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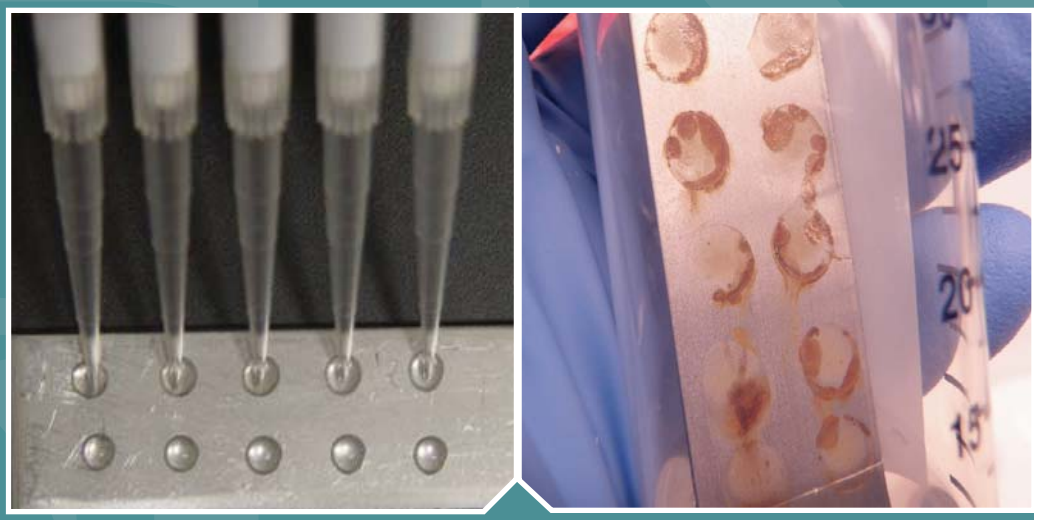
United States
Environmental Protection
Agency

Evaluation of Ethylene Oxide for the Inactivation of *Bacillus anthracis*



Office of Research and Development
National Homeland Security Research Center

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Evaluation of Ethylene Oxide for the Inactivation of *Bacillus anthracis*

U.S. Environmental Protection Agency

Research Triangle Park, NC 27711

Disclaimer

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Executive Summary

The U.S. Environmental Protection Agency, Office of Research and Development's Homeland Security Research Program is striving to protect human health and the environment from adverse impacts resulting from acts of terror by investigating the effectiveness and applicability of technologies for homeland security (HS)-related applications. The purpose of this investigation was to determine the decontamination efficacy of ethylene oxide (EtO) in inactivating *Bacillus anthracis* (causative agent for anthrax) spores on six material types typically found in museums. The objective of this study was to provide an understanding of the performance of EtO to guide its use and implementation in HS applications. In the assessment of options for decontamination following the release of *B. anthracis*, it is important to know whether and to what extent such factors can impact the decontamination efficacy.

This investigation focused on decontamination of six types of materials, including sensitive materials such as those that may typically be found in museums. The materials tested include: glass, bare pine wood, painted canvas, archival paper, silk fabric and carbon steel. Decontamination efficacy tests were conducted with spores of *B. anthracis* or *B. atrophaeus*, the latter organism included to assess its potential as a surrogate for future studies related to *B. anthracis* and EtO. Additionally, the difference between EtO efficacy against *B. atrophaeus* spores inoculated onto materials using a liquid suspension or dry method was assessed. Decontamination efficacy was quantified in terms of log reduction (LR), based on the difference in the number of bacterial spores recovered from the positive control coupons and test coupons. Tests were conducted with varying temperatures, relative humidity (RH) levels, concentrations of EtO, and contact times to assess the effect of these operational parameters on decontamination efficacy. The goal of this research was to evaluate the efficacy of EtO on a variety of materials. A LR of greater than 6 is considered to be effective [(1)]; however, results where complete spore inactivation was achieved is also reported. Inactivation or sterilization is the complete elimination of microbial viability [(2)].

Summary of Results

Various combinations of temperatures ranging from 30 °C to 50 °C, RH ranging from 30% to 75%, EtO concentrations ranging from 150 mg/L to 600 mg/L and contact times ranging from 45 minutes to 360 minutes were evaluated. The EtO gas decontamination technology provided a ≥six log reduction of *B. anthracis* on all six coupon material types under the following conditions:

- 50 °C, 50% RH, ≥600 mg/L EtO for ≥180 minutes
- 50 °C, 60% RH, ≥300 mg/L EtO for ≥180 minutes
- 50 °C, 75% RH, ≥150 mg/L EtO for ≥180 minutes
- 37 °C, 75% RH, ≥300 mg/L EtO for ≥90 minutes

Although slightly less effective against *B. atrophaeus*, complete inactivation was achieved on all six coupon types under the following conditions:

- 50 °C, 75% RH, ≥150 mg/L EtO for ≥180 minutes
- 37 °C, 75% RH, ≥300 mg/L EtO for ≥90 minutes

In general, *B. anthracis* was more difficult to inactivate on archival paper than *B. atrophaeus*. The lowest LRs were observed on this coupon type. *B. anthracis* was easier to inactivate on glass

and bare pine wood as these coupon types generally had higher LRs than the other four coupon types. In contrast, *B. atrophaeus* was easier to inactivate on archival paper and bare pine wood. Painted canvas and carbon steel were the most resistant to *B. atrophaeus* inactivation using EtO.

