide information on decontamination methods and technologies that can be used in the response and recovery efforts resulting from a CBR release over a wide area. The complexity and heterogeneity of the wide-area decontamination challenge necessitates the understanding of the effectiveness of a range of decontamination options. In addition to effective fumigation approaches, rapidly deployable or readily available surface decontamination approaches have also been recognized as a tool to enhance the capabilities to respond to and recover from such an intentional CBR dispersion.

Through working with ORD's program office partners (EPA's Office of Emergency Management and Office of Chemical Safety and Pollution Prevention) and Regional on-scene coordinators, NHSRC is attempting to understand and develop useful decontamination procedures for wide-area remediation. This report documents the results of a laboratory study designed to better understand the effectiveness of ozone fumigation to decontaminate materials contaminated with *Bacillus anthracis* spores; data are also presented on the decontamination efficacy for materials contaminated with *Bacillus subtilis* spores.

These results, coupled with additional information in separate NHSRC publications (available at www.epa.gov/nhsrc) can be used to determine whether a particular decontamination technology can be effective in a given scenario. NHSRC has made this publication available to the response community to prepare for and recover from disasters involving chemical and/or biological contamination. This research is intended to move EPA one step closer to achieving its homeland security goals and its overall mission of protecting human health and the environment while providing sustainable solutions to our environmental problems.

Jonathan Herrmann, Director
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Acknowledgments

Contributions of the following organization and individuals (reviewers of this report) are gratefully acknowledged:

United States Environmental Protection Agency (EPA)
Office of Research and Development, National Homeland Security Research Center
Joan Bursey
Sang Don Lee
Eletha Brady-Roberts

EPA Office of Pesticide Programs, Antimicrobials Division
Carlton J. Kempter

United State Department of Energy
Paula Krauter (Sandia National Laboratory)

Battelle

Executive Summary

In this study, ozone fumigation was evaluated with regard to its ability to decontaminate six materials (glass, wood, carpet, laminate, metal ductwork, and painted wallboard paper) inoculated with *Bacillus anthracis* and *Bacillus subtilis* spores (approximately 1 x 10^8 CFU). Decontamination testing was conducted at concentrations of approximately 7,000, 9,000, 9,800, 11,000 and 12,000 ppmv ozone for various contact times (4, 6, 8, 9, or 12 hours) at target levels of 22 ± 5 °C and 75% or 85% relative humidity (%RH) ± 5% RH. Decontamination results are summarized in Tables ES-1 and ES-2. Five replicates of each test material were evaluated at each condition.

Table ES-1. Inactivation (Log Reduction) of *B. anthracis* spores

Target Test Conditions, Ozone/%RH+	Contact Time, hr	Material Type*					
		Glass	Wood	Carpet	Laminate	Metal Ductwork	Wallboard Paper
7,000 ppmv, 85% RH ± 5%	4	4.39	4.13	4.10	3.11	2.33	7.60[a]
	6	2.71	5.04	6.13	2.79	1.98	6.87
	8	6.25	6.70[a]	7.82[a]	4.99	2.95	7.60[a]
7,000 ppmv, 75% RH ± 5%	4	1.86	2.53	2.06	1.51	1.48	5.21
	6	2.33	3.35	3.03	1.65	1.44	6.97
	8	2.33	2.95	4.38	1.58	1.70	6.34
9,000 ppmv,‡ 85% RH ± 5%	4	2.64	6.01	4.31	2.21	1.79	7.17
	6	3.26	6.31	7.93[a]	3.76	2.17	7.66[a]
	8	3.92	6.68[a]	7.93[a]	3.10	2.28	7.66[a]
9,000 ppmv,‡ 85% RH ± 5%	6	4.81	6.34	7.90[a]	2.75	2.81	7.74[a]
	9	5.68	6.71[a]	7.90[a]	3.78	3.90	7.74[a]
	12	7.11	6.71[a]	7.90[a]	2.74	2.55	7.74[a]
9,000 ppmv, 75% RH ± 5%	4	1.72	2.31	2.33	1.27	1.55	3.65
	6	1.70	2.21	3.16	1.27	1.40	5.32
	8	1.88	2.56	4.29	1.49	1.40	5.42
9,800 ppmv, 85% RH ± 5%	6	6.61	6.90[a]	7.92[a]	3.52	3.34	7.84[a]
	9	7.33	6.90[a]	7.92[a]	3.35	4.00	7.84[a]
	12	6.95	6.60	7.92[a]	5.39	4.97	7.84[a]
9,800 ppmv[1], 85% RH ± 5%	6	5.13	6.54[a]	7.76[a]	3.10	2.58	7.48[a]
	9	5.11	6.54[a]	7.76[a]	3.70	3.07	7.48[a]
	12	6.13	6.54[a]	7.76[a]	4.05	3.85	7.48[a]
11,000 ppmv,† 85% RH ± 5%	6	4.04	5.50	7.51	2.27	2.50	7.53[a]
	9	4.16	6.43[a]	7.82[a]	4.43	3.70	7.53[a]
	12	6.05	6.43[a]	7.82[a]	4.92	4.72	7.53[a]
12,000 ppmv,† 85% RH ± 5%	6	6.52	7.04[a]	6.21	5.05	5.88	7.49[a]
	9	6.69	6.74	7.97[a]	6.93	5.79	7.19
	12	7.66	7.04[a]	7.97[a]	5.82	6.50	7.49[a]

[1] Immediately following inoculation, all materials were kept at 85% ± 5% RH for approximately 24 hours prior to introduction of ozone.

* Data are expressed as mean log reduction.

+ Target temperature was 22 °C ± 5 °C.

† Measurements were taken using a different, high level ozone monitor as described in Section 4.2.

‡ Conditions tested twice in order to evaluate longer contact times (i.e., 9 and 12 hours).

[a] Material was completely decontaminated, i.e., no spores were detected on any of the five replicate coupons.

Table ES-2. Inactivation (Log Reduction) of *B. subtilis*

Target Test Conditions, Ozone/%RH[+]	Contact Time, hr	Material Type*					
		Glass	Wood	Carpet	Laminate	Metal Ductwork	Wallboard Paper
7,000 ppmv, 85% RH ± 5%	4	4.51	1.48	1.72	6.49	2.42	6.84
	6	6.51	4.00	2.82	6.57	2.51	7.44[a]
	8	7.60	3.30	2.01	8.02[a]	3.45	7.44[a]
7,000 ppmv, 75% RH ± 5%	4	1.18	0.38	0.83	1.01	0.39	3.07
	6	1.88	1.71	0.87	1.25	0.44	5.81
	8	2.42	0.42	1.60	1.53	0.55	5.17
9,000 ppmv,[‡] 85% RH ± 5%	4	2.84	0.74	2.06	2.78	1.23	3.17
	6	3.87	1.04	2.15	2.48	1.32	5.30
	8	4.60	1.53	2.89	2.91	2.15	5.52
9,000 ppmv,[‡] 85% RH ± 5%	6	6.53	2.62	3.84	7.59[a]	7.17	6.75[a]
	9	6.89	3.41	5.14	7.59[a]	6.86	6.75[a]
	12	7.11	5.87	5.99	7.29	7.47[a]	6.75[a]
9,000 ppmv, 75% RH ± 5%	4	2.77	0.69	1.87	2.70	1.71	5.87
	6	3.91	0.78	2.02	2.50	1.75	4.08
	8	6.28	1.14	2.32	3.84	2.80	6.67
9,800 ppmv, 85% RH ± 5%	6	5.68	4.54	4.35	7.51	6.17	6.87[a]
	9	6.59	3.64	5.34	7.82[a]	7.65[a]	6.87[a]
	12	7.08	5.70	6.69	7.82[a]	6.31	6.87[a]
9,800 ppmv[1], 85% RH ± 5%	6	7.28[a]	6.32[a]	4.97	3.23	5.86	5.97[a]
	9	6.11	6.32[a]	6.84	5.98	6.47	5.97[a]
	12	6.47	6.32[a]	7.05	7.26[a]	6.66	5.97[a]
12,000 ppmv, [†] 85% RH ± 5%	6	6.08	2.80	3.69	7.80[a]	4.69	6.57[a]
	9	7.30	3.77	4.05	7.80[a]	6.42	6.57[a]
	12	7.60[a]	3.13	4.72	7.80[a]	6.26	6.57[a]

[1] Immediately following inoculation, all materials were kept at 85% ± 5% RH for approximately 24 hours prior to introduction of ozone.

* Data are expressed as mean log reduction.

[+] Target temperature was 22 °C ± 5 °C.

[†] Measurements were taken using a different high level ozone monitor as described in Section 4.2.

[‡] Conditions tested twice in order to evaluate longer contact times (i.e., 9 and 12 hours).

[a] Material was completely decontaminated, i.e., no spores were detected on any of the five replicate coupons.

The highest log reductions achieved for *B. anthracis* were on wallboard paper, carpet, and wood, with ≥6 log reduction on over 67% of tests on these materials. (A six log reduction or greater has been proposed as a requirement by EPA for registration as a sporicidal decontaminant[(1)].) For *B. subtilis*, the highest efficacies were achieved on glass, laminate and wallboard paper, with over 54% of the tests on these materials resulting in log reductions ≥6. At the highest concentration (12,000 ppmv) and after 12 hours, greater than 6 log reduction was observed on all materials except laminate (which had a log reduction of 5.82) for *B. anthracis;* and ≥ 6 log reduction on all materials except wood and carpet for *B. subtilis*.

No visible damage to the test materials was observed following ozone fumigation.

Contents

Figures

Tables

Abbreviations/Acronyms

BSC	biological safety cabinet
CFU	colony-forming units
CI	confidence interval
EPA	U.S. Environmental Protection Agency
g/Nm^3	gram/Normal meter cubed
HEPA	high-efficiency particulate air
NHSRC	National Homeland Security Research Center
NIST	National Institute of Standards and Technology
ORD	Office of Research and Development
ppmv	parts per million by volume
QA	quality assurance
QC	quality control
QMP	quality management plan
RH	relative humidity
rpm	revolutions per minute
SE	standard error
sL/m	standard liters per minute
TSA	technical systems audit
WA	work assignment

1.0 Introduction

The U.S. Environmental Protection Agency's (EPA's) National Homeland Security Research Center (NHSRC) is helping protect human health and the environment from adverse impacts resulting from the release of chemical, biological, or radiological agents. With an emphasis on decontamination and consequence management, water infrastructure protection, and threat and consequence assessment, NHSRC is working to develop tools and information that will help detect the intentional introduction of chemical or biological contaminants in buildings or water systems, contain these contaminants, decontaminate buildings, outdoor areas and water systems, and facilitate the disposal of material resulting from cleanups.

NHSRC works in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and permitters; and with the participation of individual technology developers in carrying out performance tests on homeland security technologies. NHSRC evaluates the performance of innovative homeland security technologies by developing test plans that are responsive to the needs of stakeholders, conducting tests, collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and high quality are generated and that the results are defensible. Through this work, NHSRC provides high-quality information that is useful to decision makers in purchasing or applying the tested technologies. It provides potential users with unbiased, third-party information that can supplement vendor-provided information. Stakeholder involvement ensures that user needs and perspectives are incorporated into the test design so that useful performance information is produced for each of the tested technologies.

In this work, the efficacy of ozone fumigation against *Bacillus anthracis* Ames and *Bacillus subtilis* spores applied to glass, wood, carpet, laminate, galvanized metal, and painted wallboard paper was evaluated at elevated (75% and 85%) relative humidity (RH) and 7,000, 9,000, 9,800, 11,000 or 12,000 parts per million by volume (ppmv) ozone. Decontamination efficacy was determined based on the log reduction in viable spores recovered from the inoculated materials (with and without exposure to ozone gas).

2.0 Procedures

This section provides an overview of the procedures that were used for the bench-scale evaluation of ozone fumigation to inactivate *B. anthracis* and *B. subtilis* spores on six different test materials. Testing was performed in accordance with a peer reviewed and EPA approved test and quality assurance plan. The general test approach and methods are summarized in this section.

2.1 Biological Agent

Testing was conducted with *B. anthracis* Ames spores produced at Battelle Biomedical Research Center (West Jefferson, OH); this strain was verified via genotyping by an independent laboratory. Details of the method used to produce these *B. anthracis* spores are published in the Journal of Applied Microbiology.[(2)] Testing was also conducted with *B. subtilis* spores (ATCC 19659) from Battelle stock culture.

2.2 Test Materials

Decontamination testing was conducted using glass, wood, carpet, laminate, galvanized metal, and painted wallboard paper. Information on these materials and associated sterilization approaches is presented in Table 2-1. Coupons of the materials were cut to size (1.9 cm width by 7.5 cm length) from a larger piece of material. Coupons were then sterilized by autoclaving or gamma irradiation; the selected approach (Table 2-1) was based on cost-effectiveness and minimization of the physical alterations of the material. Autoclaving was performed at Battelle following an internal standard operating procedure, and gamma irradiation at ~40 kilogray was conducted by STERIS Isomedix Services (Libertyville, IL). Gamma-irradiated coupons were sealed in 6 mil Uline® poly tubing (Uline, Chicago, IL) to preserve sterility until the coupons were ready for use.

Table 2-1. Test Materials

Material	Lot/Batch/ Observation	Manufacturer/ Supplier Name	Coupon Size, Width x Length	Material Preparation
Glass	C1036	Brooks Brothers Glass, Columbus, OH	1.9 cm x 7.5 cm	Autoclave
Wood (untreated pine)	Generic modeling	West Jefferson Hardware, West Jefferson, OH	1.9 cm x 7.5 cm	Gamma irradiation
Carpet	Shaw EcoTek 6	Grossmans Bargain Outlet; Columbus, OH	1.9 cm x 7.5 cm	Gamma irradiation
Laminate	NA	A'Jack Inc.; Columbus, OH	1.9 cm x 7.5 cm	Gamma irradiation
Metal ductwork (galvanized metal)	NA	Adept Products; West Jefferson, OH	1.9 cm x 7.5 cm	Autoclave
Painted wallboard Paper	05-16-03; Set-E-493; Roll-3	United States Gypsum Company; Chicago, IL	1.9 cm x 7.5 cm	Gamma irradiation

2.3 Coupon Inoculation

Test and positive control coupons were placed on a flat surface within a Class II biological safety cabinet (BSC) and inoculated with approximately 1 x 10^8 colony-forming units (CFU) of viable *B. anthracis* or *B. subtilis* spores per coupon. A 100 µL aliquot of a stock suspension of approximately 1 x 10^9 CFU/mL was dispensed using a micropipette applied as 10 µL droplets across the coupon surface (see Figure 2-1). After inoculation, the coupons were held overnight in a Class III BSC to dry under ambient conditions, approximately 22 °C and 40% RH.

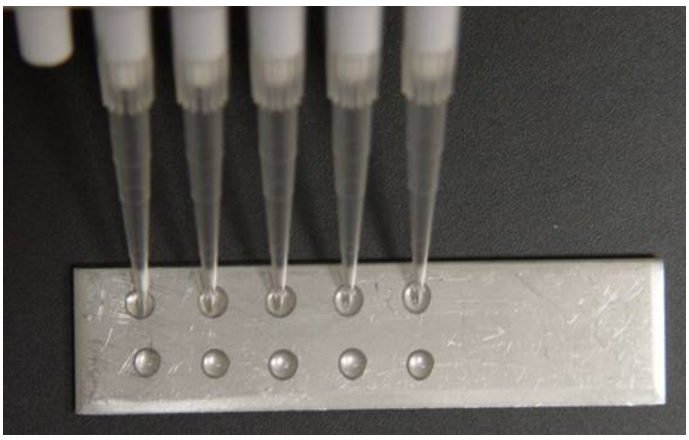

Figure 2-1. Inoculation of coupon using a micropipette.

The number and type of replicate coupons used for each combination of material, ozone concentration, and %RH included were:

- 5 replicate test coupons (inoculated with *B. anthracis* or *B. subtilis* spores and exposed to ozone) per contact time,
- 5 positive controls (inoculated with *B. anthracis* or *B. subtilis* spores, but not exposed to ozone),
- 1 laboratory blank (inoculated only with sterile water and not exposed to ozone)
- 1 procedural blank (inoculated only with sterile water and exposed to ozone for the longest contact time).

2.4 Coupon Extraction and Biological Agent Quantification

For sample extraction, test coupons, positive controls, and blanks were placed in 50 mL polypropylene conical vials containing 10 mL of sterile phosphate-buffered saline extraction buffer containing 0.1% Triton X-100 surfactant (Sigma, St. Louis, MO). The vials were capped, placed on their side and agitated on an orbital shaker for 15 minutes at approximately 200 revolutions per minute (rpm) at room temperature.

Residual viable spores were determined using a dilution plating approach. Following extraction, the extract was removed and a series of 10-fold dilutions was prepared in sterile water. An aliquot (0.1 mL) of either the undiluted extract and/or each serial dilution were plated onto tryptic soy agar in triplicate. The cultures were incubated for 18-24 hrs at 37 ºC ± 2 ºC for *B. anthracis* and 35 °C ± 2 °C for *B. subtilis*. Colonies were counted manually and CFU/mL were determined by multiplying the average number of colonies per plate by the reciprocal of the dilution. Dilution data representing the greatest number of individually definable colonies were expressed as arithmetic mean ± standard deviation of the numbers of CFU observed.

2.5 Calculations

The percent recovery of viable *B. anthracis* and *B. subtilis* spores from each test and positive control coupon was determined in order to ascertain the differential number of spores recovered from test coupons following initial inoculation and completing the fumigation process. Recovery was defined as the total number of viable CFU extracted from each test and positive control coupon relative to the number of CFU inoculated onto each coupon.

The number of CFU/mL (spore density) was calculated from plate counts as:

CFU/mL = CFU counted on plate / volume of spore suspension inoculated onto plate (1)

The number of viable CFU inoculated onto a coupon was calculated as:

CFU/coupon = spore density (CFU/mL) × 0.1 mL inoculation volume/coupon (2)

The number of detected viable CFU extracted from a coupon was calculated as:

Total CFU = [(arithmetic mean CFU plate count × 1/dilution factor)/plated volume] × (extraction buffer volume) (3)

where:

CFU plate count = arithmetic mean of triplicate plates
plated volume = portion of total extraction buffer used to prepare dilutions inoculated onto the plates (0.1 mL)
dilution factor = serial dilution having the greatest number of colonies
extraction buffer volume = 10 mL

Recovery (%) was calculated for the j^{th} individual positive control (or test) coupon within the i^{th} test material (a specific test material) as:

$$Recovery_{ij} = \frac{x_{ij0}}{x_{ij}} \text{ x } 100 \quad (4)$$

where:

X_{ij0} = CFU values in extract samples for j^{th} of five positive control (or test) coupons of i^{th} test material after drying period

X_{ij} = CFU values spiked onto j^{th} of five positive control (or test) coupons of i^{th} test material

The number of CFU of *B. anthracis* spores in extracts of test and control coupons was determined to calculate efficacy of the decontaminant. Efficacy was defined as the extent (as log reduction) by which viable *B. anthracis* spores extracted from test coupons after decontamination were less numerous than the viable *B. anthracis* spores extracted from the associated positive control coupons. The first steps in this calculation were to determine the logarithm of the CFU count value from each coupon, and then the mean of those logarithm values (geometric mean) for each set of positive control and associated test coupons. Efficacy of a decontaminant for a test organism on the i^{th} coupon material was calculated as the difference between those mean log values, i.e.,:

$$Efficacy_{ij} = \overline{(\log CFUc_{ij})} - \overline{(\log CFUt_{ij})} \quad (5)$$

where:

log $CFUc_{ij}$ = j individual logarithm values obtained from positive control coupons
log $CFUt_{ij}$ = j individual logarithm values obtained from corresponding test coupons
———— = geometric mean (the overbar designates a mean value)

In each test, five positive control coupons and five corresponding test coupons (i.e., $j = 5$) were used. In the case where no viable CFU were found in a coupon extract, a CFU count of 1 was assigned, resulting in a log CFU of zero for that coupon.

The variances (i.e., the square of the standard deviation) of the log $CFUc_{ij}$ and log $CFUt_{ij}$ values were also calculated for both the control and test coupons (i.e., S^2c_{ij} and S^2t_{ij}), and were used to calculate the pooled standard error (SE) for the efficacy value calculated in Equation 6, as follows:

$$SE = \sqrt{\frac{S^2c_{ij}}{5} + \frac{S^2t_{ij}}{5}} \quad (6)$$

where:

the number 5, again, represents the number j of coupons in both the control and test data sets.

The significance of differences in efficacy across different coupon materials or treatments was assessed based on the 95% confidence interval (CI) of each efficacy result. The 95% CI is:

$$95\%\ CI = Efficacy \pm (1.96 \times SE) \quad (7)$$

2.6 Surface Damage

The physical effect of the ozone fumigation on the materials was also qualitatively monitored during the evaluation. This approach provided a gross visual assessment of whether the ozone fumigation altered the appearance of the test materials. The procedural control (coupon that is fumigated, but has no spores applied) was visually compared to a laboratory blank coupon (a coupon not exposed to ozone and that has no spores applied). Obvious visible damage might include structural damage, surface degradation, discoloration, or other aesthetic impacts.

3.0 Quality Assurance/Quality Control

Quality assurance/quality control (QC) procedures were performed in accordance with the test/QA Plan developed for this study. The QA/QC procedures and results are summarized below.

3.1 Performance Evaluation Audit

Performance evaluation audits were conducted to assess the quality of the results obtained during these experiments. Temperatures were monitored but efforts were not undertaken to control any of the test temperatures. A performance evaluation audit was not conducted for ozone concentration as appropriate certified standards were not available. Similarly, no performance evaluation audits were performed to confirm the concentration of the *B. anthracis* or *B. subtilis* spores because quantitative standards for these biological agents do not exist. However, the control coupons and blanks support the spore measurements and the stock inoculums were confirmed on each day of testing. Both organisms were obtained in pure culture as described in Section 2.1. and were cultured according to Battelle internal SOPs. Table 3-1 summarizes the performance evaluation audits that were performed.

Table 3-1. Performance Evaluation Audits

Measurement	Audit Procedure	Allowable Tolerance	Actual Tolerance
Temperature	Compared to independent calibrated thermometer	± 2 °C	All <2 °C for 13 instances
Relative Humidity*	N/A	N/A	N/A
Time	Compare time to independent clock or watch value	± 2 sec/hr	0 second/hr for 8 instances

*Relative humidity was measured using the wet/dry bulb thermometer and verification of RH was covered under the temperature Performance Evaluation Audit.

3.2 Technical Systems Audit

Observations and findings from the technical systems audit (TSA) were documented and submitted to the test leader for response. Laboratory QA staff conducted technical systems audits on July 19 and 20, and November 4, 9 and 10, 2010 to ensure that the tests were being conducted in accordance with the appropriate test/QA plan. As part of the audit, test procedures were compared to those specified in the test/QA plan and data acquisition and handling procedures were reviewed. None of the findings of the TSA required corrective action. TSA records were permanently stored with the laboratory QA Manager.

3.3 Data Quality Audit

At least 10% of the data acquired during the evaluation were audited. A QA auditor traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting to ensure the integrity of the reported results. All calculations performed on

the data undergoing the audit were checked.

3.4 QA/QC Reporting

Each assessment and audit was documented in accordance with the test/QA plan. For these tests, findings were noted (none significant) in the data quality audit, but no follow-up corrective action was necessary. The findings were mostly minor data transcriptions errors requiring some recalculation of efficacy results, but none were gross errors in recording. Copies of the assessment reports were distributed to the EPA QA Manager and laboratory staff. QA/QC procedures were performed in accordance with the test/QA plan.

3.5 Deviations from Test/QA Plan

All mean relative humidity levels were within the target range, although there were a few measurements outside the target range; see Section 4.0, Tables 4-1 and 4-2.

A Performance Evaluation Audit was not performed for relative humidity because a hygrometer was not used. The relative humidity audit was covered by the temperature audit since the relative humidity measurements were made using the wet/dry bulb method.

Lock & Lock (HPL838P, Farmers Branch, TX) containers were used to house all positive control and test materials inside the decontamination testing chamber. This method was chosen to ensure that all materials would be open to the ozone gas at the same time instead of exposing each coupon individually.

The positive control recovery (mean CFU ≥5% and ≤120% of spike control) was not attained for the wood positive control (1.26% to 4.96%) on all occasions except for the following:

- 7,000 ppmv ozone, 85% RH, *B. anthracis*
- 9,000 ppmv ozone, 85% RH, *B. anthracis*
- 9,800 ppmv ozone, 85% RH, *B. anthracis*
- 12,000 ppmv ozone, 85% RH, *B. anthracis*

Positive control recoveries for wallboard paper were also below the recovery criterion on three occasions: 9,000 ppmv ozone, 85% RH, *B. subtilis* (3.13%), 9,800 ppmv ozone, 85% RH, *B. subtilis* (2.00%), and 12,000 ppmv ozone, 85% RH, *B. subtilis* (3.22%).

In previous studies with bare pine wood, spore recoveries were also low (9% for *B. anthracis*, 1% for *B. subtilis* spores and 2.2% for *Geobacillus stearothermophilus* spores).[1] While the mechanisms which cause spores to be difficult to recover from porous materials have not been determined, it is possible that spores applied in aqueous suspension are carried via capillary action acting on the liquid into the pits and cavities of the porous matrix thereby making mechanical extraction of the spores from these materials difficult. There were not expected to be any adverse impacts on testing as a sufficient amount of *B. anthracis* spores were recovered ($> 10^6$ CFU) from which to evaluate decontamination efficacy. Percent recovery of spores from wood and wallboard paper positive controls from all other tests was ≥5%.

4.0 Ozone Fumigation Procedures

4.1 Test Matrix and Environmental Conditions

As shown in the test matrix (Table 4-1), ozone fumigation of spores applied to the six material types (glass, wood, carpet, laminate, metal ductwork, and painted wallboard paper) was evaluated at five different ozone concentrations at ambient temperature (approximately 25 ^{o}C) and under two elevated relative humidity levels (75% RH and 85% RH) for 4, 6, 8, 9 and 12 hours. At the 9,000 ppmv concentration, 85% RH was tested twice in order to investigate longer contact times (i.e., 9 and 12 hours). At the 9,800 ppmv concentration, 85% RH was also tested twice. In one instance the testing chamber was pre-humidified and the coupons were held at this condition immediately following inoculation for approximately 24 hours prior to decontamination.

Table 4-1. Target Test Matrix for Ozone Fumigation

Ozone Concentration, Temperature	%RH ± %, full scale	Contact Time, hr
7,000 ppmv, 22 °C ± 5 °C	85% ± 5%	4, 6, 8
	75% ± 5%	4, 6, 8
9,000 ppmv, 22 °C ± 5 °C	85% ± 5%‡	4, 6, 8
	75% ± 5%	4, 6, 8
	85% ± 5%‡	6, 9, 12
9,800 ppmv, 22 °C ± 5 °C	85% ± 5%	6, 9, 12
	85% ± 5%[1]	6, 9, 12[1]
11,000 ppmv*, 22 °C ± 5 °C	85% ± 5%	6, 9, 12
12,000 ppmv, 22 °C ± 5 °C	85% ± 5%	6, 9, 12

[1] Immediately following inoculation, all materials were kept at 85% ± 5% RH for approximately 24 hours prior to introduction of ozone.
‡ Conditions tested twice in order to evaluate longer contact times (i.e., 9 and 12 hours).
* This concentration tested with *B. anthracis* spores only

Testing was performed inside a Class III BSC (The Baker Company, Sanford, ME). A 120 mm fan (Cooler Guys, UF12B12BWL, Kirkland, WA) inside the Class III BSC was used to mix the atmosphere inside the cabinet. RH was increased as needed to achieve the target RH levels by using an ultrasonic fogger system. The custom-designed ultrasonic fogger with a water trap, shown in Figure 4-1, was developed and used to humidify the test chamber. The manually controlled ultrasonic fogger was attached to a polyvinylchloride pipe. Humidified air from inside the pipe was then pumped into the test chamber until the specified RH was reached.

Figure 4-1. Ultrasonic fogging system.

A wet/dry bulb hygrometer (comprised of two National Institute of Standards and Technology [NIST]-traceable thermometers [Fisher 13-990-270]) was fabricated (see Figure 4-2) and used to monitor the temperature (and subsequently relative humidity) every 2 to 15 minutes. The tops of these two high quality thermometers were glued to the backing of an existing hygrometer in order for the measurements to be made using NIST-traceable thermometers. The wick material from the hygrometer was used to wrap one NIST-traceable thermometer bulb (on right in photograph) which was kept constantly wet by immersion in a siphon reservoir (~10 mL H_2O) while the other NIST-traceable thermometer (on left in photograph) gave a dry temperature reading. The fan described above was approximately 12 inches behind the bulbs of the thermometers and provided constant air flow. The actual bulbs of the thermometers were open to the airflow of the test chamber. Differences in the temperature readings from the wet/dry bulb thermometers were used to determine relative humidity with an online humidity calculator [(3)].

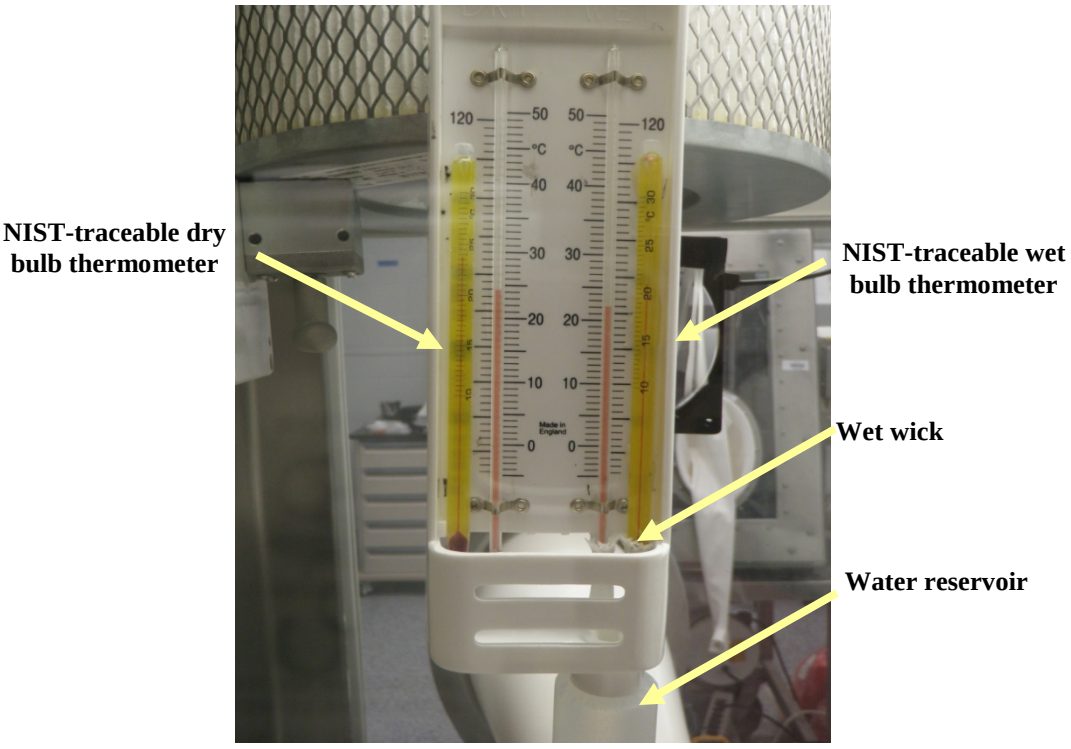

Figure 4-2. Custom-fabricated hygrometer.

Target test temperatures (22 °C ± 5 °C) were attained as measured by the dry bulb thermometer (associated with a wet/dry bulb hygrometer). Measured by the wet/dry bulb hygrometer, relative humidity levels were infrequently above the upper limits of the targeted relative humidity levels, although mean relative humidity levels were all within the target range of either 75% or 85% ± 5 %. Mean ozone concentrations met the target concentration of 7,000, 9,000, 9,800, 11,000 or 12,000 ppmv ozone (±10%). All temperature, relative humidity and concentration data for all the testing are shown in Tables 4-2 and 4-3.

Table 4-2. Summary of Temperature, Relative Humidity and Ozone Conditions for *B. anthracis* Tests

Target Test Conditions, Ozone/%RH[+]	Contact Time, hr	Dry Bulb Thermometer, °C*[†]	Wet Bulb Thermometer, °C*[†]	%RH*[†]	Ozone ppmv*[‡]
7,000 ppmv 85% RH ± 5%	4	25.3 ± 0.5	24.0 ± 0.5	90.0 ± 0.5	7,000 ± 40
	6	25.4 ± 0.5	24.1 ± 0.5	89.6 ± 0.9	7,021 ± 71
	8	25.2 ± 0.6	23.8 ± 0.8	88.4 ± 2.4	7,021 ± 102
7,000 ppmv 75% RH ± 5%	4	24.4 ± 0.4	21.4 ± 0.4	76.9 ± 2.8	7,007 ± 35
	6	24.5 ± 0.4	21.4 ± 0.5	76.3 ± 2.9	7,011 ± 43
	8	24.5 ± 0.4	21.5 ± 0.5	76.3 ± 2.6	7,011 ± 39
9,000 ppmv 85% RH ± 5%	4	24.7 ± 0.5	22.9 ± 0.5	85.6 ± 1.7	9,004 ± 43
	6	24.7 ± 0.5	22.8 ± 0.6	84.9 ± 1.9	9,002 ± 44
	8	24.6 ± 0.5	22.8 ± 0.6	85.0 ± 1.8	9,003 ± 44
9,000 ppmv 75% RH ± 5%	4	24.4 ± 0.7	21.3 ± 0.6	75.8 ± 2.0	9,007 ± 68
	6	24.3 ± 0.6	21.2 ± 0.5	75.9 ± 1.8	9,015 ± 75
	8	24.2 ± 0.6	21.1 ± 0.5	76.4 ± 2.0	9,017 ± 75
9,000 ppmv 85% RH ± 5%	6	25.8 ± 0.5	24.0 ± 0.5	85.9 ± 1.6	8,996 ± 32
	9	25.7 ± 0.5	23.8 ± 0.5	85.5 ± 1.6	8,995 ± 40
	12	25.4 ± 0.8	23.6 ± 0.8	85.4 ± 1.6	8,982 ± 73
9,800 ppmv 85% RH ± 5%	6	25.9 ± 0.5	24.0 ± 0.5	85.2 ± 1.0	9,801 ± 55
	9	25.9 ± 0.5	24.0 ± 0.5	85.3 ± 1.0	9,798 ± 54
	12	25.8 ± 0.6	23.9 ± 0.6	85.2 ± 1.1	9,798 ± 52
9,800 ppmv[1] 85% RH ± 5%	6	26.1 ± 0.4	24.2 ± 0.5	85.4 ± 1.7	9,792 ± 37
	9	26.1 ± 0.4	24.1 ± 0.5	84.7 ± 1.8	9,796 ± 43
	12	25.8 ± 0.7	23.8 ± 0.7	84.5 ± 1.7	9,798 ± 48
11,000 ppmv 85% RH ± 5%	6	25.3 ± 0.3	23.3 ± 0.3	84.4 ± 2.0	10,979 ± 85
	9	25.3 ± 0.4	23.3 ± 0.4	84.2 ± 2.1	10,967 ± 83
	12	25.0 ± 0.7	23.0 ± 0.7	84.3 ± 2.2	10,976 ± 80
12,000 ppmv 85% RH ± 5%	6	24.8 ± 0.5	22.9 ± 0.6	84.4 ± 1.8	11,982 ± 63
	9	24.8 ± 0.5	22.8 ± 0.5	84.7 ± 2.3	11,972 ± 73
	12	24.6 ± 0.6	22.6 ± 0.7	84.7 ± 2.4	11,981 ± 68

[1] Immediately following inoculation, all materials were kept at 85% ± 5% RH for approximately 24 hours prior to introduction of ozone.

*Data are presented as mean ± standard deviation.

[+] Target temperature was 22 °C ± 5 °C.

[†] Measurements were recorded manually every 2-15 minutes.

[‡] Measurements were logged automatically every minute.

Table 4-3. Summary of Temperature, Relative Humidity and Ozone Conditions for *B. subtilis* Tests

Target Test Conditions, Ozone/%RH[+]	Contact Time, hr	Dry Bulb Thermometer, °C*[†]	Wet Bulb Thermometer, °C*[†]	%RH*[†]	Ozone ppmv*[‡]
7,000 ppmv 85% RH ± 5%	4	25.6 ± 0.7	23.8 ± 0.7	86.2 ± 1.8	6,998 ± 32
	6	25.6 ± 0.6	23.8 ± 0.6	86.4 ± 2.3	7,002 ± 36
	8	25.6 ± 0.6	23.9 ± 0.7	86.2 ± 2.4	7,008 ± 51
7,000 ppmv 75% RH ± 5%	4	24.4 ± 0.4	21.3 ± 0.5	75.5 ± 1.9	7,003 ± 18
	6	24.5 ± 0.5	21.4 ± 0.5	75.5 ± 2.0	6,996 ± 33
	8	24.5 ± 0.5	21.4 ± 0.5	75.7 ± 1.9	6,996 ± 29
9,000 ppmv 85% RH ± 5%	4	24.3 ± 0.5	22.3 ± 0.6	84.2 ± 1.8	9,007 ± 33
	6	24.4 ± 0.5	22.4 ± 0.6	84.6 ± 1.7	9,006 ± 35
	8	24.3 ± 0.5	22.4 ± 0.6	85.2 ± 2.1	9,003 ± 35
9,000 ppmv 75% RH ± 5%	4	24.7 ± 0.4	21.6 ± 0.3	76.0 ± 2.2	9,011 ± 48
	6	24.7 ± 0.5	21.6 ± 0.3	76.1 ± 2.4	9,011 ± 48
	8	24.5 ± 0.6	21.5 ± 0.5	76.0 ± 2.3	9,003 ± 43
9,000 ppmv 85% RH ± 5%	6	26.0 ± 0.4	24.0 ± 0.4	85.1 ± 0.8	9,010 ± 44
	9	25.8 ± 0.5	23.8 ± 0.5	85.0 ± 0.9	9,009 ± 40
	12	25.6 ± 0.7	23.3 ± 1.0	84.8 ± 1.1	9,009 ± 38
9,800 ppmv 85% RH ± 5%	6	25.6 ± 0.5	23.7 ± 0.6	85.1 ± 1.9	9,791 ± 39
	9	25.7 ± 0.5	23.8 ± 0.6	85.4 ± 2.0	9,786 ± 39
	12	25.6 ± 0.6	23.6 ± 0.7	85.1 ± 2.0	9,785 ± 43
9,800 ppmv[1] 85% RH ± 5%	6	24.9 ± 0.4	23.4 ± 0.3	88.6 ± 0.8	9,790 ± 39
	9	25.2 ± 0.6	23.7 ± 0.6	88.6 ± 0.6	9,799 ± 40
	12	25.3 ± 0.6	23.9 ± 0.6	88.5 ± 0.6	9,799 ± 39
12,000 ppmv 85% RH ± 5%	6	24.8 ± 0.4	22.9 ± 0.5	85.2 ± 1.5	12,009 ± 106
	9	24.7 ± 0.4	22.8 ± 0.5	84.9 ± 1.4	11,999 ± 101
	12	24.6 ± 0.6	22.7 ± 0.6	85.1 ± 1.7	12,008 ± 94

[1] Immediately following inoculation, all materials were kept at 85% ± 5% RH for approximately 24 hours prior to introduction of ozone.

* Data are presented as mean ± standard deviation.

[+] Target temperature was 22 °C ± 5 °C.

[†] Measurements were recorded manually every 2-15 minutes.

[‡] Measurements were logged automatically every minute.

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4.2 Decontamination Technology Description and Procedures

An Ozone Generator AC-2045 (IN USA, Inc., Norwood, MA) and a Low Concentration Ozone Analyzer IN2000-L2-LC (IN USA, Inc.), shown in Figure 4-3, were initially used in this evaluation. Since the low level analyzer range was applicable for 0-9,999 ppmv, a Mini Hicon-LR (IN USA, Inc.) was used for the higher level concentrations (11,000 and 12,000 ppmv; these ozone levels were not initially planned for testing). In addition, a Smart-Trak® 2 flow controller (Sierra Instruments, Monterey, CA) was used to control the flow of oxygen into the ozone generator. The ozone generator was used per the manufacturer's instructions. Briefly, the oxygen gas (Praxair, Inc., Danbury, CT; with 99.5% oxygen and 0.5% nitrogen) was supplied to the generator through the flow controller at 10 standard liters/minute (sL/m) and then subjected to electrical discharge in the ozone generating cell which produced the ozone gas. The ozone generator was run at maximum power (100%) until the desired concentration was reached. At that time, the power on the generator was reduced to approximately 30% and the oxygen flow reduced to approximately 0.4-0.5 sL/m to provide a small but continuous injection of ozone to keep the concentration at the desired level (e.g. 9,000 ppmv ± 10%). Because high concentrations of ozone are dangerous to humans, ozone levels around the chamber, especially near inlets/outlets, were continuously checked using a low-level ozone monitor (Model A-22, Ozone Solutions, Inc., Hull, IA) when the generator was in use (also shown in Figure 4-3). See Figure 4-4 for a diagram of the ozone fumigation setup.

Figure 4-3. Ozone generator, low concentration ozone analyzer, and low-level ambient monitor.

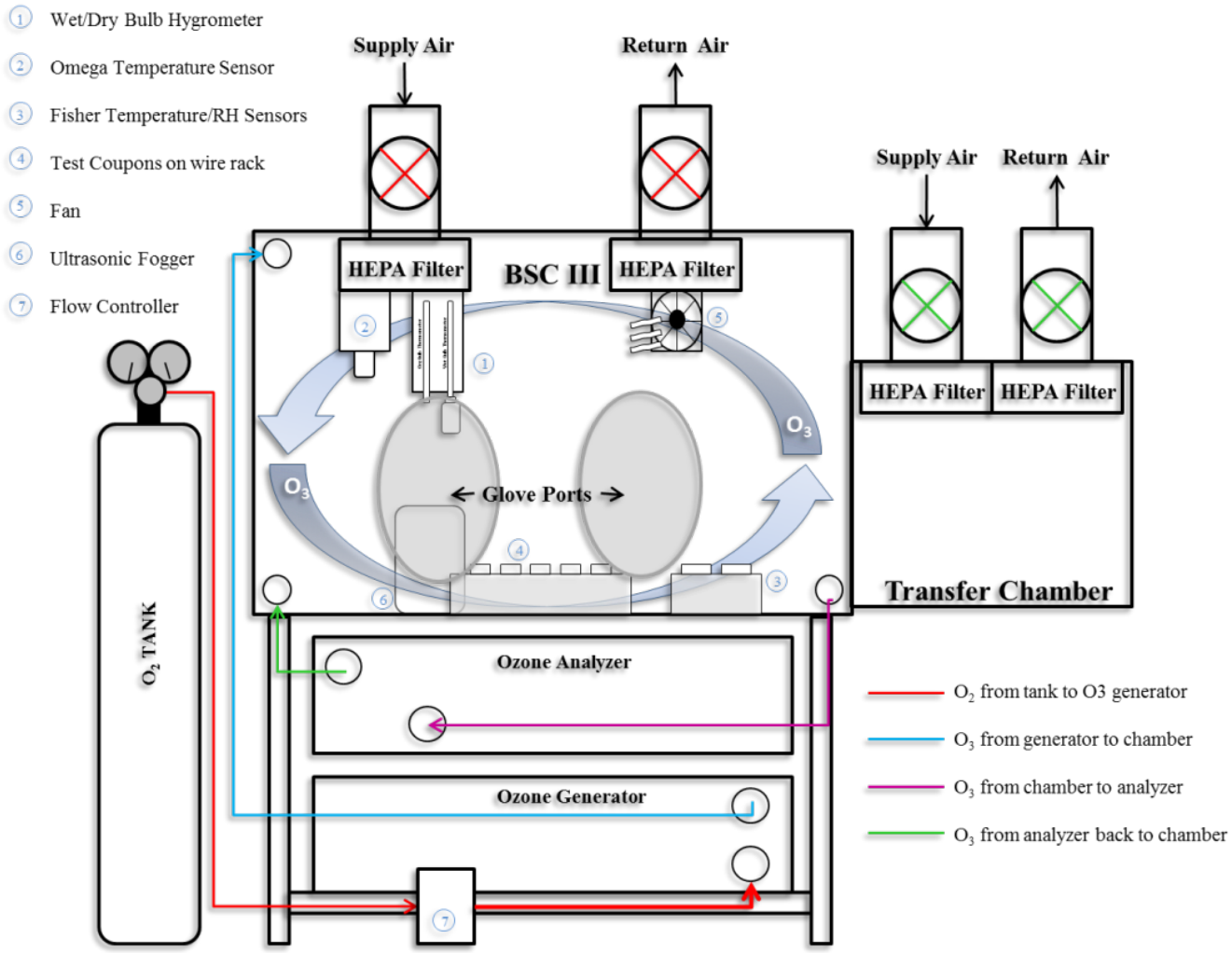


Figure 4-4. Diagram of ozone fumigation setup.