EtO fumigation against *B. atrophaeus* was evaluated using both liquid and dry coupon inoculum methods. Only one test was completed for directly comparing the two methods at 50 °C, 50% RH, and 150 mg/L EtO for a 45-minute contact time. Using these parameters, the spores inoculated as a dried formulation were slightly easier, although not significantly, to inactivate than spores applied to archival paper via liquid suspension.

EtO is an effective decontaminant against *B. anthracis* and *B. atrophaeus* under optimal combinations of concentration, contact time, temperature, and RH.

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Abbreviations/Acronyms

ADC	aerosol deposition chamber
ATCC	American Type Culture Collection
BBRC	Battelle Biomedical Research Center
BSC	biological safety cabinet
CFU	colony-forming unit(s)
CI	confidence interval
cm	centimeter(s)
°C	degree(s) Celsius
DNA	deoxyribonucleic acid
EPA	U.S. Environmental Protection Agency
EtO	ethylene oxide
HCl	hydrochloric acid
hr	hour(s)
kGy	kiloGray
L	liter(s)
LAL	Limulus amebocyte lycate
LR	log reduction
MDI	metered dose inhaler
min	minute(s)
mg	milligram(s)
mL	milliliter(s)
µL	microliter(s)
NHSRC	National Homeland Security Research Center
ORD	EPA Office of Research and Development
PBSTx	phosphate-buffered saline + 0.1% Triton X-100
PCR	polymerase chain reaction
QA	quality assurance
QC	quality control
RH	relative humidity
rpm	revolution(s) per minute
SD	standard deviation
SE	standard error
SEC	Sensor Electronics Corporation
SFW	sterile filtered water (cell-culture grade)
STS	sodium thiosulfate
TQAP	test/quality assurance plan
TSA	technical systems audit(s)
WA	work assignment

1.0 Introduction

The U.S. Environmental Protection Agency (EPA), Office of Research and Development's Homeland Security Research Program (HSRP) 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, EPA 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 or water systems, and facilitate the disposal of material resulting from cleanups.

EPA's HSRP 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 results are defensible. This program provides high-quality information that is useful to decision makers in purchasing or applying the tested technologies. 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 ethylene oxide (EtO) against *Bacillus anthracis* Ames and *B. atrophaeus* spores applied to materials, including sensitive materials such as those that may typically be found in museums or that could be sensitive to other types of decontaminants. The materials included in this testing were: glass, bare pine wood, painted canvas, archival paper, silk and carbon steel. Decontamination efficacy was determined based on the log reduction in viable spores recovered from the inoculated samples (with and without exposure to ethylene oxide). The goal of this research was to evaluate the efficacy of EtO on a variety of materials. A LR of greater than 6 is considered to be effective (USEPA, 2010).

2.0 Technology Description and Test Matrices

2.1 Technology Description

Ethylene oxide (EtO; Cat# 387614, Sigma Aldrich, St. Louis, MO, USA) is an organic compound with the formula C_2H_4O. EtO is flammable at room temperature and is a carcinogenic, mutagenic, irritant, and anesthetic gas with a misleadingly pleasant aroma. EtO is used to sterilize many things except food, drugs and liquids. Because the gas leaves no residue and does not damage the materials it contacts, EtO is widely used as a disinfectant and sterilant in hospitals and the medical equipment industry to replace steam in the sterilization of heat-sensitive tools such as disposable plastic syringes. This gas is a candidate that may possibly be used to decontaminate and sterilize sensitive materials that might be found in museums such as canvas paintings and fabrics in the event of a biological agent release.

2.2 Test Matrix

The test matrix for the EtO fumigation tests is shown in Table 2-1. The target temperature of 50 °C and target RH of 50% were based off standard EtO sterilization cycles developed by Andersen Products (Haw River, NC, USA)[4]. *B. anthracis* and *B. atrophaeus* were tested on all six material types for Tests 1 through 19. Test 20 was conducted using *B. atrophaeus* inoculated onto archival paper only to compare the efficacy for coupons inoculated with dry versus liquid inoculation methods.

Table 2-1. EtO Test Matrix

Test Number	Materials	Target Temperature (°C; ±2 °C)	Target RH (%; ±10%)	Target EtO Concentration (mg/L; ±10%)	Contact Time (min)	Sample Replicates
1	Glass Bare Pine Wood Painted Canvas Archival Paper Silk Fabric Carbon Steel	50	30	300	180	T=5 C=5 B_T=1 B_C=1
2		50	50	150	45	
3		50	50	150	90	
4		50	50	150	180	
5		50	50	300	45	
6		50	50	300	90	
7		50	50	300	180	
8*		50	50	300	180	
9		50	50	300	360	
10		50	50	600	180	
11		50	60	300	180	
12		50	60	600	360	
13		50	75	150	180	
14		50	75	300	45	
15		50	75	300	90	
16		50	75	300	180	
17		37	75	300	45	
18		37	75	300	90	
19		37	75	300	180	
20†	Archival Paper	50	50	150	45	T=6 C=6 B_T=1 B_C=1

T = Test Coupon
C = Positive Control Coupon
B_T = Procedural Blank Coupon
B_C = Laboratory Blank Coupon
*Samples subjected to a 24 hour (hr) pre-humidification cycle
†Samples inoculated with *B. atrophaeus* only; applied as a dry formulation as well as a liquid suspension

3.0 Summary of Test Procedures

This section provides an overview of the procedures that were used for the bench-scale evaluation of EtO to inactivate *B. anthracis* and *B. atrophaeus* spores on six different materials. Testing was performed in accordance with the EPA approved *Test/QA Plan for the Evaluation of Ethylene Oxide for the Inactivation of Bacillus anthracis* and associated amendments. The test/QA plan provides additional procedural details that are not included in this report [(3)].

3.1 Biological Agent

The *B. anthracis* spores used for this testing were prepared from a qualified stock of the Ames strain at the Battelle Biomedical Research Center (BBRC, Lot B21, West Jefferson, OH). All spore lots were subject to a stringent characterization and qualification process required by Battelle's standard operating procedure for spore production. Specifically, all spore lots were characterized prior to use by observation of colony morphology, direct microscopic observation of spore morphology and size and determination of percent refractivity and percent encapsulation. In addition, the number of viable spores was determined by colony count and expressed as colony forming units per milliliter (CFU/mL). Theoretically, once plated onto bacterial growth media, each viable spore germinates and yields one CFU. Variations in the expected colony phenotypes were recorded. Endotoxin concentration of each spore preparation was determined by the Limulus amebocyte lysate (LAL) assay to assess whether contamination from gram-negative bacteria occurred during the propagation and purification process of the spores.

The *B. atrophaeus* spores (Lot 19076-03268) were obtained in powder form from Dugway Proving Ground. No further activities were performed to verify the identity of the organism.

The *B. anthracis* stock spore suspensions were prepared in sterile filtered water (SFW) at an approximate concentration of 1×10^9 CFU/mL and stored under refrigeration at 2 to 8 degrees Celsius (°C). Similarly, the *B. atrophaeus* stock spore suspensions were prepared in sterile phosphate-buffered saline containing 0.1% Triton X-100 surfactant (PBSTx; Sigma, St. Louis, MO, USA) at the same concentration and stored at 2-8 °C.

3.2 Test Materials

Decontamination testing was conducted on six materials (glass, bare pine wood, painted canvas, archival paper, silk fabric and carbon steel). Information on the materials used for testing is presented in Table 3-1 and a picture of each is shown in Figure 3-1. Material coupons were cut to uniform length and width from a larger piece of stock material. Materials were prepared for testing either by sterilization via gamma irradiation at ~40 kilogray (kGy; STERIS Isomedix Services, Libertyville, IL, USA) or by autoclaving at 121 °C for 15 minutes. Gamma irradiated material coupons were sealed in 6 mil Uline Poly Tubing (Uline, Chicago, IL, USA) and autoclaved coupons were sealed in sterilization pouches (Fisher, Pittsburg, PA, USA) to preserve sterility until the coupons were ready for use.

Table 3-1. Test Materials

Material	Lot, Batch, ASTM No., or Observation	Manufacturer/ Supplier Name Location	Approximate Coupon Size, width x length	Approximate Coupon Thickness	Material Preparation
Glass	C1036	Brooks Brothers Columbus, OH	1.9 centimeter (cm) x 7.5 cm	0.3 cm	Autoclave
Bare Pine Wood	Generic Molding	Lowes Columbus, OH	1.9 cm x 7.5 cm	0.5 cm	Gamma Irradiation
Painted Canvas	NA	EPA	1.9 cm x 7.5 cm	0.1 cm	Gamma Irradiation
Archival Paper	10146, Acquerello Portofino Paper	Dick Blick Galesburg, IL	1.9 cm x 7.5 cm	0.1 cm	Gamma Irradiation
Silk Fabric	Silk Dupioni, Pewter Gray	Joann Fabrics Hudson, OH	1.9 cm x 7.5 cm	0.1 cm	Gamma Irradiation
Carbon Steel	ASTM A1008, Grade CS, Type B	Adept Products West Jefferson, OH	1.9 cm x 7.5 cm	0.1 cm	Autoclave

NA = Not Applicable

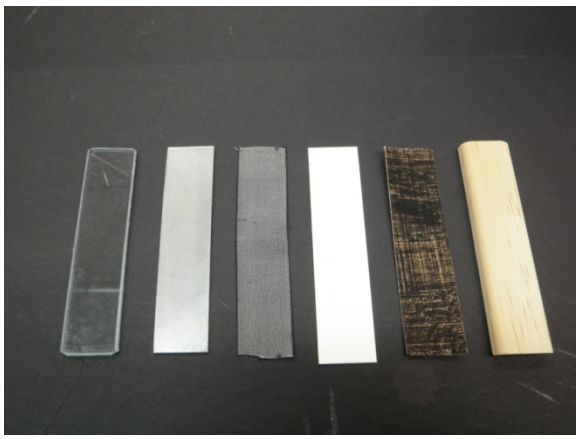


Figure 3-1. Coupon types from left to right: glass, carbon steel, silk fabric, archival paper, painted canvas and bare pine wood.