During each fumigation cycle, coupons inoculated with *B. anthracis* or *B. subtilis* spores were placed in sealed containers (Lock & Lock, HPL838P, Farmers Branch, TX) in the Class III BSC. All containers were opened in the Class III BSC until the targeted %RH was achieved. At this point, all containers were closed during the initial injection of ozone. The containers holding the test coupons were opened in sequence in order to achieve an appropriate contact time for exposure to ozone. Contact time was defined as the time from opening the container to the time ozone was exhausted from the Class III BSC. Positive controls and laboratory blanks were also kept in a sealed container (same as the test materials) in the Class III BSC for the full fumigation cycle. The procedural blank coupons were opened for exposure to ozone at the same time as the test coupons with the longest contact time (e.g. 8 or 12 hours).

At the conclusion of each fumigation cycle, the ozone generator was shut down and the ozone was rapidly exhausted from the Class III BSC.

The Low Concentration Ozone Analyzer IN-2000-L2-LC was used to measure ozone concentrations for all testing except for the 11,000 or 12,000 ppmv tests, in which the High Concentration Ozone Analyzer Mini Hicon-LR was used. The Mini Hicon-LR analyzer reads in g/Nm^3 and is calibrated for use between 0 and 50 g/Nm^3. A conversion factor (1 gram/Normal meter cubed [g/Nm^3] = 467 ppmv) was used to determine the concentration in ppmv during fumigation. After results were received from the 11,000 ppmv fumigation test (using *B. anthracis*), the analyzers did not appear to be reading the same concentration of ozone when drawing from the fumigation chamber at the same time. IN USA, Inc. was contacted regarding this issue and explained the differences in concentration readings. The Mini Hicon-LR comes with a pressure and temperature sensor built in; this temperature and pressure compensation is a process that adjusts the measurement of gas to standardize conditions for comparison with other measurements. Therefore, all measurements are normalized to 273K and 1 atm, making the conversion of 1 g/Nm^3 = 467 ppmv relevant. The IN-2000-l2-LC provides ozone concentration data directly in units of ppmv.

In addition, the IN-2000-L2-LC was initially calibrated on July 9, 2009, and again on 10/20/2010 by the manufacturer for a range of 1-9,999 ppmv, and, according to IN-USA, making the unit the most accurate at the mid-range level of between 4,000 and 6,000 ppmv. The Mini Hicon-LR was calibrated by the manufacturer on 1/28/11 for a range of ~0-50 g/Nm^3, and 11,000-12,000 ppmv (~23.55 - 25.70 g/Nm^3) falls in the middle of this range. According to the manufacturer, the issue of having one analyzer run at the high end of its calibration and the other run at the mid-range of its calibration caused the differences in concentration output between the two analyzers.

To address this discrepancy, the two analyzers were run simultaneously and data were captured from both at 7,000, 9,000 and 9,800 ppmv (at both 75% and 85% RH) as read on the IN-2000-L2-LC analyzer. The ozone was held at each target condition for approximately 15 minutes to capture sufficient data. Because of this discrepancy, the 11,000 ppmv results obtained by the high-level analyzer were found to correspond with the 9,800 ppmv results on the low-level analyzer. Due to the corresponding results, *B. subtilis* was not tested at the 11,000 ppmv concentration. Similarly, when these data were extrapolated, the 12,000 ppmv results were found to correspond to ~10,800 ppmv if read with the low-level analyzer. The results of this comparison are summarized in Table 4-4.

Table 4-4. Summary of High- and Low-Level Analyzer Comparisons

Target Condition[+]	Dry Bulb Thermometer, °C*[†]	%RH*	IN-2000-L2-LC Ozone ppmv*[‡]	Mini Hicon-LR Ozone ppmv*[‡]	Average Difference, ppmv
7,000 ppmv ozone, 85% RH ± 5% RH	23.8 (23.8-23.8)	85.3 (83.9-85.8)	7,072 (6957-7185)	8,073 (7,892-8,412)	1,001
7,000 ppmv ozone, 75% RH ± 5% RH	24.6 (24.3-24.8)	76.3 (75.0-78.4)	7,081 (6,985-7,191)	8,013 (7,929-8,145)	932
9,000 ppmv ozone, 85% RH ± 5% RH	24.1 (24.0-24.3)	82.2 (80.2-84.1)	9,030 (8,669-9,121)	10,264 (10,138-10,355)	1,234
9,000 ppmv ozone, 75% RH ± 5% RH	24.7 (24.5-24.8)	75.4 (71.4-78.5)	9,053 (8,973-9,199)	10,248 (10,168-10,325)	1,195
9,800 ppmv ozone, 85% RH ± 5% RH	24.4 (24.3-24.5)	85.6 (84.2-86.1)	9825 (9,742-9,966)	11,123 (11,045-11,276)	1,298
9,800 ppmv ozone, 75% RH ± 5% RH	24.6 (24.5-24.8)	73.2 (73.1-73.2)	9,795 (9,736-9,885)	11,107 (10,858-11,485)	1,312

*Data are presented as mean (range).
[+] Target temperature was 22 °C ± 5 °C.
[†] Measurements were taken every 5 minutes.
[‡] Measurements were taken every minute.

5.0 Results

5.1 Inactivation of *B. anthracis* Spores

7,000 ppmv Ozone. Ozone fumigation results for *B. anthracis* spores at 7,000 ppmv ozone are presented in Table 5-1 and Figures 5-1 through 5-6. At 85% RH, no viable *B. anthracis* spores were recovered from wood or carpet following 8 hours of contact time, or from painted wallboard paper following the 4 or 8 hour contact times. Viable *B. anthracis* spores were recovered from the materials at all other contact times and at both 75% and 85% at this concentration (log reductions for all materials ranged from 1.44 on metal ductwork to 6.97 on wallboard paper).

9,000 ppmv Ozone. Ozone fumigation results for *B. anthracis* spores at 9,000 ppmv ozone are presented in Tables 5-2 and 5-3 and Figures 5-1 through 5-6. At 85% RH, no viable *B. anthracis* spores were recovered from carpet or painted wallboard paper following contact times of 6, 8, 9 or 12 hours or from wood after 8, 9 or 12 hours. Viable *B. anthracis* spores were recovered from the materials at all other contact times and at both 75% and 85% at this concentration (log reductions for all materials ranged from 1.17 on metal ductwork to 7.17 on wallboard paper).

9,800 ppmv Ozone (with and without 24 hour pre-humidification). Ozone fumigation results for *B. anthracis* spores at 9,800 ppmv ozone are presented in Tables 5-4 and 5-5 and Figures 5-1 through 5-6. At 85% RH, no viable *B. anthracis* spores were recovered from carpet or painted wallboard paper following any contact time (6, 9 or 12 hours). After subjecting the test materials to 85% RH for ~24 hours and then introducing 9,800 ppmv ozone, no viable *B. anthracis* spores were recovered from wood, carpet or painted wallboard paper following 6, 9 or 12 hour contact times. Viable *B. anthracis* spores were recovered from the materials at all other contact times at this concentration (log reductions for all materials ranged from 2.58 on metal ductwork to 7.33 on glass). This concentration of ozone was not tested at 75% RH. Pre-humidification of the testing chamber for 24 hours did not improve efficacy and even reduced efficacy in most instances for glass, laminate and metal ductwork.

11,000 ppmv Ozone. Results from fumigation of *B. anthracis* spores with 11,000 ppmv ozone are presented in Table 5-6 and Figures 5-1 through 5-6. At 85% RH, no viable *B. anthracis* spores were recovered from wood or carpet following 9 and 12 hour contact times. No spores were recovered from wallboard paper following any contact time (6, 9 or 12 hours). Viable *B. anthracis* spores were recovered from the materials at all other contact times at this concentration (log reductions for all materials ranged from 2.27 on laminate to 7.51 on carpet). The concentration for this test was measured by the high-level analyzer which corresponds to ~9,800 ppmv as measured with the low-level analyzer (Reference Section 4.2 and Table 4-4). The results from this test are similar to the results obtained in the 9,800 ppmv test in that wood, carpet and wallboard paper had the highest log

reductions. This concentration of ozone was not tested at 75% RH.

12,000 ppmv Ozone. Ozone fumigation results for *B. anthracis* spores at 12,000 ppmv ozone are presented in Table 5-7 and Figures 5-1 through 5-6. At 85% RH, no viable *B. anthracis* spores were recovered from wallboard paper and wood at 6 or 12 hours, carpet at 9 or 12 hours, and glass at 12 hours. Viable *B. anthracis* spores were recovered from the materials at all other contact times at this concentration (log reductions for all materials ranged from 5.05 on laminate to 6.93 on laminate). This concentration of ozone was not tested at 75% RH.

All associated laboratory and procedural blanks resulted in 0 CFU recovered for all *B. anthracis* tests.

Note that for test conditions in which no viable spores were recovered from all five replicate test coupons for a given material, log reduction results are reported using a "≥" symbol, indicating the potential for having a higher log reduction had there been a greater amount of spores inoculated onto the coupons. Additionally, we caution the reader to avoid comparing log reduction results for two materials when both materials have been completely decontaminated, as the difference in log reduction may be attributed to a difference in extraction recovery.

Table 5-1. Ozone Fumigation Results for *B. anthracis* Spores at 7,000 ppmv Ozone, 22 °C, 85% RH and 75% RH*

Contact Time, hr	Material	Inoculation Amount, CFU	Positive Control[‡]	Test Material[§]	Inactivation Efficacy [#]
			Mean Recovered *B. anthracis* Spores, CFU[†]		
			85% RH		
4	Glass	8.20×10^7	$3.27 \pm 4.25 \times 10^7$	$1.03 \pm 1.10 \times 10^4$	4.39 ± 1.29
	Wood		$5.16 \pm 1.61 \times 10^6$	$2.27 \pm 2.40 \times 10^3$	4.13 ± 1.36
	Carpet		$6.73 \pm 1.43 \times 10^7$	$1.23 \pm 0.917 \times 10^4$	4.10 ± 0.86
	Laminate		$4.28 \pm 0.508 \times 10^7$	$3.55 \pm 1.47 \times 10^4$	3.11 ± 0.16
	Metal Ductwork		$4.90 \pm 0.818 \times 10^7$	$2.43 \pm 0.911 \times 10^5$	2.33 ± 0.17
	Wallboard Paper		$4.13 \pm 0.997 \times 10^7$	0.00 ± 0.00	$\geq 7.60 \pm 0.10$
6	Glass	8.20×10^7	$3.27 \pm 4.25 \times 10^7$	$9.72 \pm 1.04 \times 10^4$	2.71 ± 0.39
	Wood		$5.16 \pm 1.61 \times 10^6$	$4.47 \pm 6.19 \times 10^2$	5.04 ± 1.35
	Carpet		$6.73 \pm 1.43 \times 10^7$	$9.27 \pm 13.9 \times 10^2$	6.13 ± 1.46
	Laminate		$4.28 \pm 0.508 \times 10^7$	$7.50 \pm 3.07 \times 10^4$	2.79 ± 0.17
	Metal Ductwork		$4.90 \pm 0.818 \times 10^7$	$6.71 \pm 4.60 \times 10^5$	1.98 ± 0.35
	Wallboard Paper		$4.13 \pm 0.997 \times 10^7$	$2.68 \pm 3.67 \times 10^1$	6.87 ± 0.88
8	Glass	8.20×10^7	$3.27 \pm 4.25 \times 10^7$	$6.54 \pm 10.4 \times 10^2$	6.25 ± 1.53
	Wood		$5.16 \pm 1.61 \times 10^6$	0.00 ± 0.00	$\geq 6.70 \pm 0.11$
	Carpet		$6.73 \pm 1.43 \times 10^7$	0.00 ± 0.00	$\geq 7.82 \pm 0.08$
	Laminate		$4.28 \pm 0.508 \times 10^7$	$2.46 \pm 2.65 \times 10^3$	4.99 ± 1.35
	Metal Ductwork		$4.90 \pm 0.818 \times 10^7$	$1.86 \pm 2.52 \times 10^5$	2.95 ± 0.72
	Wallboard Paper		$4.13 \pm 0.997 \times 10^7$	0.00 ± 0.00	$\geq 7.60 \pm 0.10$
			75% RH		
4	Glass	1.15×10^8	$8.03 \pm 10.3 \times 10^7$	$7.13 \pm 1.91 \times 10^5$	1.86 ± 0.39
	Wood		$3.74 \pm 0.592 \times 10^6$	$1.46 \pm 1.41 \times 10^4$	2.53 ± 0.31
	Carpet		$6.34 \pm 1.72 \times 10^7$	$5.90 \pm 3.00 \times 10^5$	2.06 ± 0.23
	Laminate		$3.15 \pm 0.402 \times 10^7$	$9.75 \pm 1.87 \times 10^5$	1.51 ± 0.08
	Metal Ductwork		$5.35 \pm 0.336 \times 10^7$	$1.89 \pm 0.820 \times 10^6$	1.48 ± 0.16
	Wallboard Paper		$2.80 \pm 0.826 \times 10^7$	$2.73 \pm 3.19 \times 10^2$	5.21 ± 0.46
6	Glass	1.15×10^8	$8.03 \pm 10.3 \times 10^7$	$2.89 \pm 2.06 \times 10^5$	2.33 ± 0.47
	Wood		$3.74 \pm 0.592 \times 10^6$	$1.08 \pm 0.809 \times 10^4$	3.35 ± 1.59
	Carpet		$6.34 \pm 1.72 \times 10^7$	$8.60 \pm 10.5 \times 10^4$	3.03 ± 0.36
	Laminate		$3.15 \pm 0.402 \times 10^7$	$7.05 \pm 1.06 \times 10^5$	1.65 ± 0.08
	Metal Ductwork		$5.35 \pm 0.336 \times 10^7$	$2.19 \pm 1.04 \times 10^6$	1.44 ± 0.23
	Wallboard Paper		$2.80 \pm 0.826 \times 10^7$	$4.00 \pm 8.94 \times 10^1$	6.97 ± 0.91
8	Glass	1.15×10^8	$8.03 \pm 10.3 \times 10^7$	$3.32 \pm 2.66 \times 10^5$	2.33 ± 0.52
	Wood		$3.74 \pm 0.592 \times 10^6$	$1.67 \pm 3.00 \times 10^4$	2.95 ± 0.70
	Carpet		$6.34 \pm 1.72 \times 10^7$	$1.25 \pm 1.55 \times 10^4$	4.38 ± 1.07
	Laminate		$3.15 \pm 0.402 \times 10^7$	$9.97 \pm 5.21 \times 10^5$	1.58 ± 0.30
	Metal Ductwork		$5.35 \pm 0.336 \times 10^7$	$1.09 \pm 0.227 \times 10^6$	1.70 ± 0.08
	Wallboard Paper		$2.80 \pm 0.826 \times 10^7$	$4.21 \pm 8.68 \times 10^1$	6.34 ± 1.36

[†] Data are expressed as mean ± standard deviation of five replicates.
[‡] Positive control coupons were inoculated but not exposed to ozone.
[§] Test materials were inoculated and exposed to ozone for the contact time.
[#] Data are expressed as mean log reduction ± 95% CI of the SE.
* Ozone concentration, temperature and relative humidity listed are target levels. Refer to Table 4-2 for actual levels.

Table 5-2. Ozone Fumigation Results for *B. anthracis* Spores at 9,000 ppmv Ozone, 22 °C, 85% RH and 75% RH*

Contact Time, hr	Material	Inoculation Amount, CFU	Positive Control‡	Test Material§	Inactivation Efficacy#
			Mean Recovered *B. anthracis* Spores, CFU†		
			85% RH		
4	Glass	1.08×10^8	$1.00 \pm 1.10 \times 10^8$	$1.67 \pm 0.277 \times 10^5$	2.64 ± 0.31
	Wood		$4.89 \pm 1.29 \times 10^6$	$4.34 \pm 9.70 \times 10^2$	6.01 ± 1.31
	Carpet		$8.69 \pm 1.38 \times 10^7$	$4.49 \pm 1.83 \times 10^3$	4.31 ± 0.16
	Laminate		$5.38 \pm 3.23 \times 10^7$	$2.88 \pm 0.818 \times 10^5$	2.21 ± 0.30
	Metal Ductwork		$7.18 \pm 1.69 \times 10^7$	$1.17 \pm 0.341 \times 10^6$	1.79 ± 0.16
	Wallboard Paper		$4.63 \pm 1.03 \times 10^7$	$5.34 \pm 11.9 \times 10^1$	7.17 ± 0.96
6	Glass	1.08×10^8	$1.00 \pm 1.10 \times 10^8$	$9.53 \pm 5.72 \times 10^4$	3.26 ± 0.97
	Wood		$4.89 \pm 1.29 \times 10^6$	$1.34 \pm 3.00 \times 10^1$	6.31 ± 0.72
	Carpet		$8.69 \pm 1.38 \times 10^7$	0.00 ± 0.00	$\geq 7.93 \pm 0.06$
	Laminate		$5.38 \pm 3.23 \times 10^7$	$7.83 \pm 1.15 \times 10^3$	3.76 ± 0.28
	Metal Ductwork		$7.18 \pm 1.69 \times 10^7$	$6.39 \pm 4.96 \times 10^6$	2.17 ± 0.37
	Wallboard Paper		$4.63 \pm 1.03 \times 10^7$	0.00 ± 0.00	$\geq 7.66 \pm 0.09$
8	Glass	1.08×10^8	$1.00 \pm 1.10 \times 10^8$	$6.79 \pm 4.20 \times 10^4$	3.92 ± 1.95
	Wood		$4.89 \pm 1.29 \times 10^6$	0.00 ± 0.00	$\geq 6.68 \pm 0.10$
	Carpet		$8.69 \pm 1.38 \times 10^7$	0.00 ± 0.00	$\geq 7.93 \pm 0.06$
	Laminate		$5.38 \pm 3.23 \times 10^7$	$3.64 \pm 0.747 \times 10^4$	3.10 ± 0.28
	Metal Ductwork		$7.18 \pm 1.69 \times 10^7$	$3.77 \pm 1.21 \times 10^5$	2.28 ± 0.15
	Wallboard Paper		$4.63 \pm 1.03 \times 10^7$	0.00 ± 0.00	$\geq 7.66 \pm 0.09$
			75% RH		
4	Glass	1.11×10^8	$3.78 \pm 0.718 \times 10^7$	$8.17 \pm 5.00 \times 10^5$	1.72 ± 0.24
	Wood		$5.41 \pm 1.69 \times 10^6$	$4.32 \pm 3.88 \times 10^4$	2.31 ± 0.52
	Carpet		$7.05 \pm 1.10 \times 10^7$	$3.54 \pm 1.43 \times 10^5$	2.33 ± 0.19
	Laminate		$3.77 \pm 0.481 \times 10^7$	$2.04 \pm 0.181 \times 10^6$	1.27 ± 0.06
	Metal Ductwork		$5.98 \pm 1.08 \times 10^7$	$1.84 \pm 0.909 \times 10^6$	1.55 ± 0.20
	Wallboard Paper		$3.55 \pm 0.534 \times 10^7$	$2.35 \pm 2.90 \times 10^4$	3.65 ± 0.69
6	Glass	1.11×10^8	$3.78 \pm 0.718 \times 10^7$	$7.83 \pm 2.82 \times 10^5$	1.70 ± 0.14
	Wood		$5.41 \pm 1.69 \times 10^6$	$3.64 \pm 2.12 \times 10^4$	2.21 ± 0.24
	Carpet		$7.05 \pm 1.10 \times 10^7$	$5.23 \pm 2.43 \times 10^4$	3.16 ± 0.18
	Laminate		$3.77 \pm 0.481 \times 10^7$	$2.03 \pm 0.128 \times 10^6$	1.27 ± 0.06
	Metal Ductwork		$5.98 \pm 1.08 \times 10^7$	$2.50 \pm 0.930 \times 10^6$	1.40 ± 0.18
	Wallboard Paper		$3.55 \pm 0.534 \times 10^7$	$3.42 \pm 3.79 \times 10^3$	5.32 ± 1.79
8	Glass	1.11×10^8	$3.78 \pm 0.718 \times 10^7$	$6.41 \pm 4.12 \times 10^5$	1.88 ± 0.37
	Wood		$5.41 \pm 1.69 \times 10^6$	$2.29 \pm 4.59 \times 10^4$	2.56 ± 0.55
	Carpet		$7.05 \pm 1.10 \times 10^7$	$9.18 \pm 11.5 \times 10^3$	4.29 ± 0.76
	Laminate		$3.77 \pm 0.481 \times 10^7$	$1.24 \pm 0.247 \times 10^6$	1.49 ± 0.09
	Metal Ductwork		$5.98 \pm 1.08 \times 10^7$	$3.85 \pm 4.91 \times 10^6$	1.40 ± 0.40
	Wallboard Paper		$3.55 \pm 0.534 \times 10^7$	$2.79 \pm 4.09 \times 10^3$	5.42 ± 1.72

† Data are expressed as mean ± standard deviation of five replicates.
‡ Positive control coupons were inoculated but not exposed to ozone.
§ Test materials were inoculated and exposed to ozone for the contact time.
# Data are expressed as mean log reduction ± 95% CI of the SE.
* Ozone concentration, temperature and relative humidity listed are target levels. Refer to Table 4-2 for actual levels.

Table 5-3. Ozone Fumigation Results for *B. anthracis* Spores at 9,000 ppmv Ozone, 22 °C, 85% RH*

Contact Time, hr	Material	Inoculation Amount, CFU	Positive Control‡	Test Material§	Inactivation Efficacy #
			Mean Recovered *B. anthracis* Spores, CFU†		
			85% RH		
6	Glass	7.57×10^7	$4.13 \pm 0.702 \times 10^7$	$1.71 \pm 3.01 \times 10^4$	4.81 ± 1.67
	Wood		$5.20 \pm 1.09 \times 10^6$	$1.34 \pm 3.00 \times 10^1$	6.34 ± 0.72
	Carpet		$8.02 \pm 1.43 \times 10^7$	0.00 ± 0.00	$\geq 7.90 \pm 0.07$
	Laminate		$4.86 \pm 0.791 \times 10^7$	$8.72 \pm 2.25 \times 10^4$	2.75 ± 0.13
	Metal Ductwork		$4.36 \pm 1.71 \times 10^7$	$8.90 \pm 7.00 \times 10^4$	2.81 ± 0.41
	Wallboard Paper		$5.55 \pm 0.824 \times 10^7$	0.00 ± 0.00	$\geq 7.74 \pm 0.05$
9	Glass	7.57×10^7	$4.13 \pm 0.702 \times 10^7$	$8.17 \pm 18.2 \times 10^3$	5.68 ± 1.47
	Wood		$5.20 \pm 1.09 \times 10^6$	0.00 ± 0.00	$\geq 6.71 \pm 0.08$
	Carpet		$8.02 \pm 1.43 \times 10^7$	0.00 ± 0.00	$\geq 7.90 \pm 0.07$
	Laminate		$4.86 \pm 0.791 \times 10^7$	$1.74 \pm 1.66 \times 10^4$	3.78 ± 0.72
	Metal Ductwork		$4.36 \pm 1.71 \times 10^7$	$5.38 \pm 1.55 \times 10^3$	3.90 ± 0.19
	Wallboard Paper		$5.55 \pm 0.824 \times 10^7$	0.00 ± 0.00	$\geq 7.74 \pm 0.05$
12	Glass	7.57×10^7	$4.13 \pm 0.702 \times 10^7$	$6.66 \pm 14.9 \times 10^1$	7.11 ± 0.99
	Wood		$5.20 \pm 1.09 \times 10^6$	0.00 ± 0.00	$\geq 6.71 \pm 0.08$
	Carpet		$8.02 \pm 1.43 \times 10^7$	0.00 ± 0.00	$\geq 7.90 \pm 0.07$
	Laminate		$4.86 \pm 0.791 \times 10^7$	$8.80 \pm 1.16 \times 10^4$	2.74 ± 0.08
	Metal Ductwork		$4.36 \pm 1.71 \times 10^7$	$1.47 \pm 0.758 \times 10^5$	2.55 ± 0.40
	Wallboard Paper		$5.55 \pm 0.824 \times 10^7$	0.00 ± 0.00	$\geq 7.74 \pm 0.05$

† Data are expressed as mean ± standard deviation of five replicates.
‡ Positive control coupons were inoculated but not exposed to ozone.
§ Test materials were inoculated and exposed to ozone for the contact time.
# Data are expressed as mean log reduction ± 95% CI of the SE.
* Ozone concentration, temperature and relative humidity listed are target levels. Refer to Table 4-2 for actual levels.

Table 5-4. Ozone Fumigation Results for *B. anthracis* Spores at 9,800 ppmv Ozone, 22 °C, 85% RH*

Contact Time, hr	Material	Inoculation Amount, CFU	Positive Control[‡]	Test Material[§]	Inactivation Efficacy [#]
			Mean Recovered *B. anthracis* Spores, CFU[†]		
6	Glass	1.04×10^8	$5.41 \pm 2.53 \times 10^7$	$7.93 \pm 17.4 \times 10^2$	6.61 ± 1.42
	Wood		$1.02 \pm 0.958 \times 10^7$	0.00 ± 0.00	$\geq 6.90 \pm 0.27$
	Carpet		$8.37 \pm 1.63 \times 10^7$	0.00 ± 0.00	$\geq 7.92 \pm 0.08$
	Laminate		$4.25 \pm 1.06 \times 10^7$	$1.31 \pm 0.447 \times 10^4$	3.52 ± 0.17
	Metal Ductwork		$6.19 \pm 1.41 \times 10^7$	$4.70 \pm 4.77 \times 10^4$	3.34 ± 0.48
	Wallboard Paper		$1.10 \pm 0.917 \times 10^8$	0.00 ± 0.00	$\geq 7.84 \pm 0.48$
9	Glass	1.04×10^8	$5.41 \pm 2.53 \times 10^7$	$1.34 \pm 3.00 \times 10^1$	7.33 ± 0.74
	Wood		$1.02 \pm 0.958 \times 10^7$	0.00 ± 0.00	$\geq 6.90 \pm 0.27$
	Carpet		$8.37 \pm 1.63 \times 10^7$	0.00 ± 0.00	$\geq 7.92 \pm 0.08$
	Laminate		$4.25 \pm 1.06 \times 10^7$	$2.28 \pm 1.69 \times 10^4$	3.35 ± 0.30
	Metal Ductwork		$6.19 \pm 1.41 \times 10^7$	$3.17 \pm 6.23 \times 10^4$	4.00 ± 0.75
	Wallboard Paper		$1.10 \pm 0.917 \times 10^8$	0.00 ± 0.00	$\geq 7.84 \pm 0.48$
12	Glass	1.04×10^8	$5.41 \pm 2.53 \times 10^7$	$1.14 \pm 2.55 \times 10^3$	6.95 ± 1.48
	Wood		$1.02 \pm 0.958 \times 10^7$	6.60 ± 14.8	6.60 ± 0.65
	Carpet		$8.37 \pm 1.63 \times 10^7$	0.00 ± 0.00	$\geq 7.92 \pm 0.08$
	Laminate		$4.25 \pm 1.06 \times 10^7$	$5.27 \pm 3.91 \times 10^2$	5.39 ± 1.11
	Metal Ductwork		$6.19 \pm 1.41 \times 10^7$	$1.31 \pm 1.77 \times 10^3$	4.97 ± 0.51
	Wallboard Paper		$1.10 \pm 0.917 \times 10^8$	0.00 ± 0.00	$\geq 7.84 \pm 0.48$

[†] Data are expressed as mean ± standard deviation of five replicates.
[‡] Positive control coupons were inoculated but not exposed to ozone.
[§] Test materials were inoculated and exposed to ozone for the contact time.
[#] Data are expressed as mean log reduction ± 95% CI of the SE.
* Ozone concentration, temperature and relative humidity listed are target levels. Refer to Table 4-2 for actual levels.

Table 5-5. Ozone Fumigation Results for *B. anthracis* Spores at 9,800 ppmv Ozone, 22 °C, 85% RH (with pre-humidification)*[1]

Contact Time, hr	Material	Inoculation Amount, CFU	Positive Control[‡]	Test Material[§]	Inactivation Efficacy [#]
			Mean Recovered *B. anthracis* Spores, CFU[†]		
6	Glass	9.03×10^7	$2.74 \pm 1.10 \times 10^7$	$2.47 \pm 1.43 \times 10^2$	5.13 ± 0.42
	Wood		$3.77 \pm 1.48 \times 10^6$	0.00 ± 0.00	$\geq 6.54 \pm 0.19$
	Carpet		$5.87 \pm 1.30 \times 10^7$	0.00 ± 0.00	$\geq 7.76 \pm 0.09$
	Laminate		$2.20 \pm 1.17 \times 10^7$	$1.81 \pm 1.24 \times 10^4$	3.10 ± 0.30
	Metal Ductwork		$2.47 \pm 1.46 \times 10^7$	$5.61 \pm 2.02 \times 10^4$	2.58 ± 0.32
	Wallboard Paper		$3.20 \pm 1.09 \times 10^7$	0.00 ± 0.00	$\geq 7.48 \pm 0.17$
9	Glass	9.03×10^7	$2.74 \pm 1.10 \times 10^7$	$9.54 \pm 12.4 \times 10^2$	5.11 ± 1.20
	Wood		$3.77 \pm 1.48 \times 10^6$	0.00 ± 0.00	$\geq 6.54 \pm 0.19$
	Carpet		$5.87 \pm 1.30 \times 10^7$	0.00 ± 0.00	$\geq 7.76 \pm 0.09$
	Laminate		$2.20 \pm 1.17 \times 10^7$	$4.29 \pm 1.77 \times 10^3$	3.70 ± 0.28
	Metal Ductwork		$2.47 \pm 1.46 \times 10^7$	$1.00 \pm 2.07 \times 10^5$	3.07 ± 0.77
	Wallboard Paper		$3.20 \pm 1.09 \times 10^7$	0.00 ± 0.00	$\geq 7.48 \pm 0.17$
12	Glass	9.03×10^7	$2.74 \pm 1.10 \times 10^7$	$9.34 \pm 11.2 \times 10^1$	6.13 ± 1.05
	Wood		$3.77 \pm 1.48 \times 10^6$	0.00 ± 0.00	$\geq 6.54 \pm 0.19$
	Carpet		$5.87 \pm 1.30 \times 10^7$	0.00 ± 0.00	$\geq 7.76 \pm 0.09$
	Laminate		$2.20 \pm 1.17 \times 10^7$	$1.88 \pm 0.720 \times 10^3$	4.05 ± 0.26
	Metal Ductwork		$2.47 \pm 1.46 \times 10^7$	$1.12 \pm 1.57 \times 10^4$	3.85 ± 0.97
	Wallboard Paper		$3.20 \pm 1.09 \times 10^7$	0.00 ± 0.00	$\geq 7.48 \pm 0.17$

[1]Immediately following inoculation, all materials were kept at 85% ± 5% RH for approximately 24 hours prior to introduction of ozone.

[†] Data are expressed as mean ± standard deviation of five replicates.

[‡] Positive control coupons were inoculated but not exposed to ozone.

[§] Test materials were inoculated and exposed to ozone for the contact time.

[#] Data are expressed as mean log reduction ± 95% CI of the SE.

* Ozone concentration, temperature and relative humidity listed are target levels. Refer to Table 4-2 for actual levels.

Table 5-6. Ozone Fumigation Results for *B. anthracis* Spores at 11,000[+] ppmv Ozone, 22 °C, 85% RH*

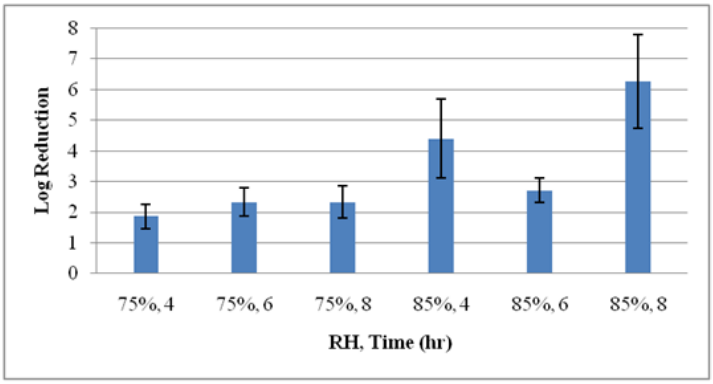
Contact Time, hr	Material	Inoculation Amount, CFU	Positive Control[‡] Mean Recovered *B. anthracis* Spores, CFU[†]	Test Material[§] Mean Recovered *B. anthracis* Spores, CFU[†]	Inactivation Efficacy [#]
6	Glass	8.60×10^7	$3.21 \pm 0.476 \times 10^7$	$8.15 \pm 7.61 \times 10^3$	4.04 ± 0.82
	Wood		$2.87 \pm 1.07 \times 10^6$	$1.07 \pm 1.88 \times 10^2$	5.50 ± 1.14
	Carpet		$6.57 \pm 0.745 \times 10^7$	6.60 ± 14.8	7.51 ± 0.60
	Laminate		$3.16 \pm 0.516 \times 10^7$	$1.81 \pm 0.853 \times 10^5$	2.27 ± 0.16
	Metal Ductwork		$4.02 \pm 0.583 \times 10^7$	$1.74 \pm 1.44 \times 10^5$	2.50 ± 0.35
	Wallboard Paper		$3.43 \pm 0.720 \times 10^7$	0.00 ± 0.00	$\geq 7.53 \pm 0.08$
9	Glass	8.60×10^7	$3.21 \pm 0.476 \times 10^7$	$3.59 \pm 3.29 \times 10^3$	4.16 ± 0.50
	Wood		$2.87 \pm 1.07 \times 10^6$	0.00 ± 0.00	$\geq 6.43 \pm 0.16$
	Carpet		$6.57 \pm 0.745 \times 10^7$	0.00 ± 0.00	$\geq 7.82 \pm 0.04$
	Laminate		$3.16 \pm 0.516 \times 10^7$	$1.43 \pm 0.901 \times 10^3$	4.43 ± 0.31
	Metal Ductwork		$4.02 \pm 0.583 \times 10^7$	$1.12 \pm 1.13 \times 10^4$	3.70 ± 0.35
	Wallboard Paper		$3.43 \pm 0.720 \times 10^7$	0.00 ± 0.00	$\geq 7.53 \pm 0.08$
12	Glass	8.60×10^7	$3.21 \pm 0.476 \times 10^7$	$2.00 \pm 2.74 \times 10^2$	6.05 ± 1.18
	Wood		$2.87 \pm 1.07 \times 10^6$	0.00 ± 0.00	$\geq 6.43 \pm 0.16$
	Carpet		$6.57 \pm 0.745 \times 10^7$	0.00 ± 0.00	$\geq 7.82 \pm 0.04$
	Laminate		$3.16 \pm 0.516 \times 10^7$	$5.93 \pm 5.28 \times 10^2$	4.92 ± 0.46
	Metal Ductwork		$4.02 \pm 0.583 \times 10^7$	$1.59 \pm 1.81 \times 10^3$	4.72 ± 0.57
	Wallboard Paper		$3.43 \pm 0.720 \times 10^7$	0.00 ± 0.00	$\geq 7.53 \pm 0.08$

[†] Data are expressed as mean ± standard deviation of five replicates.
[‡] Positive control coupons were inoculated but not exposed to ozone.
[§] Test materials were inoculated and exposed to ozone for the contact time.
[#] Data are expressed as mean log reduction ± 95% CI of the SE.
[+] This concentration was measured by the Mini Hicon-LR analyzer and corresponds to ~9,800 ppmv on the IN2000-L2-LC analyzer.
* Ozone concentration, temperature and relative humidity listed are target levels. Refer to Table 4-2 for actual levels.

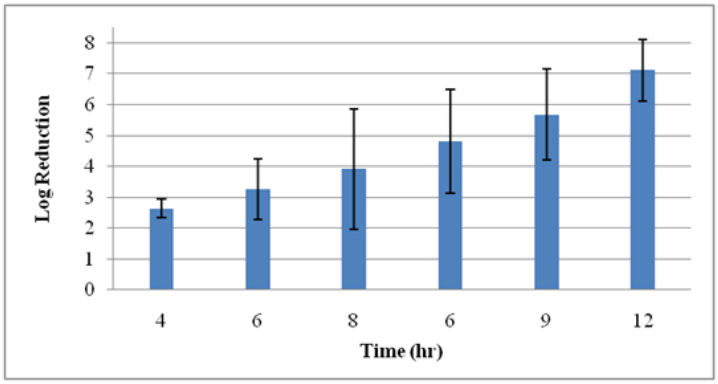
Table 5-7. Ozone Fumigation Results for *B. anthracis* Spores at 12,000[+] ppmv Ozone, 22 °C, 85% RH*

Contact Time, hr	Material	Inoculation Amount, CFU	Positive Control[‡]	Test Material[§]	Inactivation Efficacy [#]
			Mean Recovered *B. anthracis* Spores, CFU[†]		
6	Glass	1.29×10^8	$6.19 \pm 3.29 \times 10^7$	$6.01 \pm 7.24 \times 10^1$	6.52 ± 1.03
	Wood		$2.21 \pm 3.32 \times 10^7$	0.00 ± 0.00	$\geq 7.04 \pm 0.46$
	Carpet		$9.38 \pm 0.750 \times 10^7$	$1.11 \pm 1.60 \times 10^3$	6.21 ± 1.50
	Laminate		$5.93 \pm 0.727 \times 10^7$	$9.12 \pm 9.45 \times 10^2$	5.05 ± 0.48
	Metal Ductwork		$5.29 \pm 2.00 \times 10^7$	$2.06 \pm 1.88 \times 10^2$	5.88 ± 0.98
	Wallboard Paper		$3.13 \pm 0.268 \times 10^7$	0.00 ± 0.00	$\geq 7.49 \pm 0.03$
9	Glass	1.29×10^8	$6.19 \pm 3.29 \times 10^7$	$2.67 \pm 2.79 \times 10^1$	6.69 ± 0.89
	Wood		$2.21 \pm 3.32 \times 10^7$	6.60 ± 14.8	6.74 ± 0.75
	Carpet		$9.38 \pm 0.750 \times 10^7$	0.00 ± 0.00	$\geq 7.97 \pm 0.03$
	Laminate		$5.93 \pm 0.727 \times 10^7$	$1.00 \pm 2.06 \times 10^2$	6.93 ± 1.07
	Metal Ductwork		$5.29 \pm 2.00 \times 10^7$	$3.12 \pm 3.66 \times 10^2$	5.79 ± 1.03
	Wallboard Paper		$3.13 \pm 0.268 \times 10^7$	6.60 ± 14.8	7.19 ± 0.60
12	Glass	1.29×10^8	$6.19 \pm 3.29 \times 10^7$	0.00 ± 0.00	$\geq 7.66 \pm 0.41$
	Wood		$2.21 \pm 3.32 \times 10^7$	0.00 ± 0.00	$\geq 7.04 \pm 0.46$
	Carpet		$9.38 \pm 0.750 \times 10^7$	0.00 ± 0.00	$\geq 7.97 \pm 0.03$
	Laminate		$5.93 \pm 0.727 \times 10^7$	$9.61 \pm 19.1 \times 10^2$	5.82 ± 1.16
	Metal Ductwork		$5.29 \pm 2.00 \times 10^7$	$7.33 \pm 8.94 \times 10^1$	6.50 ± 1.00
	Wallboard Paper		$3.13 \pm 0.268 \times 10^7$	0.00 ± 0.00	$\geq 7.49 \pm 0.03$

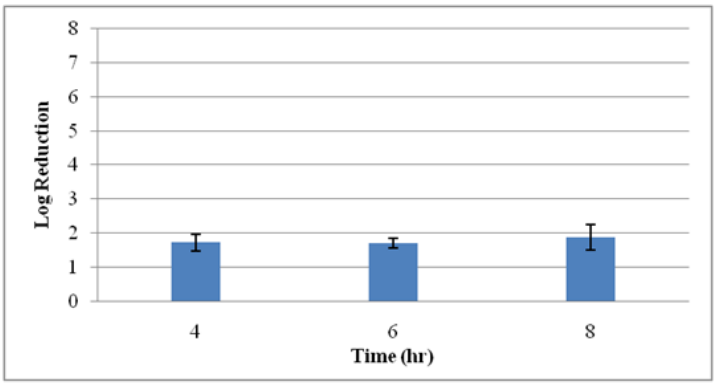
[†] Data are expressed as mean ± standard deviation of five replicates.
[‡] Positive control coupons were inoculated but not exposed to ozone.
[§] Test materials were inoculated and exposed to ozone for the contact time.
[#] Data are expressed as mean log reduction ± 95% CI of the SE.
[+] This concentration was measured by the Mini Hicon-LR analyzer and corresponds to ~10,800 ppmv on the IN2000-L2-LC analyzer.
* Ozone concentration, temperature and relative humidity listed are target levels. Refer to Table 4-2 for actual levels.



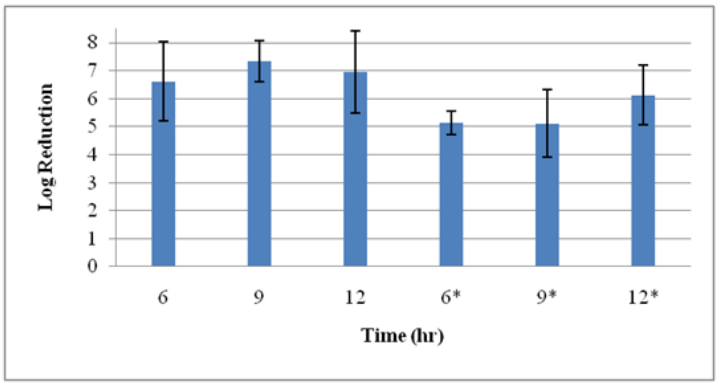
7,000 ppm O_3, 75% and 85% RH



9,000 ppm O_3, 85% RH

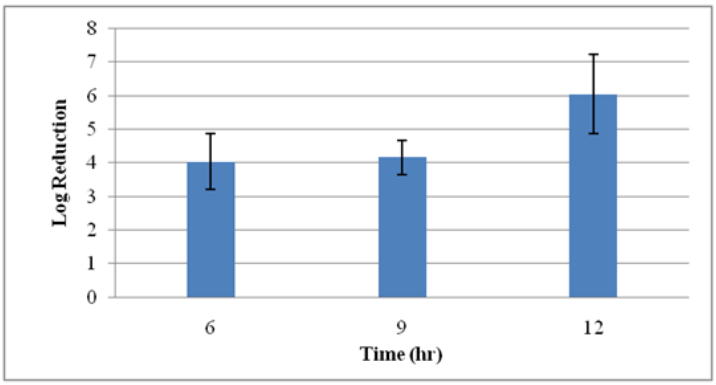


9,000 ppm O_3, 75% RH

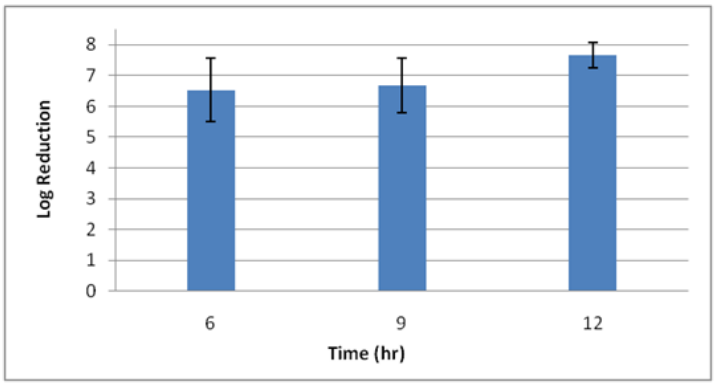


*Coupons were pre-humidified for ~24 hours

9,800 ppm O_3, 85% RH

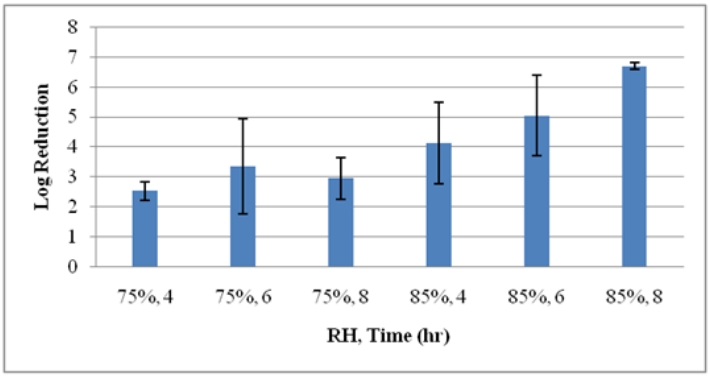


11,000 ppm O_3, 85% RH

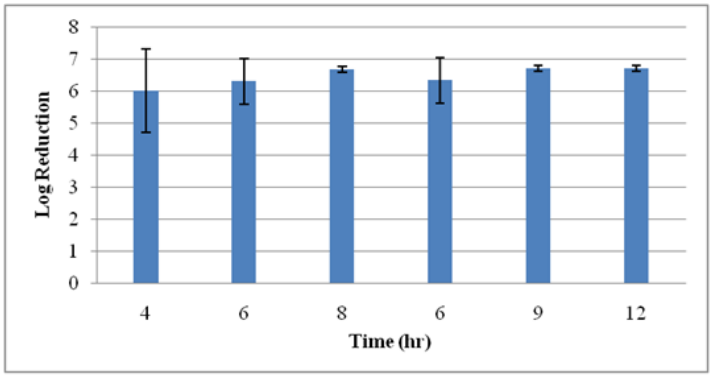


12,000 ppm O_3, 85% RH

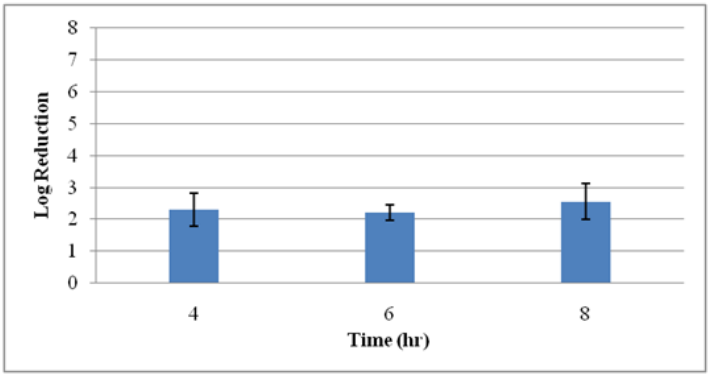
Figure 5-1. Efficacy (log reduction) against *B. anthracis* on glass.
(Data are expressed as mean log reduction ± 95% CI of the SE)