3.3 Preparation of Coupons

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 CFU of viable *B. anthracis* or *B. atrophaeus* spores per coupon. A 100 microliter (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 3-2). This approach provided a more uniform distribution of spores across the coupon surface than would be obtained through a single drop of the suspension. After inoculation, the coupons were left undisturbed overnight in a Class III BSC (used for the containment of dry spores) to dry under ambient conditions, approximately 22 °C and 40% RH.

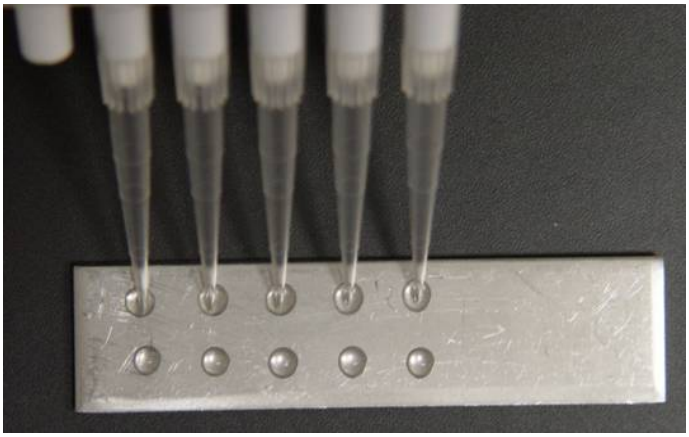


Figure 3-2. Liquid inoculation of coupon using a micropipette.

The number and type of replicate coupons used for each combination of material, decontaminant, concentration (Tests 1-19 only), and environmental condition included were:

- five test coupons (inoculated with *B. anthracis* or *B. atrophaeus* spores and exposed to decontaminant)
- five positive controls (inoculated with *B. anthracis* or *B. atrophaeus* spores but not exposed to decontaminant)
- one laboratory blank (inoculated only with sterile water and not exposed to the decontaminant)
- one procedural blank (inoculated only with sterile water and exposed to the decontaminant).

For Test 20, two inoculation methods (liquid and dry inoculation) were compared using *B. atrophaeus* inoculated onto archival paper only. Archival paper was chosen as this material had been the hardest to decontaminate and it was desirable to determine if the inoculation method had a correlation with the LR. Six test and six positive controls were inoculated with liquid suspension as described above. For dry inoculations, an additional six test and positive control paper coupons were loaded into an aerosol deposition chamber (ADC) following an established method.[(5)] A metered dose inhaler (MDI) of desired spore concentration was agitated using a vortex mixer (set to high) for 30 seconds to mix the contents fully. The MDI was then inserted into a stainless steel actuator which was connected to the ADC. The aerosol dose was administered by depressing the MDI with one firm swift actuation and holding in the depressed position for five seconds. The MDI actuator was decoupled from the lid, and the ADC remained undisturbed on the coupon surface for ≥18 hours to allow gravitational deposition of the particles. The coupons were inoculated at the EPA facility in Research Triangle Park, NC. The coupons were then removed from the ADC, placed in 50 mL conical tubes and shipped to the BBRC via common carrier. Laboratory and procedural blanks were not inoculated, but treated in the same manner as the test or positive controls as detailed.

On the day following liquid spore inoculation, coupons intended for decontamination (including blanks) were transferred into a test chamber and exposed to the EtO fumigant using the apparatus and application conditions specified in Section 3.4. Control coupons were added to the control chamber as described in Section 3.4. For Test 20, three positive controls were processed

immediately to assess any potential loss of bacteria during transit from the EPA to the BBRC.. The remaining three controls were added to the control chamber and processed as described in the following section.

3.4 EtO Fumigation Test and Control Chambers and Procedures

Figure 3-3 shows a schematic drawing of the EtO test chamber and containment system. EtO decontamination testing was conducted inside a 23-L glass test chamber developed by Battelle. As a means of secondary containment and laboratory personnel safety, this test chamber was housed inside a BSC III cabinet. Once injected into the test chamber, the EtO gas was measured continuously using an EtO Signature Process Gas Analyzer (Part No: 142-0597; Sensor Electronics Corporation [SEC], Minneapolis, MN, USA) during the entire contact time. This sensor was calibrated by SEC and was capable of measuring EtO gas between 0 and 2000 milligram/liter (mg/L). A low-speed fan was placed inside the test chamber to ensure a homogeneous mixture of EtO was achieved. When required, temperature was controlled using a heated/cooled water bath and RH was elevated using a Nafion (Permapure, Toms River, NJ, USA) tube pervaporation system. Temperature and RH in the EtO test chamber was measured using an HMT368 temperature and humidity probe (Vaisala, Inc, Woburn, MA, USA). Temperature, RH and EtO concentration were controlled with a CNI-822 controller (Omega, Stamford, CT, USA) and data were logged using the associated iLOG software.

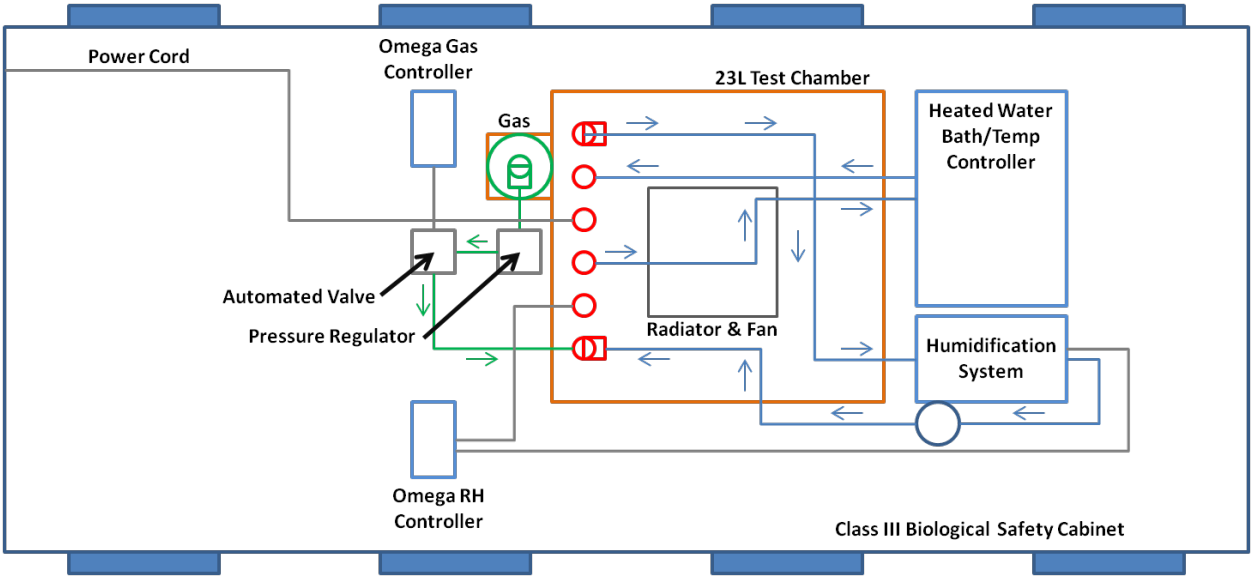


Figure 3-3. Schematic of EtO decontamination test chamber.

During a test run, inoculated test coupons were placed inside the EtO test chamber and the chamber was sealed. The chamber was allowed sufficient time to equilibrate to the required temperature and RH prior to start of the run. Once the temperature and RH were stable, 100% EtO was slowly injected into the chamber until the target concentration was reached. The test chamber remained sealed until the end of the required contact time (e.g., 45 minutes). At this time, the EtO was turned off and the seal of the test chamber broken by removing the lid. The test chamber and BSC III were allowed to off-gas until the EtO levels in the chamber reached 0 mg/L. At this time the samples were removed and processed as stated in Section 3.5.

A 9-L Lock&Lock container (Lock&Lock, Farmers Branch, TX, USA) was used as the control chamber. Fixed humidity point salts[6] were added as a slurry to a separate container placed in the bottom of the EtO control chamber. Sodium chloride was used to control the RH at 75%, potassium iodide for 60% RH, sodium bromide for 50% RH, and magnesium chloride was used to control the RH at 30%. The control chamber was placed in an incubator (Thermo Scientific, Waltham, MA, USA) for all tests and set to the appropriate temperature (i.e., 37 °C or 50 °C).

As in previous studies,[7] multiple inoculated coupons of each material were placed on a wire rack inside the test or control chamber. Blank (i.e., not inoculated) and positive control (i.e., inoculated but not decontaminated) coupons were also prepared for each test material and were utilized along with data from the test coupons (inoculated and decontaminated) to determine decontamination efficacy. This procedure provides a highly controlled, reproducible approach to assess sensitivity of the fumigation decontamination efficacy to temperature, RH, concentration and contact time.

3.5 Coupon Extraction and Biological Agent Quantification

After fumigation, test coupons, positive controls, and blanks were placed in 50 mL polypropylene conical vials containing 10 mL of sterile PBSTx. 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 tenfold dilutions was prepared in SFW. An aliquot (0.1 mL) of either the undiluted extract and/or each serial dilution was plated onto tryptic soy agar in triplicate and incubated for 18-24 hours (hrs) at 35 -37 ºC. 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. Laboratory blanks controlled for sterility and procedural blanks controlled for viable spores inadvertently introduced to test coupons. The blanks were spiked with an equivalent amount of 0.1 mL of SFW. The target acceptance criterion was that extracts of laboratory or procedural blanks were to contain no CFU.