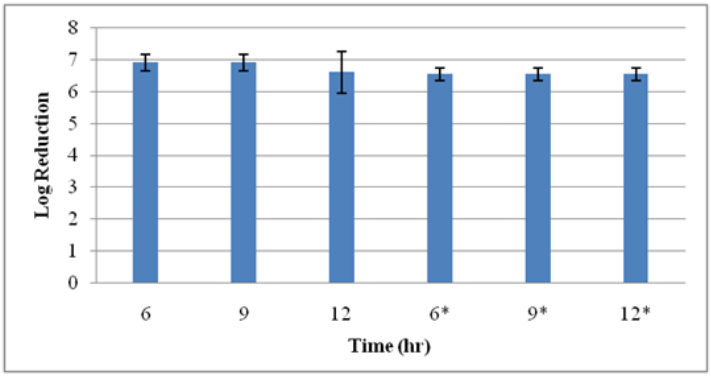
7,000 ppm O_3, 75% and 85% RH



9,000 ppm O_3, 85% RH

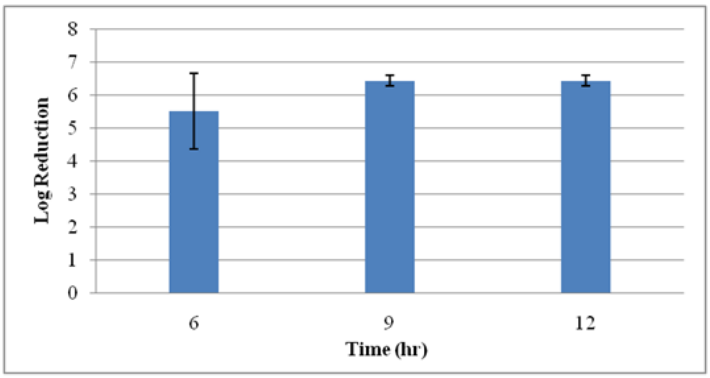


9,000 ppm O_3, 75% RH

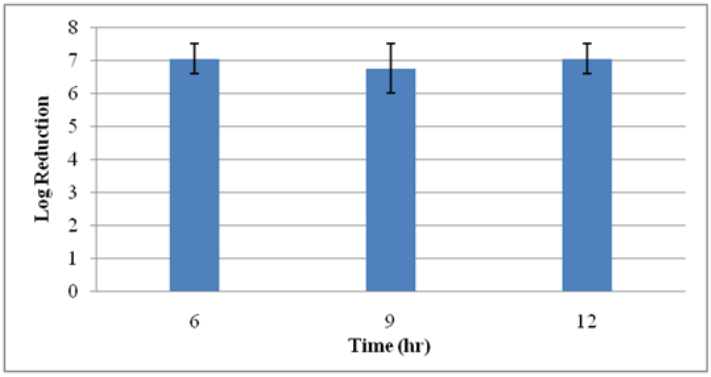


*Coupons were pre-humidified for ~24 hours

9,800 ppm O_3, 85% RH

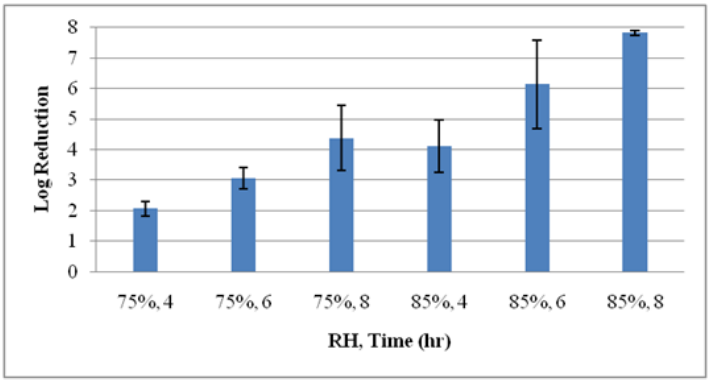


11,000 ppm O_3, 85% RH

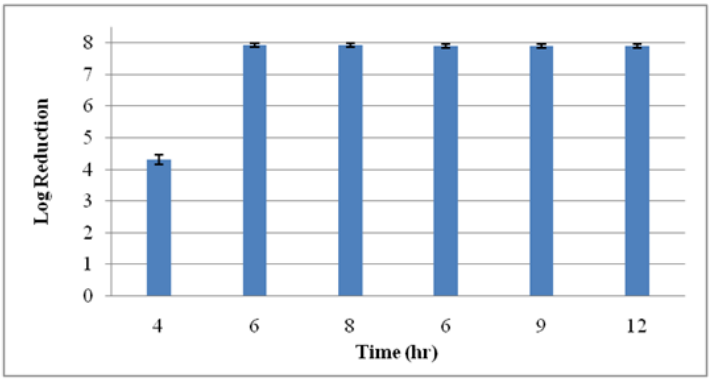


12,000 ppm O_3, 85% RH

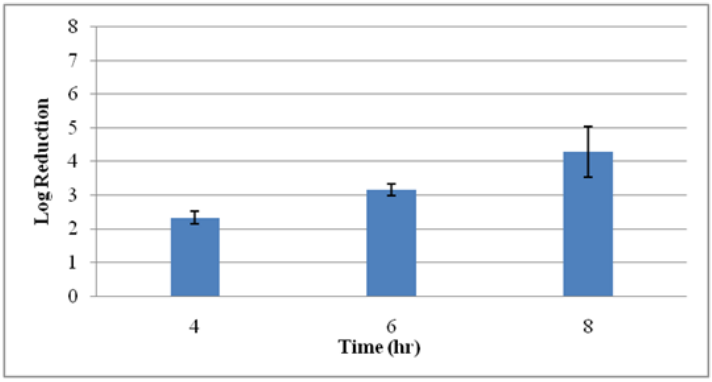
Figure 5-2. Efficacy (log reduction) against *B. anthracis* on wood.
(Data are expressed as mean log reduction ± 95% CI of the SE)



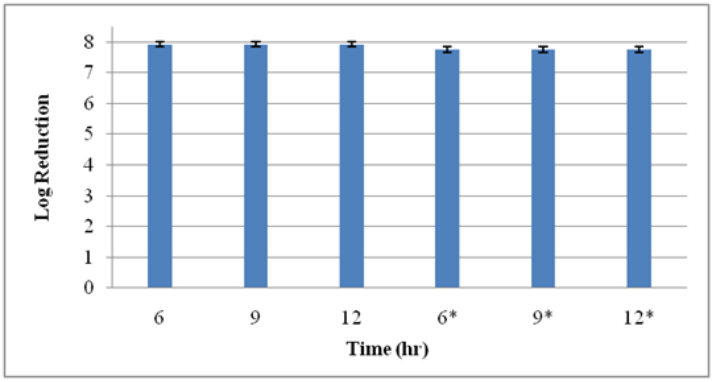
7,000 ppm O_3, 75% and 85% RH



9,000 ppm O_3, 85% RH

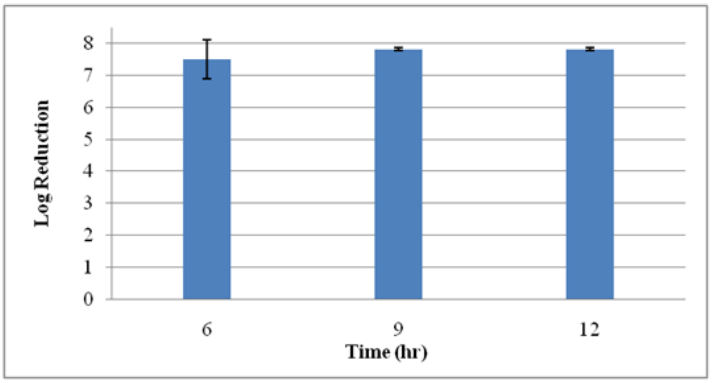


9,000 ppm O_3, 75% RH

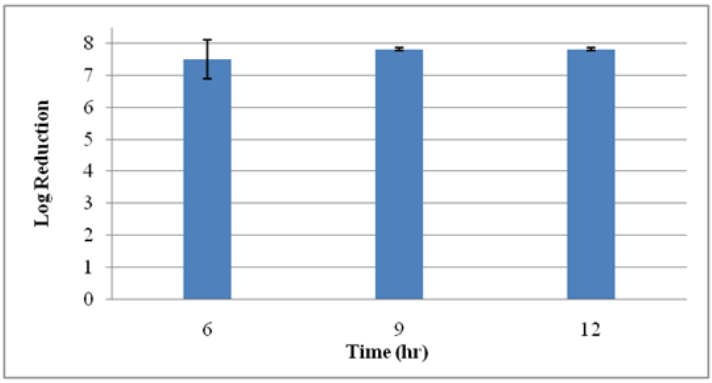


*Coupons were pre-humidified for ~24 hours

9,800 ppm O_3, 85% RH

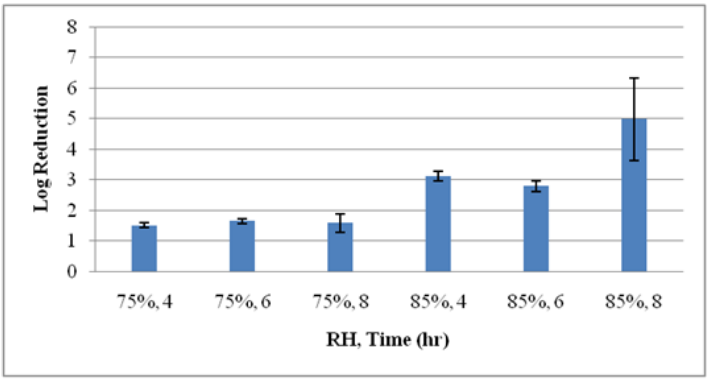


11,000 ppm O_3, 85% RH

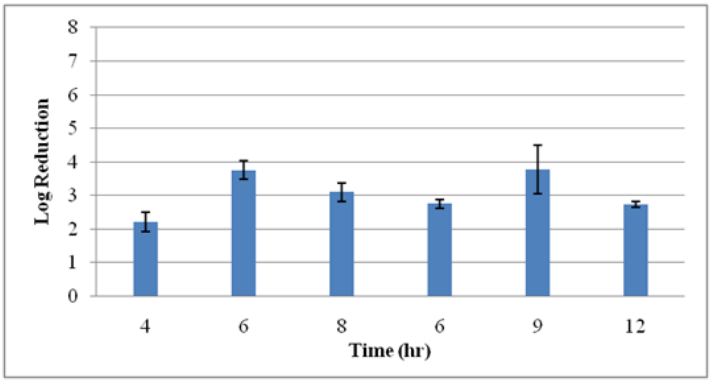


12,000 ppm O_3, 85% RH

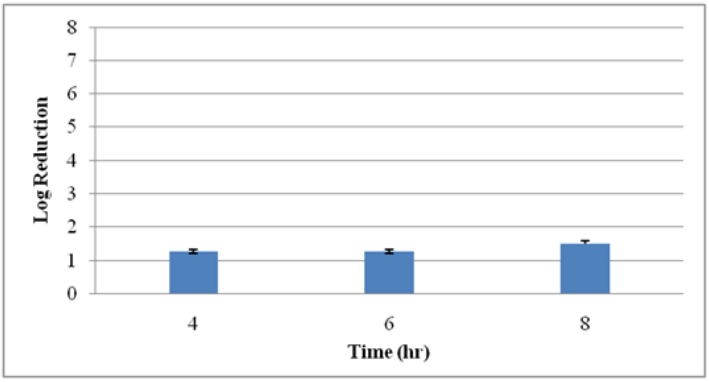
Figure 5-3. Efficacy (log reduction) against *B. anthracis* on carpet.
(Data are expressed as mean log reduction ± 95% CI of the SE)



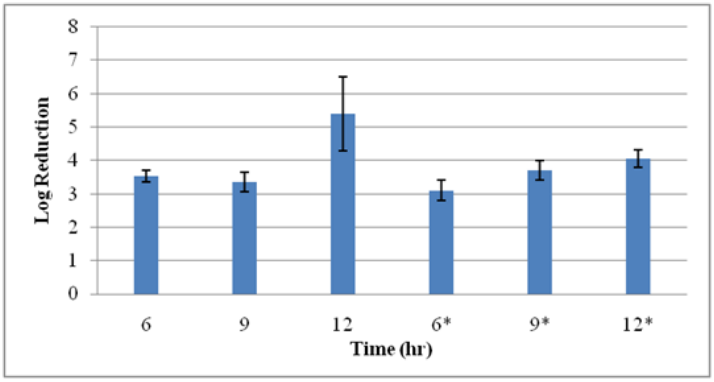
7,000 ppm O_3, 75% and 85% RH



9,000 ppm O_3, 85% RH

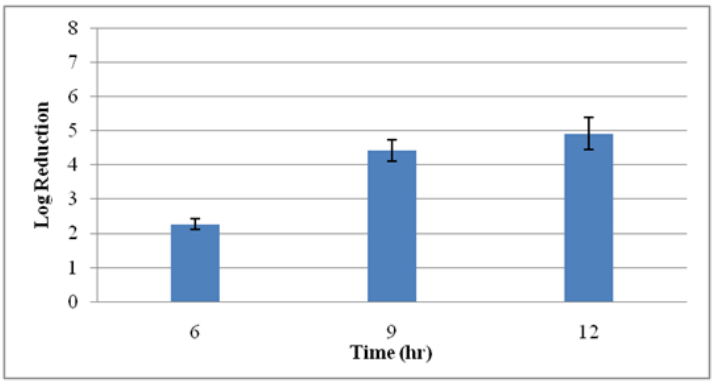


9,000 ppm O_3, 75% RH

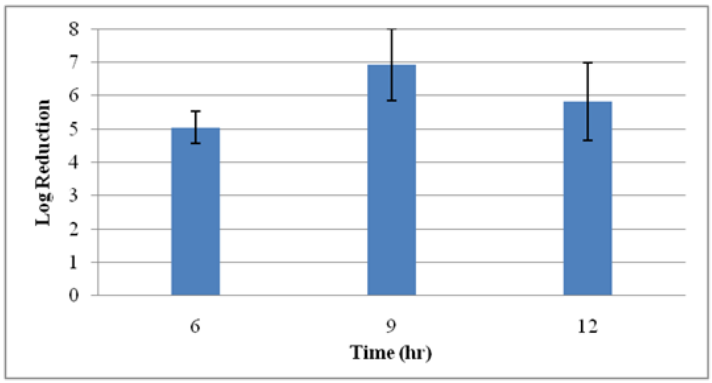


*Coupons were pre-humidified for ~24 hours

9,800 ppm O_3, 85% RH

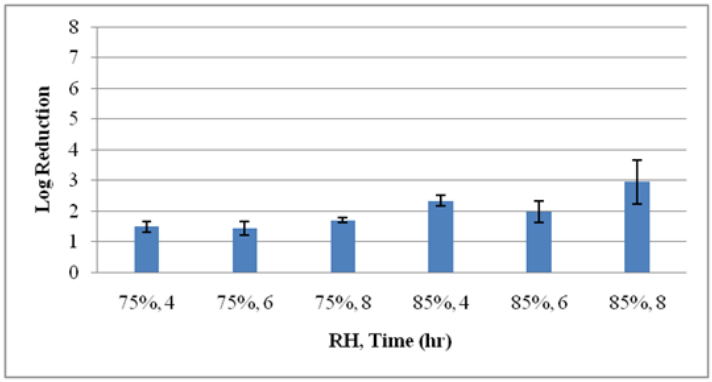


11,000 ppm O_3, 85% RH

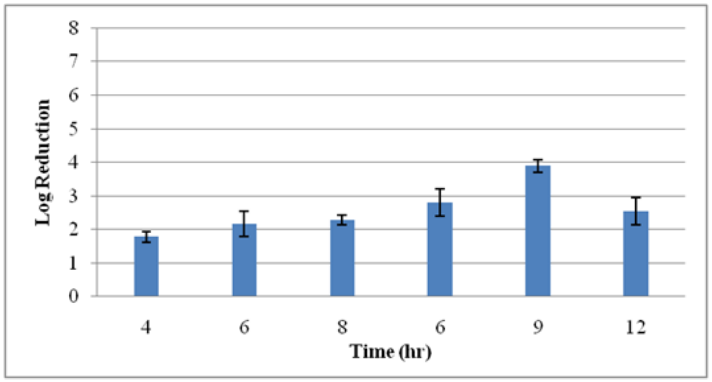


12,000 ppm O_3, 85% RH

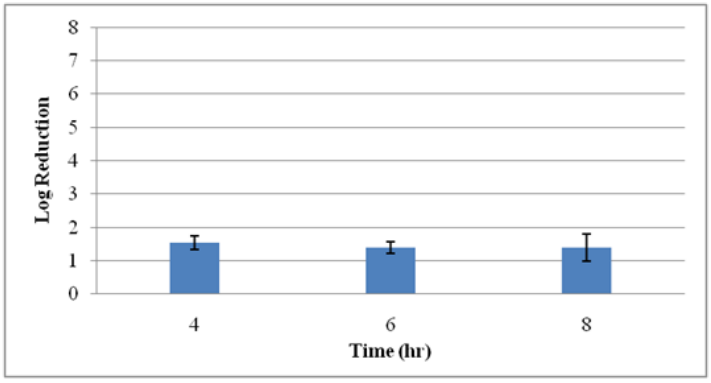
Figure 5-4. Efficacy (log reduction) against *B. anthracis* on laminate.
(Data are expressed as mean log reduction ± 95% CI of the SE)



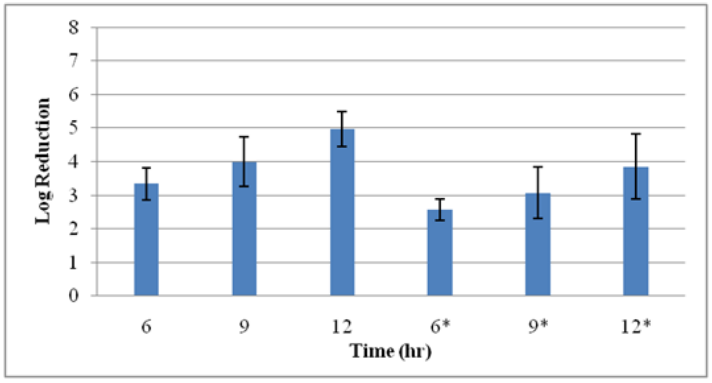
7,000 ppm O_3, 75% and 85% RH



9,000 ppm O_3, 85% RH

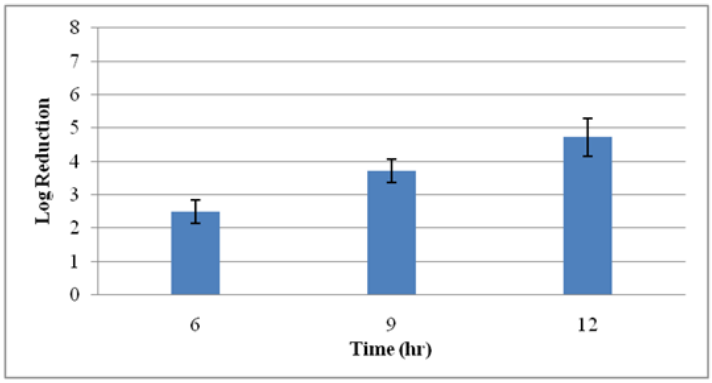


9,000 ppm O_3, 75% RH

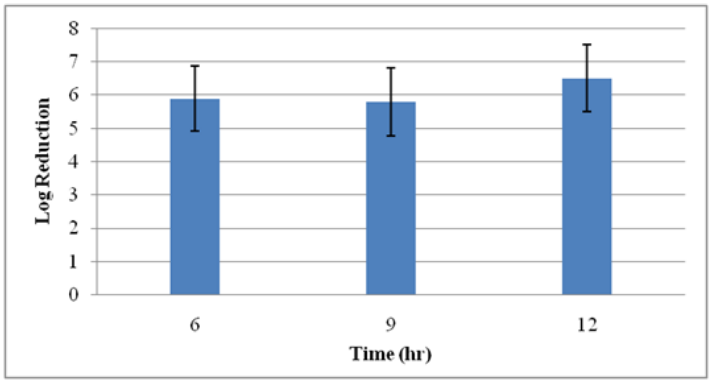


*Coupons were pre-humidified for ~24 hours

9,800 ppm O_3, 85% RH

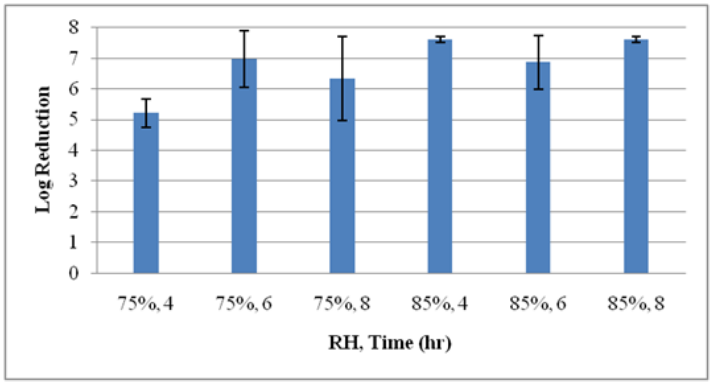


11,000 ppm O_3, 85% RH

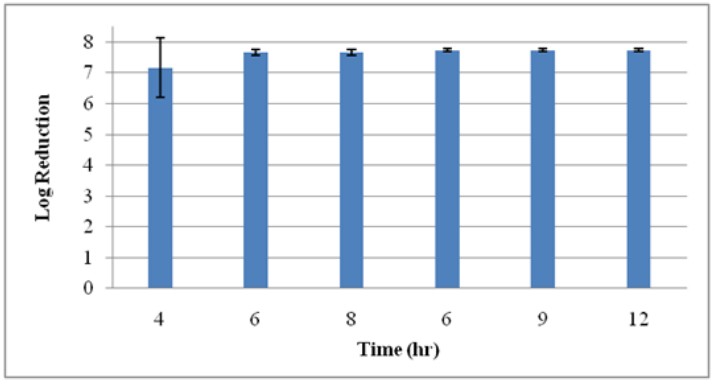


12,000 ppm O_3, 85% RH

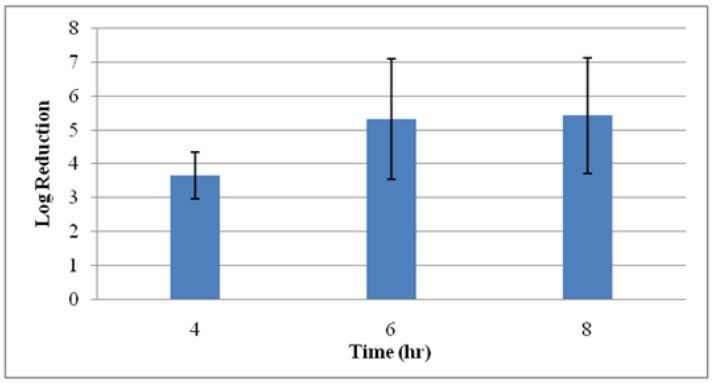
Figure 5-5. Efficacy (log reduction) against *B. anthracis* on metal ductwork.
(Data are expressed as mean log reduction ± 95% CI of the SE)



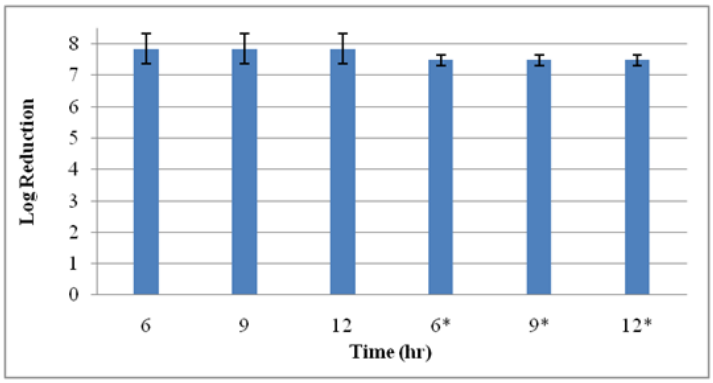
7,000 ppm O_3, 75% and 85% RH



9,000 ppm O_3, 85% RH

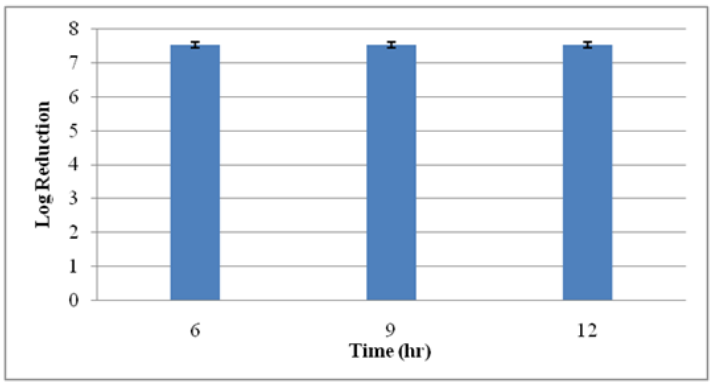


9,000 ppm O_3, 75% RH

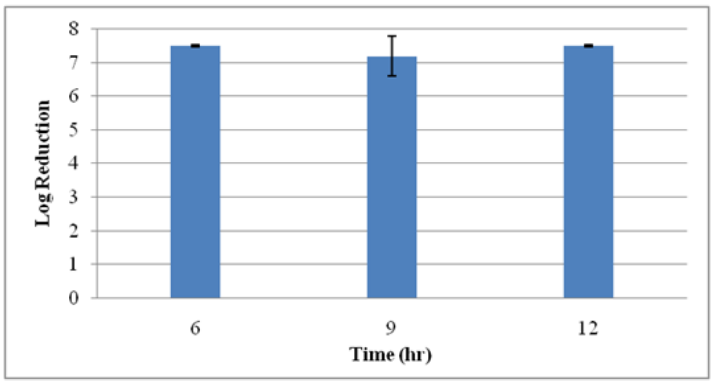


*Coupons were pre-humidified for ~24 hours

9,800 ppm O_3, 85% RH



11,000 ppm O_3, 85% RH



12,000 ppm O_3, 85% RH

Figure 5-6. Efficacy (log reduction) against *B. anthracis* on wallboard paper.
(Data are expressed as mean log reduction ± 95% CI of the SE)

5.2 Inactivation of *B. subtilis* Spores

7,000 ppmv Ozone. Ozone fumigation results for *B. subtilis* spores at 7,000 ppmv ozone are presented in Table 5-8 and Figures 5-7 through 5-12. At 85% RH, no viable *B. subtilis* spores were recovered from wallboard paper at 6 or 8 hours or on laminate at 8 hours. Viable *B. subtilis* spores were recovered from the materials at all other contact times at this concentration and at both 85% and 75% RH (log reductions for all materials ranged from 0.38 on wood to 7.60 on glass).

9,000 ppmv Ozone. Ozone fumigation results for *B. subtilis* spores at 9,000 ppmv ozone are presented in Tables 5-9 and 5-10 and Figures 5-7 through 5-12. At 85% RH, no viable *B. subtilis* spores were recovered from metal ductwork at 12 hours, from laminate at 6 or 9 hours, or from wallboard paper at 6, 9 or 12 hours. Viable *B. subtilis* spores were recovered from the materials at all other contact times at this concentration and at both 85% and 75% RH (log reductions for all materials ranged from 0.69 on wood to 7.17 on metal ductwork).

9,800 ppmv Ozone (with and without 24 hour pre-humidification). Ozone fumigation results for *B. subtilis* spores at 9,800 ppmv ozone are presented in Tables 5-11 and 5-12 and Figures 5-7 through 5-12. At 85% RH, no viable *B. subtilis* spores were recovered from painted wallboard paper following the 6, 9 and 12 hour contact times, laminate following the 9 and 12 hour contact times, and galvanized metal following the 9 hour contact time. After subjecting the test materials to 85% RH for ~24 hours and then introducing 9,800 ppmv, no viable *B. subtilis* spores were recovered from wood or pained wallboard paper after 6, 9 or 12 hours, glass after 6 hours or laminate after 12 hours. Spores were recovered from the materials at all other contact times at this concentration (log reductions for all materials ranged from 3.23 to 7.82 on laminate). Pre-humidification of the chamber for 24 hours increased the efficacy of the ozone gas on both wood and carpet, but did not increase the efficacy on the other materials in most instances.

12,000 ppmv Ozone. Ozone fumigation results for *B. subtilis* spores at 12,000 ppmv ozone are presented in Table 5-13 and Figures 5-7 through 5-12. At 85% RH, no viable *B. subtilis* spores were recovered from glass at 12 hours or from laminate and wallboard paper at 6, 9 or 12 hours. Viable *B. subtilis* spores were recovered from the materials at all other contact times at this concentration (log reductions for all materials ranged from 2.80 on wood to 7.80 on laminate).

All associated laboratory and procedural blanks resulted in 0 CFU recovered for all *B. subtilis* tests.

Table 5-8. Ozone Fumigation Results for *B. subtilis* Spores at 7,000 ppmv Ozone, 22 °C, 85% RH and 75% RH

Contact Time, hr	Material	Inoculation Amount, CFU	Positive Control[‡]	Test Material[§]	Inactivation Efficacy [#]
			Mean Recovered *B. anthracis* Spores, CFU[†]		
			85% RH		
4	Glass	3.17×10^8	$1.30 \pm 1.46 \times 10^8$	$1.54 \pm 2.74 \times 10^4$	4.51 ± 0.93
	Wood		$1.42 \pm 2.04 \times 10^7$	$2.84 \pm 1.00 \times 10^5$	1.48 ± 0.44
	Carpet		$1.61 \pm 1.87 \times 10^8$	$3.12 \pm 2.40 \times 10^6$	1.72 ± 0.56
	Laminate		$1.07 \pm 0.252 \times 10^8$	$6.67 \pm 12.1 \times 10^2$	6.49 ± 1.37
	Metal Ductwork		$2.36 \pm 0.811 \times 10^8$	$9.68 \pm 5.36 \times 10^5$	2.42 ± 0.27
	Wallboard Paper		$3.08 \pm 1.53 \times 10^7$	$1.32 \pm 1.81 \times 10^1$	6.84 ± 0.76
6	Glass	3.17×10^8	$1.30 \pm 1.46 \times 10^8$	$1.05 \pm 2.34 \times 10^5$	6.51 ± 2.19
	Wood		$1.42 \pm 2.04 \times 10^7$	$9.65 \pm 12.3 \times 10^3$	4.00 ± 1.66
	Carpet		$1.61 \pm 1.87 \times 10^8$	$4.22 \pm 3.13 \times 10^5$	2.82 ± 0.99
	Laminate		$1.07 \pm 0.252 \times 10^8$	$5.67 \pm 11.8 \times 10^2$	6.57 ± 1.28
	Metal Ductwork		$2.36 \pm 0.811 \times 10^8$	$7.53 \pm 3.13 \times 10^5$	2.51 ± 0.23
	Wallboard Paper		$3.08 \pm 1.53 \times 10^7$	0.00 ± 0.00	$\geq 7.44 \pm 0.20$
8	Glass	3.17×10^8	$1.30 \pm 1.46 \times 10^8$	$1.34 \pm 3.00 \times 10^1$	7.60 ± 0.78
	Wood		$1.42 \pm 2.04 \times 10^7$	$1.30 \pm 1.58 \times 10^4$	3.30 ± 0.87
	Carpet		$1.61 \pm 1.87 \times 10^8$	$1.17 \pm 0.498 \times 10^6$	2.01 ± 0.38
	Laminate		$1.07 \pm 0.252 \times 10^8$	0.00 ± 0.00	$\geq 8.02 \pm 0.09$
	Metal Ductwork		$2.36 \pm 0.811 \times 10^8$	$1.05 \pm 1.06 \times 10^5$	3.45 ± 0.32
	Wallboard Paper		$3.08 \pm 1.53 \times 10^7$	0.00 ± 0.00	$\geq 7.44 \pm 0.20$
			75% RH		
4	Glass	7.67×10^7	$4.31 \pm 1.07 \times 10^7$	$3.08 \pm 1.36 \times 10^6$	1.18 ± 0.22
	Wood		$1.49 \pm 0.794 \times 10^6$	$5.83 \pm 1.56 \times 10^5$	0.38 ± 0.21
	Carpet		$3.02 \pm 1.18 \times 10^7$	$4.22 \pm 1.22 \times 10^6$	0.83 ± 0.24
	Laminate		$4.77 \pm 0.425 \times 10^7$	$4.63 \pm 0.534 \times 10^6$	1.01 ± 0.06
	Metal Ductwork		$3.23 \pm 3.17 \times 10^7$	$8.31 \pm 1.22 \times 10^6$	0.39 ± 0.42
	Wallboard Paper		$4.17 \pm 1.25 \times 10^6$	$5.37 \pm 4.27 \times 10^3$	3.07 ± 0.49
6	Glass	7.67×10^7	$4.31 \pm 1.07 \times 10^7$	$7.30 \pm 5.52 \times 10^5$	1.88 ± 0.35
	Wood		$1.49 \pm 0.794 \times 10^6$	$3.82 \pm 1.05 \times 10^5$	1.71 ± 2.17
	Carpet		$3.02 \pm 1.18 \times 10^7$	$3.70 \pm 0.0915 \times 10^6$	0.87 ± 0.21
	Laminate		$4.77 \pm 0.425 \times 10^7$	$3.57 \pm 2.44 \times 10^6$	1.25 ± 0.36
	Metal Ductwork		$3.23 \pm 3.17 \times 10^7$	$7.56 \pm 1.58 \times 10^6$	0.44 ± 0.42
	Wallboard Paper		$4.17 \pm 1.25 \times 10^6$	$2.08 \pm 4.11 \times 10^3$	5.81 ± 1.56
8	Glass	7.67×10^7	$4.31 \pm 1.07 \times 10^7$	$1.63 \pm 0.267 \times 10^5$	2.42 ± 0.11
	Wood		$1.49 \pm 0.794 \times 10^6$	$5.54 \pm 2.17 \times 10^5$	0.42 ± 0.25
	Carpet		$3.02 \pm 1.18 \times 10^7$	$7.11 \pm 1.78 \times 10^5$	1.60 ± 0.23
	Laminate		$4.77 \pm 0.425 \times 10^7$	$1.43 \pm 0.210 \times 10^6$	1.53 ± 0.06
	Metal Ductwork		$3.23 \pm 3.17 \times 10^7$	$5.80 \pm 1.21 \times 10^6$	0.55 ± 0.42
	Wallboard Paper		$4.17 \pm 1.25 \times 10^6$	$2.97 \pm 6.61 \times 10^3$	5.17 ± 1.50

[†] Data are expressed as mean ± standard deviation of five replicates.
[‡] Positive control coupons were inoculated but not exposed to ozone.
[§] Test materials were inoculated and exposed to ozone for the contact time.
[#] Data are expressed as mean log reduction ± 95% CI of the SE.
* Ozone concentration, temperature and relative humidity listed are target levels. Refer to Table 4-3 for actual levels.

Table 5-9. Ozone Fumigation Results for *B. subtilis* Spores at 9,000 ppmv Ozone, 22 °C, 85% RH and 75% RH

Contact Time, hr	Material	Inoculation Amount, CFU	Positive Control[‡]	Test Material[§]	Inactivation Efficacy [#]
			Mean Recovered *B. anthracis* Spores, CFU[†]		
			85% RH		
4	Glass	1.04×10^8	$3.96 \pm 0.646 \times 10^7$	$8.19 \pm 6.28 \times 10^4$	2.84 ± 0.42
	Wood		$1.31 \pm 0.106 \times 10^6$	$2.72 \pm 1.46 \times 10^5$	0.74 ± 0.23
	Carpet		$3.67 \pm 0.655 \times 10^7$	$3.86 \pm 2.53 \times 10^6$	2.06 ± 0.29
	Laminate		$4.39 \pm 0.270 \times 10^7$	$7.49 \pm 1.64 \times 10^4$	2.78 ± 0.09
	Metal Ductwork		$5.63 \pm 1.51 \times 10^7$	$4.28 \pm 3.71 \times 10^6$	1.23 ± 0.34
	Wallboard Paper		$3.25 \pm 0.666 \times 10^6$	$1.16 \pm 1.79 \times 10^4$	3.17 ± 0.99
6	Glass	1.04×10^8	$3.96 \pm 0.646 \times 10^7$	$6.55 \pm 4.08 \times 10^3$	3.87 ± 0.30
	Wood		$1.31 \pm 0.106 \times 10^6$	$1.25 \pm 0.456 \times 10^5$	1.04 ± 0.14
	Carpet		$3.67 \pm 0.655 \times 10^7$	$2.94 \pm 1.58 \times 10^5$	2.15 ± 0.25
	Laminate		$4.39 \pm 0.270 \times 10^7$	$1.55 \pm 0.631 \times 10^5$	2.48 ± 0.14
	Metal Ductwork		$5.63 \pm 1.51 \times 10^7$	$2.82 \pm 1.01 \times 10^6$	1.32 ± 0.21
	Wallboard Paper		$3.25 \pm 0.666 \times 10^6$	$4.72 \pm 6.23 \times 10^2$	5.30 ± 1.45
8	Glass	1.04×10^8	$3.96 \pm 0.646 \times 10^7$	$1.09 \pm 0.614 \times 10^3$	4.60 ± 0.21
	Wood		$1.31 \pm 0.106 \times 10^6$	$6.33 \pm 4.78 \times 10^4$	1.53 ± 0.51
	Carpet		$3.67 \pm 0.655 \times 10^7$	$5.02 \pm 2.17 \times 10^4$	2.89 ± 0.18
	Laminate		$4.39 \pm 0.270 \times 10^7$	$5.59 \pm 1.59 \times 10^4$	2.91 ± 0.13
	Metal Ductwork		$5.63 \pm 1.51 \times 10^7$	$5.79 \pm 5.45 \times 10^5$	2.15 ± 0.43
	Wallboard Paper		$3.25 \pm 0.666 \times 10^6$	$1.87 \pm 3.64 \times 10^2$	5.52 ± 1.22
			75% RH		
4	Glass	8.83×10^7	$3.05 \pm 0.890 \times 10^7$	$1.23 \pm 1.60 \times 10^5$	2.77 ± 0.67
	Wood		$1.52 \pm 0.236 \times 10^6$	$3.50 \pm 1.55 \times 10^5$	0.69 ± 0.24
	Carpet		$3.63 \pm 1.02 \times 10^7$	$6.16 \pm 4.45 \times 10^5$	1.87 ± 0.35
	Laminate		$4.43 \pm 0.328 \times 10^7$	$9.61 \pm 4.05 \times 10^4$	2.70 ± 0.17
	Metal Ductwork		$6.50 \pm 1.69 \times 10^7$	$1.46 \pm 0.938 \times 10^6$	1.71 ± 0.26
	Wallboard Paper		$1.59 \pm 1.31 \times 10^7$	$1.18 \pm 2.54 \times 10^3$	5.87 ± 1.55
6	Glass	8.83×10^7	$3.05 \pm 0.890 \times 10^7$	$4.49 \pm 2.57 \times 10^3$	3.91 ± 0.35
	Wood		$1.52 \pm 0.236 \times 10^6$	$2.74 \pm 1.14 \times 10^5$	0.78 ± 0.23
	Carpet		$3.63 \pm 1.02 \times 10^7$	$4.40 \pm 2.90 \times 10^5$	2.02 ± 0.37
	Laminate		$4.43 \pm 0.328 \times 10^7$	$1.86 \pm 1.47 \times 10^5$	2.50 ± 0.34
	Metal Ductwork		$6.50 \pm 1.69 \times 10^7$	$1.33 \pm 0.938 \times 10^6$	1.75 ± 0.26
	Wallboard Paper		$1.59 \pm 1.31 \times 10^7$	$3.47 \pm 6.30 \times 10^3$	4.08 ± 0.74
8	Glass	8.83×10^7	$3.05 \pm 0.890 \times 10^7$	$1.00 \pm 1.70 \times 10^2$	6.28 ± 1.02
	Wood		$1.52 \pm 0.236 \times 10^6$	$1.25 \pm 0.795 \times 10^5$	1.14 ± 022
	Carpet		$3.63 \pm 1.02 \times 10^7$	$2.03 \pm 1.35 \times 10^5$	2.32 ± 0.29
	Laminate		$4.43 \pm 0.328 \times 10^7$	$1.19 \pm 1.65 \times 10^4$	3.84 ± 0.44
	Metal Ductwork		$6.50 \pm 1.69 \times 10^7$	$1.19 \pm 0.909 \times 10^5$	2.80 ± 0.26
	Wallboard Paper		$1.59 \pm 1.31 \times 10^7$	$2.00 \pm 4.47 \times 10^1$	6.67 ± 0.86

[†] Data are expressed as mean ± standard deviation of five replicates.
[‡] Positive control coupons were inoculated but not exposed to ozone.
[§] Test materials were inoculated and exposed to ozone for the contact time.
[#] Data are expressed as mean log reduction ± 95% CI of the SE.
* Ozone concentration, temperature and relative humidity listed are target levels. Refer to Table 4-3 for actual levels.

Table 5-10. Ozone Fumigation Results for *B. subtilis* Spores at 9,000 ppmv Ozone, 22 °C, 85% RH

Contact Time, hr	Material	Inoculation Amount, CFU	Positive Control[‡]	Test Material[§]	Inactivation Efficacy [#]
			Mean Recovered *B. anthracis* Spores, CFU[†]		
			85% RH		
6	Glass	9.03×10^7	$2.71 \pm 0.741 \times 10^7$	$1.00 \pm 1.88 \times 10^2$	6.53 ± 1.11
	Wood		$1.54 \pm 0.366 \times 10^6$	$6.63 \pm 8.04 \times 10^3$	2.62 ± 0.49
	Carpet		$4.98 \pm 1.06 \times 10^7$	$1.30 \pm 1.51 \times 10^4$	3.84 ± 0.52
	Laminate		$3.95 \pm 0.498 \times 10^7$	0.00 ± 0.00	$\geq 7.59 \pm 0.05$
	Metal Ductwork		$3.09 \pm 0.945 \times 10^7$	6.60 ± 14.8	7.17 ± 0.61
	Wallboard Paper		$5.90 \pm 2.32 \times 10^6$	0.00 ± 0.00	$\geq 6.75 \pm 0.14$
9	Glass	9.03×10^7	$2.71 \pm 0.741 \times 10^7$	$8.66 \pm 19.4 \times 10^1$	6.89 ± 1.04
	Wood		$1.54 \pm 0.366 \times 10^6$	$6.51 \pm 8.38 \times 10^3$	3.41 ± 1.53
	Carpet		$4.98 \pm 1.06 \times 10^7$	$9.32 \pm 12.9 \times 10^2$	5.14 ± 0.65
	Laminate		$3.95 \pm 0.498 \times 10^7$	0.00 ± 0.00	$\geq 7.59 \pm 0.05$
	Metal Ductwork		$3.09 \pm 0.945 \times 10^7$	$1.32 \pm 1.81 \times 10^1$	6.86 ± 0.74
	Wallboard Paper		$5.90 \pm 2.32 \times 10^6$	0.00 ± 0.00	$\geq 6.75 \pm 0.14$
12	Glass	9.03×10^7	$2.71 \pm 0.741 \times 10^7$	6.60 ± 14.8	7.11 ± 0.61
	Wood		$1.54 \pm 0.366 \times 10^6$	6.60 ± 14.8	5.87 ± 0.60
	Carpet		$4.98 \pm 1.06 \times 10^7$	$1.93 \pm 3.72 \times 10^3$	5.99 ± 1.56
	Laminate		$3.95 \pm 0.498 \times 10^7$	6.60 ± 14.8	7.29 ± 0.60
	Metal Ductwork		$3.09 \pm 0.495 \times 10^7$	0.00 ± 0.00	$\geq 7.47 \pm 0.13$
	Wallboard Paper		$5.90 \pm 2.32 \times 10^6$	0.00 ± 0.00	$\geq 6.75 \pm 0.14$

[†] Data are expressed as mean ± standard deviation of five replicates.
[‡] Positive control coupons were inoculated but not exposed to ozone.
[§] Test materials were inoculated and exposed to ozone for the contact time.
[#] Data are expressed as mean log reduction ± 95% CI of the SE.
* Ozone concentration, temperature and relative humidity listed are target levels. Refer to Table 4-3 for actual levels.