After each decontamination test, the BSC III and the EtO test and control chambers were thoroughly cleaned (using separate steps involving bleach, ethanol, water, then drying) following procedures established under the BBRC Facility Safety Plan.

3.6 Decontamination Efficacy

The mean percent spore recovery from each coupon was calculated using results from positive control coupons (inoculated, not decontaminated), by means of the following equation:

$$\text{Mean \% Recovery} = [\text{Mean } CFU_{pc}/CFU_{spike}] \times 100 \qquad (1)$$

where Mean CFU_{pc} is the mean number of CFU recovered from five replicate positive control coupons of a single material, and CFU_{spike} is the number of CFU spiked onto each of those coupons. The value of CFU_{spike} is known from enumeration of the stock spore suspension. Spore recovery was calculated for *B. anthracis* or *B. atrophaeus* on each coupon, and the results are included in Section 5.

The performance or efficacy of EtO was assessed by determining the number of viable organisms remaining on each test coupon after decontamination. Those numbers were compared to the number of viable organisms extracted from the positive control coupons.
The number of viable spores of *B. anthracis* or *B. atrophaeus* in extracts of test and positive control coupons was determined to calculate efficacy of the decontaminant. Efficacy is defined as the extent (as $\log_{10}$ reduction, LR) to which viable spores extracted from test coupons after decontamination were less numerous than the viable spores extracted from positive control coupons. The logarithm of the CFU abundance from each coupon extract was determined, and the mean of those logarithm values was then determined for each set of control and associated test coupons, respectively. Efficacy of a decontaminant for a test organism/test condition on the i^{th} coupon material was calculated as the difference between those mean log values, i.e.:

$$Efficacy_i = \overline{(\log_{10} CFUc_{ij})} - \overline{(\log_{10} CFUt_{ij})} \quad (2)$$

where $\log_{10} CFUc_{ij}$ refers to the j individual logarithm values obtained from the positive control coupons and $\log_{10} CFUt_{ij}$ refers to the j individual logarithm values obtained from the corresponding test coupons, and the overbar designates a mean value. In tests conducted under this plan, there were five positive controls and five corresponding test coupons (i.e., $j = 5$) for each coupon. Test 20 utilized six positive controls and six corresponding test coupons (i.e., $j = 6$).

In the case where no viable spores were found in any of the five test coupon extracts after decontamination, a CFU abundance of 1 was assigned, resulting in a $\log_{10}$ CFU of zero for that material. This situation occurred when the decontaminant was highly effective, and no viable spores were found on the decontaminated test coupons. In such cases, the final efficacy on that material was reported as greater than or equal to (≥) the value calculated by Equation 2.

The variances (i.e., the square of the standard deviation [SD]) of the $\log_{10} CFUc_{ij}$ and $\log_{10} CFUt_{ij}$ values were also calculated for both the control and test coupons (i.e., SD^2c_{ij} and SD^2t_{ij}), and were used to calculate the pooled standard error (SE) for the efficacy value calculated in Equation 2, as follows:

$$SE = \sqrt{\frac{SD^2c_{ij}}{5} + \frac{SD^2t_{ij}}{5}} \quad (3)$$

where the number 5 again represents the number of coupons in both the control and test data sets (this number was 6 for Test 20). Each efficacy result was reported as a log reduction value with an associated 95% confidence interval (CI), calculated as:

$$95\%\ CI = Efficacy \pm (1.96 \times SE) \quad (4)$$

The significance of differences in efficacy across different test conditions and spore types was assessed based on the 95% confidence interval of each efficacy result. Differences in efficacy were judged to be significant if the 95% CIs of the two efficacy results did not overlap. Any results based on this formula are hereafter noted as significantly different. Note this comparison is not applicable when the two efficacy results being compared are both reported with log reductions as ≥ some value.

3.7 Surface Damage

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

4.0 Quality Assurance/Quality Control

Quality assurance/quality control (QC) procedures were performed in accordance with the *Quality Management Plan* (QMP) and the test/QA Plan (TQAP).[3] The QA/QC procedures and results are summarized below.

4.1 Audits

4.1.1 *Performance Evaluation Audit*

Performance evaluation audits were conducted to assess the quality of the results obtained during these experiments. Table 4-1 summarizes the performance evaluation audits that were performed.

No performance evaluation audits were performed to confirm the concentration and purity of *B. anthracis* or *B. atrophaeus* spores because quantitative standards do not exist for these organisms. The control coupons and blanks support the spore measurements.