Table 5-11. Ozone Fumigation Results for *B. subtilis* Spores at 9,800 ppmv Ozone, 22 °C, 85% RH

Contact Time, hr	Material	Inoculation Amount, CFU	Positive Control[‡]	Test Material[§]	Inactivation Efficacy [#]
			Mean Recovered *B. anthracis* Spores, CFU[†]		
6	Glass	9.47×10^7	$2.88 \pm 0.800 \times 10^7$	$1.87 \pm 2.18 \times 10^2$	5.68 ± 0.94
	Wood		$2.53 \pm 0.958 \times 10^6$	$3.21 \pm 6.71 \times 10^3$	4.54 ± 1.61
	Carpet		$4.63 \pm 1.18 \times 10^7$	$3.07 \pm 2.59 \times 10^3$	4.35 ± 0.47
	Laminate		$6.65 \pm 1.30 \times 10^7$	6.60 ± 14.8	7.51 ± 0.60
	Metal Ductwork		$4.69 \pm 1.77 \times 10^7$	$6.68 \pm 6.25 \times 10^1$	6.17 ± 0.77
	Wallboard Paper		$7.79 \pm 2.69 \times 10^6$	0.00 ± 0.00	$\geq 6.87 \pm 0.14$
9	Glass	9.47×10^7	$2.88 \pm 0.800 \times 10^7$	$1.20 \pm 2.50 \times 10^2$	6.59 ± 1.10
	Wood		$2.53 \pm 0.958 \times 10^6$	$9.34 \pm 16.7 \times 10^3$	3.64 ± 1.58
	Carpet		$4.63 \pm 1.18 \times 10^7$	$4.19 \pm 5.31 \times 10^2$	5.34 ± 0.54
	Laminate		$6.65 \pm 1.30 \times 10^7$	0.00 ± 0.00	$\geq 7.82 \pm 0.08$
	Metal Ductwork		$4.69 \pm 1.77 \times 10^7$	0.00 ± 0.00	$\geq 7.65 \pm 0.15$
	Wallboard Paper		$7.79 \pm 2.69 \times 10^6$	0.00 ± 0.00	$\geq 6.87 \pm 0.14$
12	Glass	9.47×10^7	$2.88 \pm 0.800 \times 10^7$	$1.34 \pm 3.00 \times 10^1$	7.08 ± 0.73
	Wood		$2.53 \pm 0.958 \times 10^6$	$4.86 \pm 10.9 \times 10^2$	5.70 ± 1.34
	Carpet		$4.63 \pm 1.18 \times 10^7$	$4.07 \pm 8.91 \times 10^2$	6.69 ± 1.29
	Laminate		$6.65 \pm 1.30 \times 10^7$	0.00 ± 0.00	$\geq 7.82 \pm 0.08$
	Metal Ductwork		$4.69 \pm 1.77 \times 10^7$	$4.00 \pm 2.81 \times 10^1$	6.31 ± 0.69
	Wallboard Paper		$7.79 \pm 2.69 \times 10^6$	0.00 ± 0.00	$\geq 6.87 \pm 0.14$

[†] Data are expressed as mean ± standard deviation of five replicates.
[‡] Positive control coupons were inoculated but not exposed to ozone.
[§] Test materials were inoculated and exposed to ozone for the contact time.
[#] Data are expressed as mean log reduction ± 95% CI of the SE.
* Ozone concentration, temperature and relative humidity listed are target levels. Refer to Table 4-3 for actual levels.

Table 5-12. Ozone Fumigation Results for *B. subtilis* Spores at 9,800 ppmv Ozone, 22 °C, 85% RH (with pre-humidification) [1]

Contact Time, hr	Material	Inoculation Amount, CFU	Positive Control[‡] Mean Recovered *B. anthracis* Spores, CFU[†]	Test Material[§] Mean Recovered *B. anthracis* Spores, CFU[†]	Inactivation Efficacy [#]
6	Glass	4.87×10^7	$2.01 \pm 0.794 \times 10^7$	0.00 ± 0.00	$\geq 7.28 \pm 0.15$
	Wood		$2.41 \pm 1.27 \times 10^6$	0.00 ± 0.00	$\geq 6.32 \pm 0.25$
	Carpet		$3.02 \pm 1.16 \times 10^7$	$5.34 \pm 5.76 \times 10^2$	4.97 ± 0.52
	Laminate		$1.93 \pm 0.781 \times 10^7$	$1.19 \pm 0.613 \times 10^4$	3.23 ± 0.26
	Metal Ductwork		$1.40 \pm 0.198 \times 10^7$	$1.07 \pm 1.52 \times 10^2$	5.86 ± 1.05
	Wallboard Paper		$9.74 \pm 3.26 \times 10^5$	0.00 ± 0.00	$\geq 5.97 \pm 0.13$
9	Glass	4.87×10^7	$2.01 \pm 0.794 \times 10^7$	$6.66 \pm 8.50 \times 10^1$	6.11 ± 0.97
	Wood		$2.41 \pm 1.27 \times 10^6$	0.00 ± 0.00	$\geq 6.32 \pm 0.25$
	Carpet		$3.02 \pm 1.16 \times 10^7$	$1.32 \pm 1.81 \times 10^1$	6.84 ± 0.75
	Laminate		$1.93 \pm 0.781 \times 10^7$	$3.32 \pm 2.37 \times 10^1$	5.98 ± 0.65
	Metal Ductwork		$1.40 \pm 0.198 \times 10^7$	$2.00 \pm 2.99 \times 10^1$	6.47 ± 0.81
	Wallboard Paper		$9.74 \pm 3.26 \times 10^5$	0.00 ± 0.00	$\geq 5.97 \pm 0.13$
12	Glass	4.87×10^7	$2.01 \pm 0.794 \times 10^7$	$4.68 \pm 7.32 \times 10^1$	6.47 ± 0.99
	Wood		$2.41 \pm 1.27 \times 10^6$	0.00 ± 0.00	$\geq 6.32 \pm 0.25$
	Carpet		$3.02 \pm 1.16 \times 10^7$	$2.00 \pm 4.47 \times 10^1$	7.05 ± 0.80
	Laminate		$1.93 \pm 0.781 \times 10^7$	0.00 ± 0.00	$\geq 7.26 \pm 0.15$
	Metal Ductwork		$1.40 \pm 0.198 \times 10^7$	$5.34 \pm 11.9 \times 10^1$	6.66 ± 0.95
	Wallboard Paper		$9.74 \pm 3.26 \times 10^5$	0.00 ± 0.00	$\geq 5.97 \pm 0.13$

[1] Immediately following inoculation, all materials were kept at 85% ± 5% RH for approximately 24 hours prior to introduction of ozone.

[†] Data are expressed as mean ± standard deviation of five replicates.

[‡] Positive control coupons were inoculated but not exposed to ozone.

[§] Test materials were inoculated and exposed to ozone for the contact time.

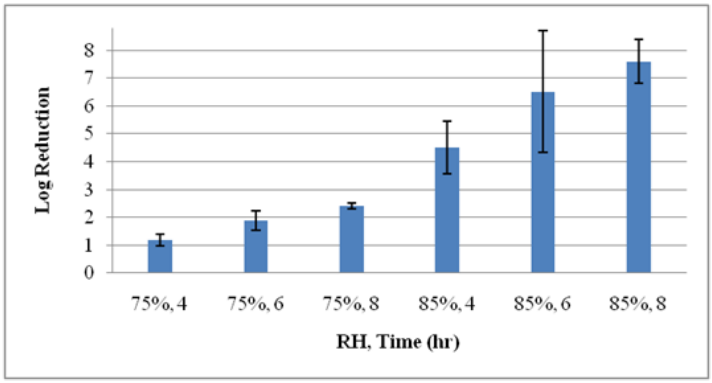
[#] Data are expressed as mean log reduction ± 95% CI of the SE.

* Ozone concentration, temperature and relative humidity listed are target levels. Refer to Table 4-3 for actual levels.

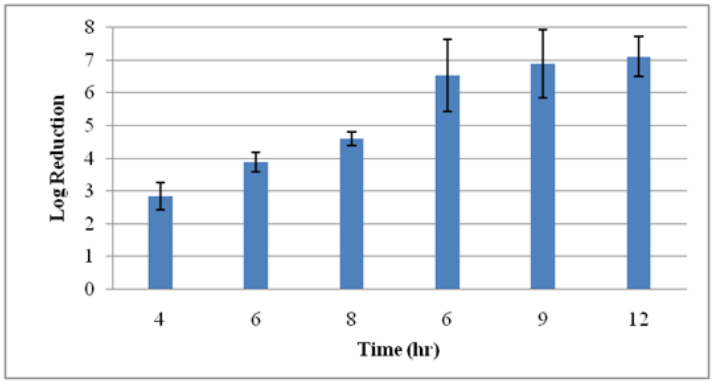
Table 5-13. Ozone Fumigation Results for *B. subtilis* Spores at 12,000[+] ppmv Ozone, 22 °C, 85% RH

Contact Time, hr	Material	Inoculation Amount, CFU	Positive Control[‡] Mean Recovered *B. anthracis* Spores, CFU[†]	Test Material[§] Mean Recovered *B. anthracis* Spores, CFU[†]	Inactivation Efficacy [#]
6	Glass	1.17×10^8	$4.08 \pm 0.829 \times 10^7$	$3.06 \pm 4.37 \times 10^2$	6.08 ± 1.26
	Wood		$5.09 \pm 2.75 \times 10^6$	$7.45 \pm 1.42 \times 10^3$	2.80 ± 0.19
	Carpet		$5.56 \pm 1.49 \times 10^7$	$1.11 \pm 0.254 \times 10^4$	3.69 ± 0.14
	Laminate		$6.41 \pm 1.12 \times 10^7$	0.00 ± 0.00	$\geq 7.80 \pm 0.07$
	Metal Ductwork		$5.50 \pm 1.32 \times 10^7$	$1.29 \pm 0.707 \times 10^3$	4.69 ± 0.29
	Wallboard Paper		$3.76 \pm 0.634 \times 10^6$	0.00 ± 0.00	$\geq 6.57 \pm 0.06$
9	Glass	1.17×10^8	$4.08 \pm 0.829 \times 10^7$	6.66 ± 14.9	7.30 ± 0.60
	Wood		$5.09 \pm 2.75 \times 10^6$	$5.01 \pm 8.01 \times 10^3$	3.77 ± 0.95
	Carpet		$5.56 \pm 1.49 \times 10^7$	$7.58 \pm 6.84 \times 10^3$	4.05 ± 0.47
	Laminate		$6.41 \pm 1.12 \times 10^7$	0.00 ± 0.00	$\geq 7.80 \pm 0.07$
	Metal Ductwork		$5.50 \pm 1.32 \times 10^7$	$1.47 \pm 2.04 \times 10^2$	6.42 ± 1.12
	Wallboard Paper		$3.76 \pm 0.634 \times 10^6$	0.00 ± 0.00	$\geq 6.57 \pm 0.06$
12	Glass	1.17×10^8	$4.08 \pm 0.829 \times 10^7$	0.00 ± 0.00	$\geq 7.60 \pm 0.08$
	Wood		$5.09 \pm 2.75 \times 10^6$	$4.20 \pm 2.42 \times 10^3$	3.13 ± 0.35
	Carpet		$5.56 \pm 1.49 \times 10^7$	$2.11 \pm 2.21 \times 10^3$	4.72 ± 0.65
	Laminate		$6.41 \pm 1.12 \times 10^7$	0.00 ± 0.00	$\geq 7.80 \pm 0.07$
	Metal Ductwork		$5.50 \pm 1.32 \times 10^7$	$1.29 \pm 2.82 \times 10^3$	6.26 ± 1.39
	Wallboard Paper		$3.76 \pm 0.634 \times 10^6$	0.00 ± 0.00	$\geq 6.57 \pm 0.06$

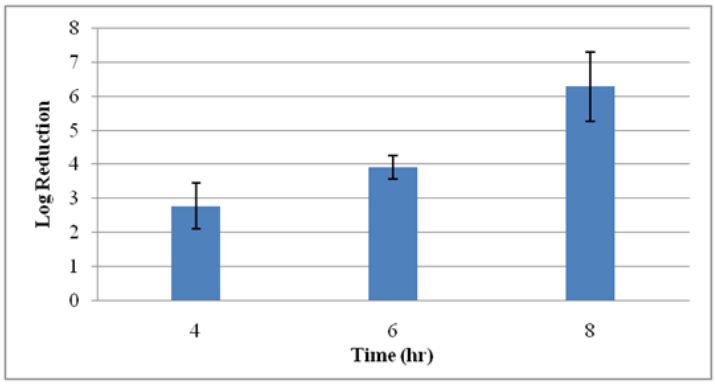
[†] Data are expressed as mean ± standard deviation of five replicates.
[‡] Positive control coupons were inoculated but not exposed to ozone.
[§] Test materials were inoculated and exposed to ozone for the contact time.
[#] Data are expressed as mean log reduction ± 95% CI of the SE.
[+] This concentration was measured by the Mini Hicon-LR analyzer and corresponds to ~10,800 ppmv on the IN2000-L2-LC analyzer.
* Ozone concentration, temperature and relative humidity listed are target levels. Refer to Table 4-3 for actual levels.



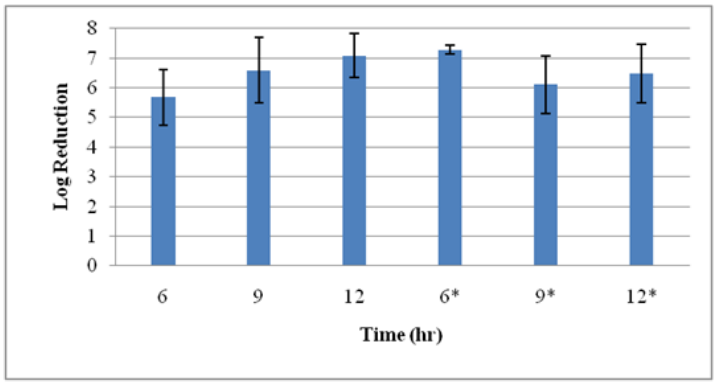
7,000 ppm O_3, 75% and 85% RH



9,000 ppm O_3, 85% RH

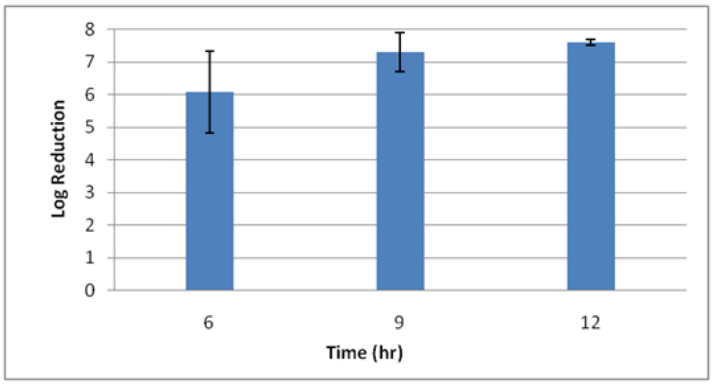


9,000 ppm O_3, 75% RH



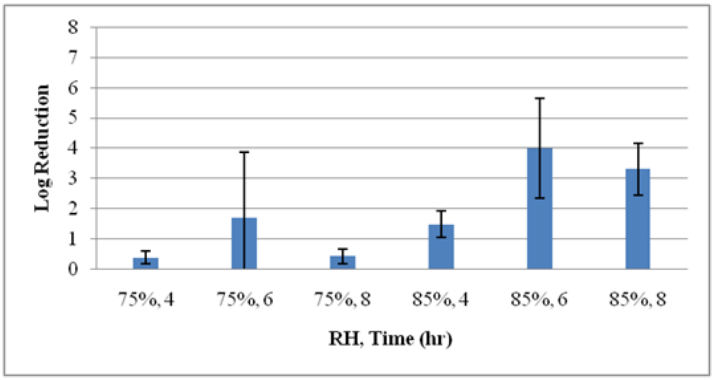
*Coupons were pre-humidified for ~24 hours

9,800 ppm O_3, 85% RH

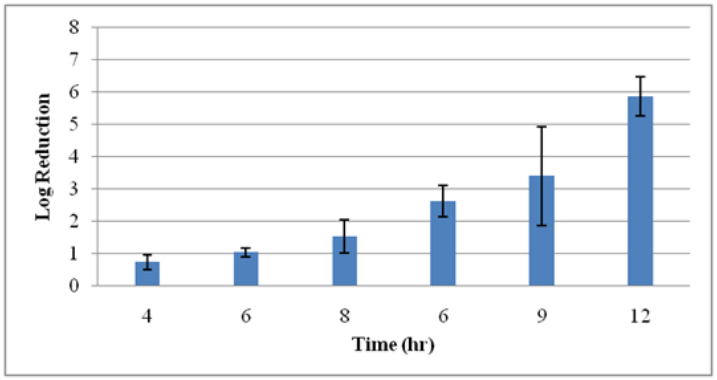


12,000 ppm O_3, 85% RH

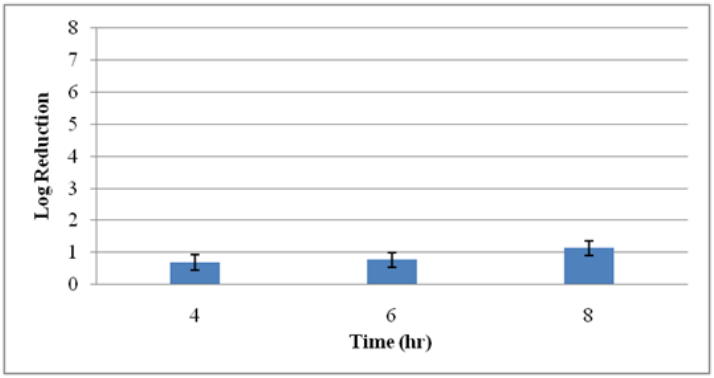
Figure 5-7. Efficacy (log reduction) against *B. subtilis* on glass.
(Data are expressed as mean log reduction ± 95% CI of the SE)



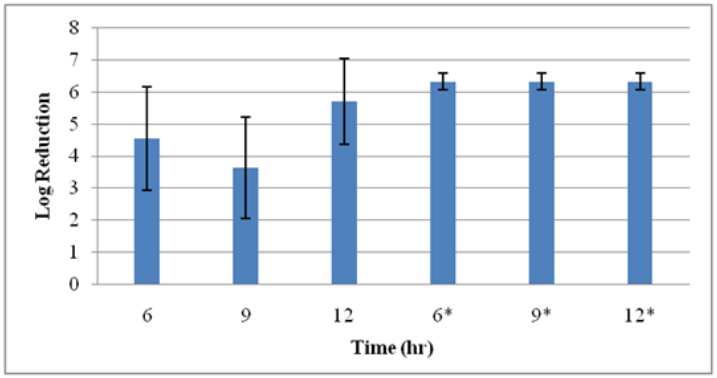
7,000 ppm O_3, 75% and 85% RH



9,000 ppm O_3, 85% RH

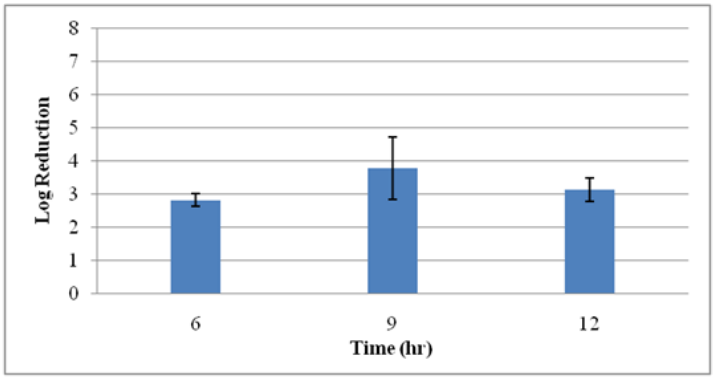


9,000 ppm O_3, 75% RH



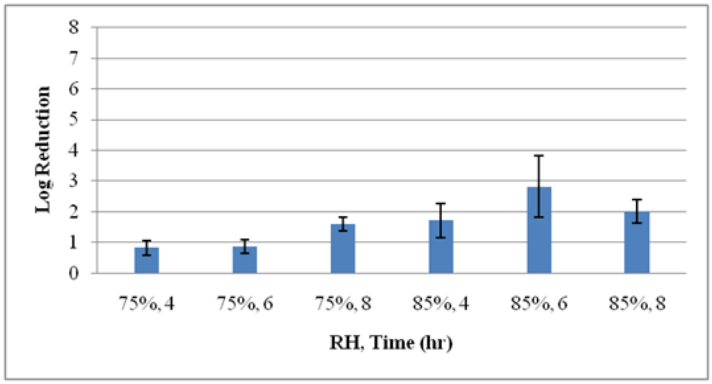
*Coupons were pre-humidified for ~24 hours

9,800 ppm O_3, 85% RH

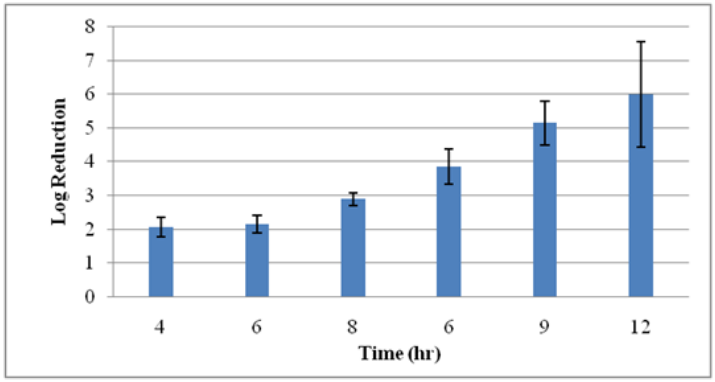


12,000 ppm O_3, 85% RH

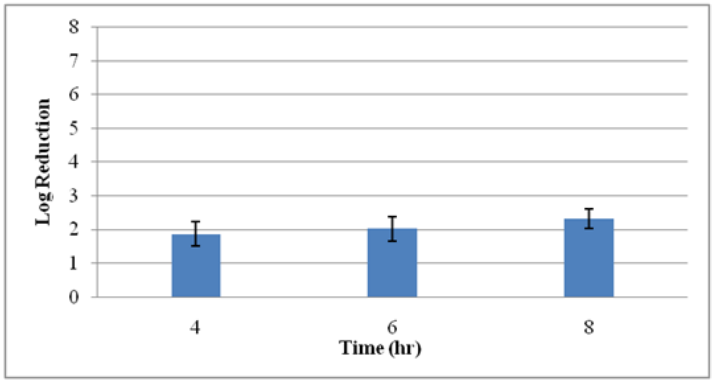
Figure 5-8. Efficacy (log reduction) against *B. subtilis* on wood.
(Data are expressed as mean log reduction ± 95% CI of the SE)