Table 4-1. Performance Evaluation Audits

Measurement	Audit Procedure	Allowable Tolerance	Actual Tolerance
Volume of liquid from micropipettes	Gravimetric evaluation	± 10%	±0.15% to 2.5%
Time	Compared to independent clock	± 2 sec/hr	0 sec/hr
Temperature	Compared to independent calibrated thermometer	± 2 °C	± 1.02 °C
Relative Humidity	Compare to independent calibrated hygrometer	± 10%	± 3.14%
EtO Concentration	SEC Gas Analyzer Calibrated once annually by SEC and checked by technician once prior to start of testing	± 10%	± 10%
Balance	Compared to independent calibrated weight sets	± 0.5 g	± 0.1 g

4.1.2 Technical Systems Audit

Observations and findings from the technical systems audit (TSA) were documented and submitted to the Battelle Work Assignment (WA) Leader for response. Battelle QA staff conducted a TSA on October 30, 2012, to ensure that the tests were being conducted in accordance with the appropriate test/QA plan and QMP. 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.

4.1.3 Data Quality Audit

At least 10% of the data acquired during the evaluation were audited. A Battelle 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. Minor data issues were noted and corrected before data were used in reporting.

4.2 Test/Quality Assurance Plan Deviations

Section 2.1 of the TQAP states "Five positive controls and one procedural blank will be similarly handled compared to the test coupons that undergo EtO efficacy testing. The positive controls and laboratory blank will be placed in the control chamber and extracted/cultured at the completion of the decontamination efficacy test." Test and control samples were inoculated on October 29, 2012, and allowed to dry overnight for testing on October 30, 2012 (Test 2). Laboratory blank coupons were inadvertently forgotten and were not run for this test. This is not expected to have an impact on the data quality as previous blank coupons were negative.

Section 3.2 of the TQAP states "The temperature and RH of the control chamber (Lock & Lock, Farmers Branch, TX, USA) will be measured with a thermometer/hygrometer (Fisher Scientific Cat. No. S66283, Pittsburgh, PA, USA) and the data will be recorded using a HOBO data logger (Onset Part No. U12-001, Bourne, MA, USA)." For Test 8 started on 1/30/13, the HOBO was inadvertently not launched inside the control chamber, resulting in no temperature or RH data at the end of the contact period. The parameters for Test 8 were as follows: 300 mg/L EtO; 50 °C; 50% RH; 180 minute contact time with a 24-hour pre-humidification period.This is not expected to have an impact on data quality as the temperature in the room did not vary significantly.

4.3 QA/QC Reporting

Each assessment and audit was documented in accordance with the TQAP and QMP. 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 transcription errors requiring some recalculation of efficacy results, but none were gross errors in recording. QA/QC procedures were performed in accordance with the TQAP.

4.4 Data Review

Records and data generated in the evaluation received a QC/technical review before they were utilized in calculating or evaluating results and prior to incorporation in reports. The staff member performing the QC/technical review was involved in the experiments and added his/her initials and the date to a hard copy of the record being reviewed. This hard copy was returned to the staff member who stored the record.

5.0 Results

5.1 EtO Results

The efficacy of EtO against *B. anthracis* and *B. atrophaeus* was evaluated using the quantitative method at concentrations ranging from 150 to 600 mg/L, temperatures ranging from 37 °C to 50 °C, and RH from 30% to 75%. Contact times ranged from 45 to 360 min. The results of these tests are shown in Tables 5-1 and 5-2 and Figures 5-1 through 5-6.

Additionally, the efficacy of EtO against *B. atrophaeus* was evaluated using both liquid and dry inoculum methods at a concentration of 150 mg/L EtO, 50 °C and 50% RH for a contact time of 45 minutes. These methods are described in Section 3.3. The results of this test are shown in Table 5-3.

The temperature, RH, and EtO concentrations listed Tables 5-1, 5-2 and 5-3 are target values. Actual values for temperature, RH and EtO concentration all fell within target tolerance ranges specified in Table 2-1.