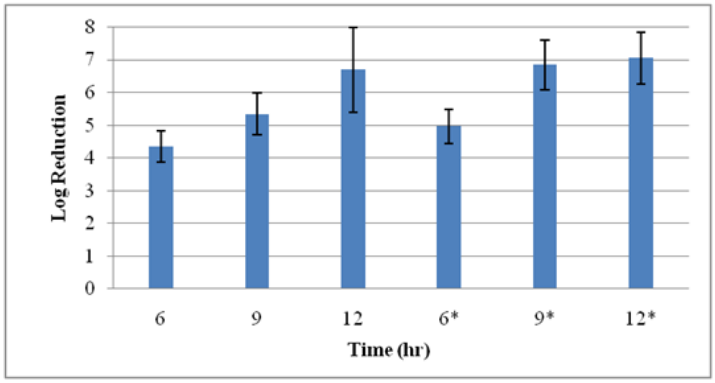
7,000 ppm O_3, 75% and 85% RH



9,000 ppm O_3, 85% RH

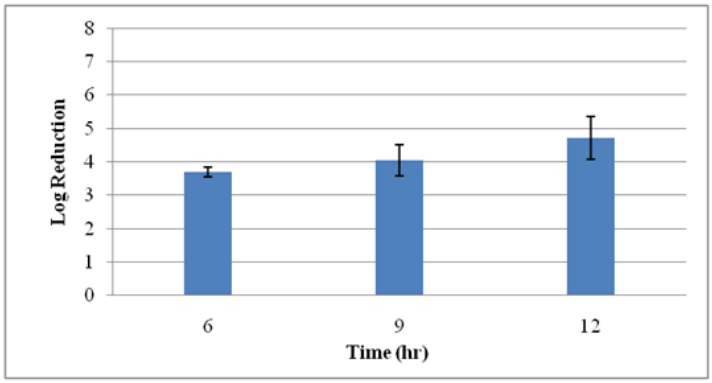


9,000 ppm O_3, 75% RH



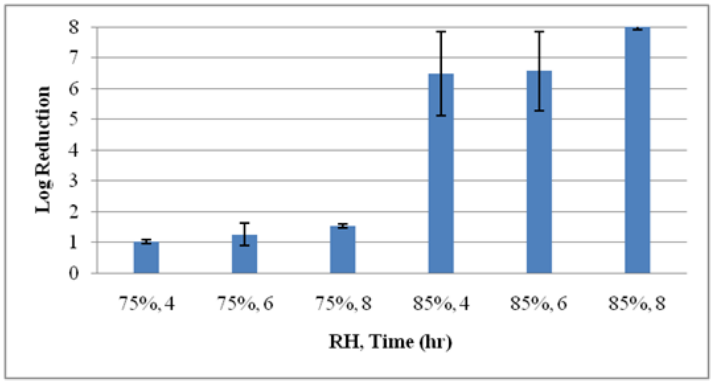
*Coupons were pre-humidified for ~24 hours

9,800 ppm O_3, 85% RH

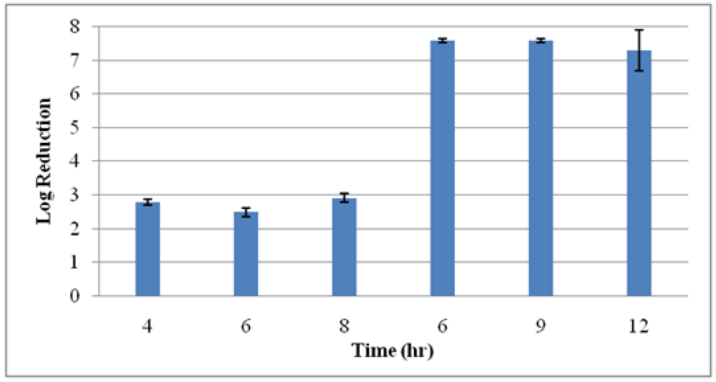


12,000 ppm O_3, 85% RH

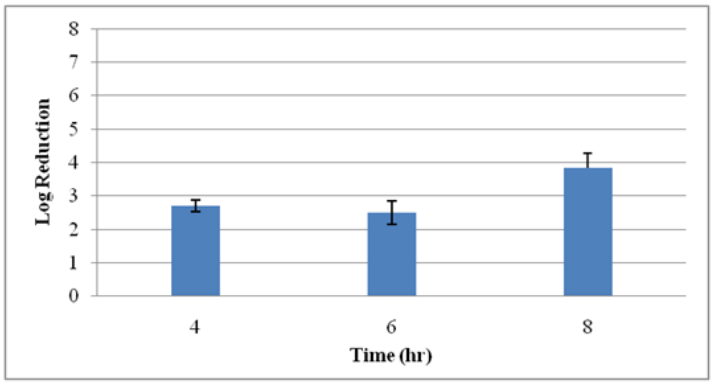
Figure 5-9. Efficacy (log reduction) against *B. subtilis* on carpet.
(Data are expressed as mean log reduction ± 95% CI of the SE)



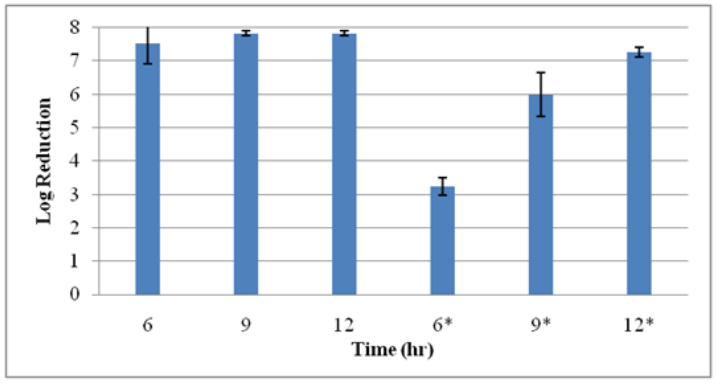
7,000 ppm O_3, 75% and 85% RH



9,000 ppm O_3, 85% RH

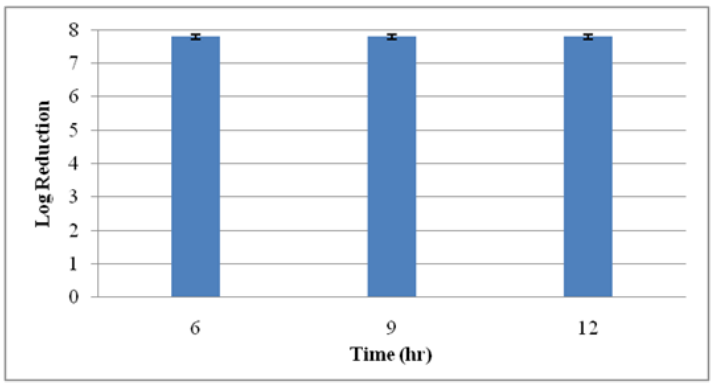


9,000 ppm O_3, 75% RH



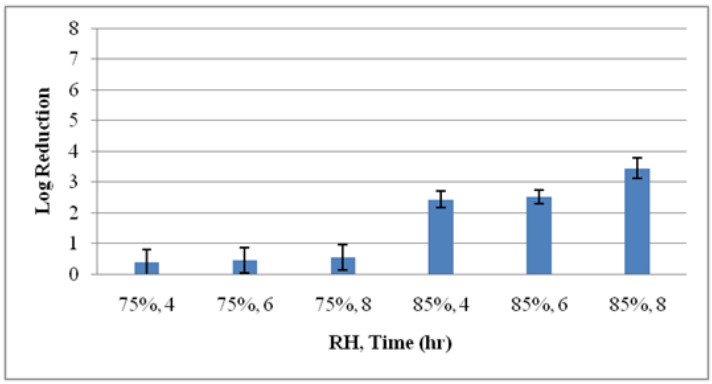
*Coupons were pre-humidified for ~24 hours

9,800 ppm O_3, 85% RH

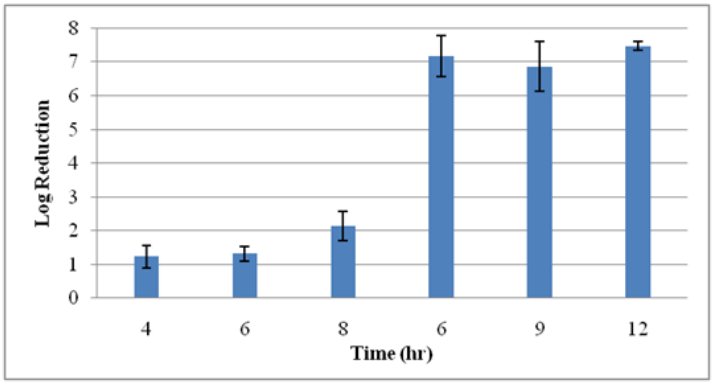


12,000 ppm O_3, 85% RH

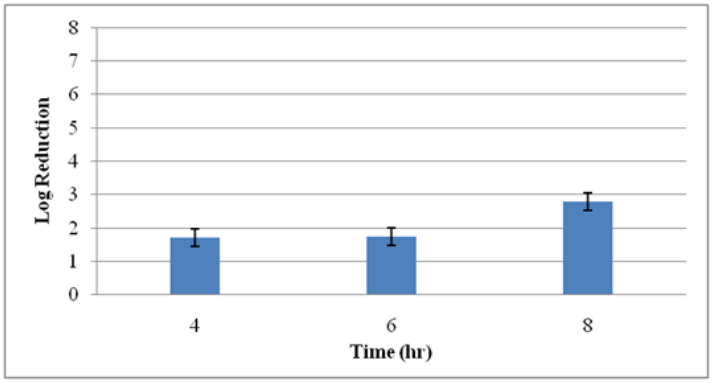
Figure 5-10. Efficacy (log reduction) against *B. subtilis* on laminate.
(Data are expressed as mean log reduction ± 95% CI of the SE)



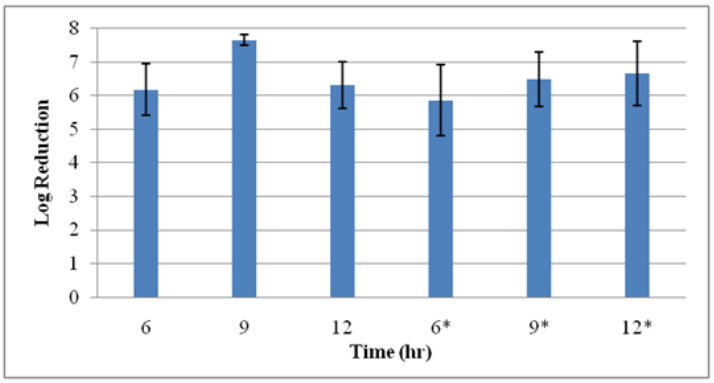
7,000 ppm O_3, 75% and 85% RH



9,000 ppm O_3, 85% RH

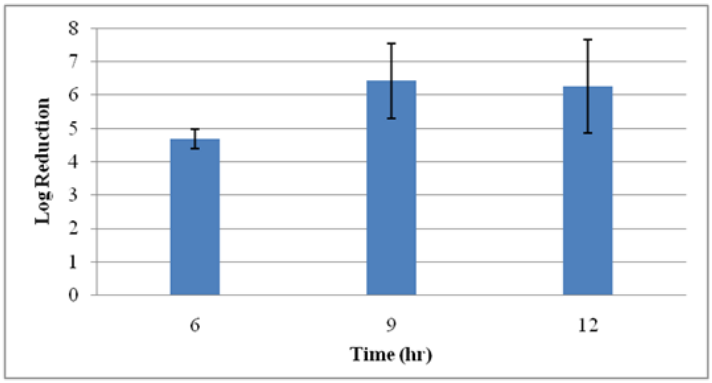


9,000 ppm O_3, 75% RH



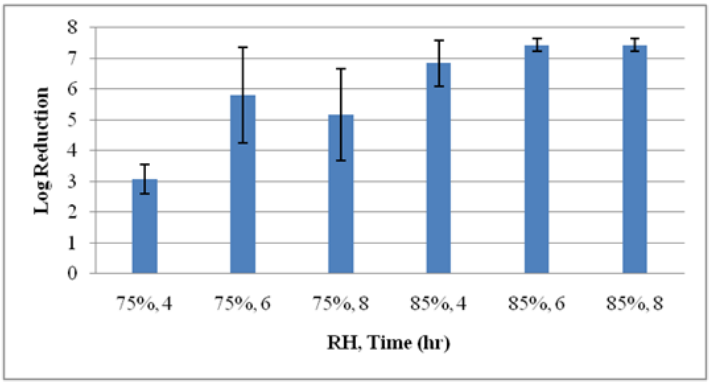
*Coupons were pre-humidified for ~24 hours

9,800 ppm O_3, 85% RH

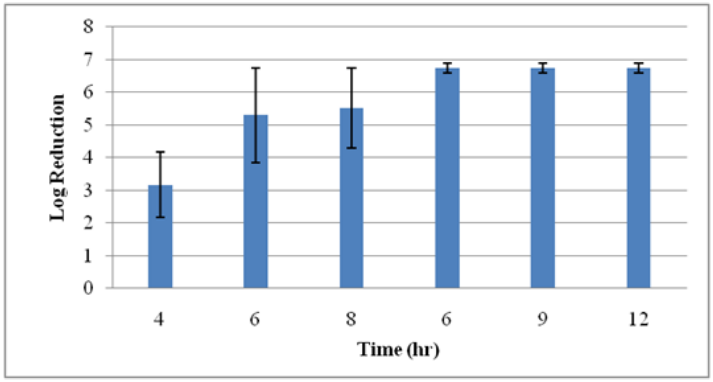


12,000 ppm O_3, 85% RH

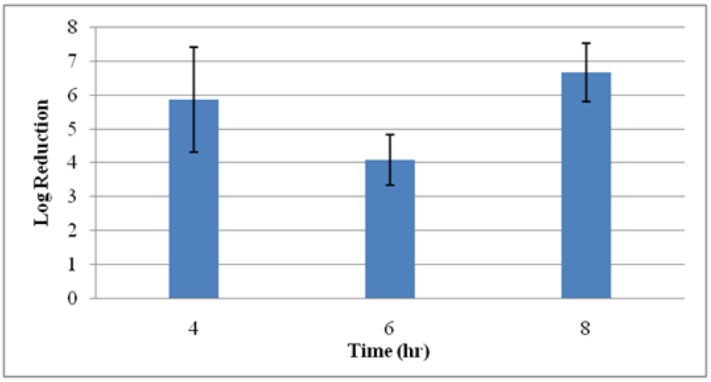
Figure 5-11. Efficacy (log reduction) against *B. subtilis* on metal ductwork.
(Data are expressed as mean log reduction ± 95% CI of the SE)



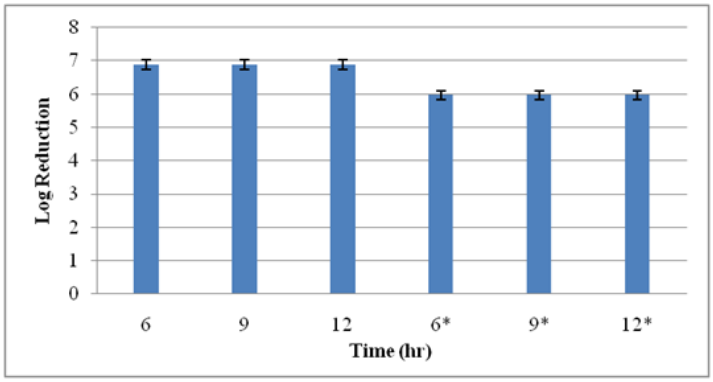
7,000 ppm O_3, 75% and 85% RH



9,000 ppm O_3, 85% RH

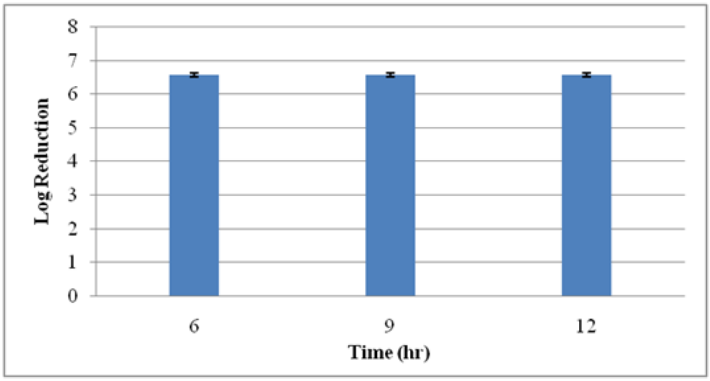


9,000 ppm O_3, 75% RH



*Coupons were pre-humidified for ~24 hours

9,800 ppm O_3, 85% RH



12,000 ppm O_3, 85% RH

Figure 5-12. Efficacy (log reduction) against *B. subtilis* on wallboard paper.
(Data are expressed as mean log reduction ± 95% CI of the SE)

5.3 Surface Damage to Materials

At the end of each fumigation test, the procedural blanks were visually compared to the laboratory blanks, and test coupons were visually compared to positive controls, to assess any impact the ozone gas may have had on each material type. Based on the visual appearance of the decontaminated coupons, there were no apparent changes in the color, reflectivity, or roughness of any of the six material surfaces after being exposed to ozone gas for up to 12,000 ppmv and 12 hours.

6.0 Summary

Viable *B. anthracis* and *B. subtilis* spores were recovered from all coupon types at 7,000 and 9,000 ppmv ozone when tested at 75% RH. At these same concentrations and contact times, but at 85% RH, there were several materials that were completely decontaminated, and generally higher log reductions were achieved when compared to testing at 75% RH. Following inoculation and prior to decontamination using 9,800 ppmv, a set of samples was held at ~85% RH for ~24 hours. Log reductions for these pre-humidified samples were slightly lower in most instances compared to samples tested at the same concentration (9,800 ppmv ozone) and contact times, but not pre-humidified for the 24 hours at 85% RH.

For *B. anthracis*, wallboard paper, carpet and wood were easiest to decontaminate with ozone gas (85%, 70% and 67% of tests yielded ≥6 log reduction, respectively) versus glass, metal ductwork, and laminate (37%, 4% and 4% of tests yielded ≥6 log reduction, respectively). *B. anthracis* spores were recovered from laminate and metal ductwork following fumigation at all ozone concentrations and contact times (log reductions ranged from 1.27 to 6.93) and from glass at all concentrations and contact times except for 12,000 ppmv and 12 hours (log reductions ranged from 1.70 to 7.66). No *B. anthracis* spores were recovered from carpet at contact times ≥8 hours. There were also no *B. anthracis* spores recovered from wood or carpet at 9,800 ppmv or 12,000 ppmv after 6 hours. Few to no *B. anthracis* spores were recovered from wallboard paper at all concentrations and contact times tested at 85% RH (0 to 5.34 x 10^1 CFU recovered).

For *B. subtilis*, glass, wallboard paper and laminate were easiest to decontaminate with ozone gas (58%, 54% and 54% of tests yielded ≥6 log reduction, respectively) versus metal ductwork, wood and carpet (42%, 13% and 13% of tests yielded ≥6 log reduction, respectively). *B. subtilis* spores were recovered from glass following fumigation at all concentrations except for 12,000 ppmv after a 12 hour contact time (log reductions ranged from 1.18 to 7.60). *B. subtilis* spores were recovered from wood at every contact time and every concentration (log reductions ranged from 0.38 to 5.87) except for the samples subjected to the 24 hour elevated %RH in which no viable spores were recovered. Spores were recovered from carpet at every concentration and contact time (log reductions ranged from 0.83 to 7.05). At 85% RH, no viable *B. subtilis* spores were recovered from the laminate coupons at 7,000 ppmv after 8 hours, 9,000 and 12,000 ppmv after 6, 9 or 12 hours or at 9,800 ppmv after 9 (not subjected to the 24 hour elevated %RH) or 12 hours. *B. subtilis* spores were also recovered at all contact times and concentrations from metal ductwork except for 9,000 ppmv after 12 hours (85% RH) and 9,800 ppmv after 9 hours (log reductions ranged from 0.39 to 7.17). Very few to no *B. subtilis* spores were recovered from wallboard paper at all concentrations and contact times tested at 85% RH (overall, 0 to 1.16 x 10^4 CFU recovered).

Two different analyzers were used to measure the target ozone concentrations at low (7,000 to 9,800 ppmv) and high (11,000 and 12,000 ppmv) levels. A comparison of these analyzers indicated that the high concentration analyzer yielded measurements approximately 1,000 to 1,300 ppmv higher than those measured by the low level analyzer. This may explain the observed lack of improvement in efficacy for some materials when increasing the ozone concentration from 9,800 to 11,000 ppmv (or from 9,800 to 12,000 ppmv for *B. subtilis*).

Ozone fumigation did not cause visible damage to any of the materials tested (glass, wood, carpet, laminate, metal ductwork and painted wallboard paper) for any test, including exposure for up to 12 hours at 12,000 ppmv ozone.

In comparing the decontamination efficacies (log reduction) for *B. subtilis* and *B. anthracis* for each test condition, contact time and material, in approximately 23% out of a total of 144 tests, the log reductions were significantly higher for *B. subtilis*. Differences were determined to be significant if the 95% confidence intervals for the log reduction results for the two microorganisms did not overlap.

In conclusion, ozone gas is a promising fumigant decontamination technology for the inactivation of anthrax spores on building materials, provided that sufficient concentration, contact time, temperature and relative humidity are achieved for the various materials being decontaminated. In general, decontamination efficacy improved with increasing ozone concentration and RH, and was affected by the material.

7.0 References

1. US Environmental Protection Agency. Product Performance Test Guidelines, OPPTS 810.2000: General Considerations for Public Health Uses of Antimicrobial Agents, Public Review Draft. EPA 712-C-07-005. November 5, 2009.

2. Rogers, J.V., C.L. Sabourin, Y.W. Choi, W.R. Richter, D.C. Rudnicki, K.B. Riggs, M.L. Taylor, and J. Chang, *Decontamination assessment of Bacillus anthracis, Bacillus subtilis, and Geobacillus stearothermophilus spores on indoor surfaces using a hydrogen peroxide gas generator.* Journal of Applied Microbiology, 2005(99): p. 739-748.

3. http://www.ringbell.co.uk/info/humid.htm

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