Table 5-1. EtO Efficacy Against *Bacillus anthracis*[a] Spores

Test Number	Temp (°C) / RH (%)	Concentration (mg/L) / Contact Time (min)	Material	Inoculum (CFU/coupon)	Mean Recovered *B. anthracis* (CFU/coupon)		Decontamination Efficacy ± CI[d]
					Positive Control[b]	Test Coupon[c]	
1	50/30	300/180	Glass	7.60×10^7	$2.55 \pm 0.78 \times 10^7$	$2.82 \pm 5.43 \times 10^3$	4.77 ± 0.91
			Bare Pine Wood		$4.19 \pm 0.52 \times 10^7$	$3.96 \pm 4.42 \times 10^3$	4.26 ± 0.47
			Painted Canvas		$5.84 \pm 0.67 \times 10^7$	$3.40 \pm 3.22 \times 10^3$	4.54 ± 0.59
			Archival Paper		$4.05 \pm 0.39 \times 10^7$	$7.11 \pm 2.89 \times 10^5$	1.78 ± 0.14
			Silk Fabric		$1.37 \pm 0.24 \times 10^7$	$1.43 \pm 0.93 \times 10^4$	3.04 ± 0.23
			Carbon Steel		$4.59 \pm 0.73 \times 10^7$	$1.20 \pm 1.40 \times 10^3$	4.99 ± 0.71
2	50/50	150/45	Glass	9.07×10^7	$5.85 \pm 0.56 \times 10^7$	$9.70 \pm 5.98 \times 10^3$	3.89 ± 0.35
			Bare Pine Wood		$7.83 \pm 1.34 \times 10^6$	$8.56 \pm 11.6 \times 10^3$	3.31 ± 0.59
			Painted Canvas		$5.09 \pm 0.58 \times 10^7$	$4.00 \pm 0.80 \times 10^4$	3.11 ± 0.09
			Archival Paper		$4.10 \pm 0.40 \times 10^7$	$3.27 \pm 0.58 \times 10^5$	2.10 ± 0.07
			Silk Fabric		$2.84 \pm 0.28 \times 10^7$	$5.13 \pm 2.01 \times 10^4$	2.77 ± 0.17
			Carbon Steel		$5.79 \pm 1.21 \times 10^7$	$9.17 \pm 3.86 \times 10^3$	3.83 ± 0.20
3	50/50	150/90	Glass	1.17×10^8	$5.67 \pm 0.56 \times 10^7$	$6.61 \pm 11.2 \times 10^3$	5.68 ± 1.82
			Bare Pine Wood		$4.35 \pm 1.32 \times 10^6$	0.00 ± 0.00	≥6.62 ± 0.12
			Painted Canvas		$4.73 \pm 0.43 \times 10^7$	$5.66 \pm 4.56 \times 10^3$	4.65 ± 1.50
			Archival Paper		$3.93 \pm 0.53 \times 10^7$	$1.96 \pm 1.29 \times 10^4$	3.38 ± 0.28
			Silk Fabric		$2.62 \pm 0.71 \times 10^7$	$9.07 \pm 9.47 \times 10^2$	4.65 ± 0.47
			Carbon Steel		$3.69 \pm 1.59 \times 10^7$	$1.36 \pm 0.90 \times 10^4$	3.45 ± 0.31
4	50/50	150/180	Glass	9.37×10^7	$5.55 \pm 0.81 \times 10^7$	$4.99 \pm 8.65 \times 10^2$	5.84 ± 1.07
			Bare Pine Wood		$5.96 \pm 0.96 \times 10^6$	$1.27 \pm 1.73 \times 10^2$	5.77 ± 1.20
			Painted Canvas		$8.03 \pm 0.56 \times 10^7$	$2.74 \pm 5.90 \times 10^1$	7.48 ± 0.83
			Archival Paper		$4.74 \pm 0.40 \times 10^7$	$7.19 \pm 3.21 \times 10^4$	2.86 ± 0.21
			Silk Fabric		$2.20 \pm 0.72 \times 10^7$	$9.08 \pm 10.8 \times 10^2$	4.85 ± 0.79
			Carbon Steel		$4.99 \pm 0.37 \times 10^7$	$6.05 \pm 10.1 \times 10^1$	6.86 ± 1.02
5	50/50	300/45	Glass	8.63×10^7	$2.37 \pm 0.90 \times 10^7$	$1.60 \pm 1.50 \times 10^2$	5.61 ± 0.93
			Bare Pine Wood		$8.93 \pm 4.28 \times 10^6$	$4.72 \pm 6.43 \times 10^1$	6.09 ± 1.01
			Painted Canvas		$6.60 \pm 1.35 \times 10^7$	$3.39 \pm 5.72 \times 10^1$	7.08 ± 0.90
			Archival Paper		$3.28 \pm 0.21 \times 10^7$	$9.75 \pm 2.23 \times 10^4$	2.54 ± 0.09
			Silk Fabric		$2.59 \pm 0.64 \times 10^7$	$8.27 \pm 2.90 \times 10^2$	4.51 ± 0.19
			Carbon Steel		$2.59 \pm 2.21 \times 10^7$	$5.37 \pm 8.34 \times 10^1$	6.22 ± 0.96
6	50/50	300/90	Glass	7.17×10^7	$2.67 \pm 0.93 \times 10^7$	$3.39 \pm 4.66 \times 10^1$	6.64 ± 0.93
			Bare Pine Wood		$4.49 \pm 1.07 \times 10^6$	7.46 ± 14.4 x 10	6.34 ± 0.60
			Painted Canvas		$6.66 \pm 1.20 \times 10^7$	$2.08 \pm 4.43 \times 10^1$	7.42 ± 0.79
			Archival Paper		$4.01 \pm 0.43 \times 10^7$	$1.07 \pm 1.42 \times 10^3$	5.28 ± 1.21
			Silk Fabric		$2.36 \pm 0.75 \times 10^7$	$2.73 \pm 4.30 \times 10^1$	6.65 ± 0.87
			Carbon Steel		$3.96 \pm 1.16 \times 10^7$	7.46 ± 14.4 x 10	7.28 ± 0.61
7	50/50	300/180	Glass	9.20×10^7	$4.37 \pm 1.02 \times 10^7$	0.00 ± 0.00	≥7.63 ± 0.09
			Bare Pine Wood		$4.15 \pm 2.23 \times 10^6$	0.00 ± 0.00	≥6.56 ± 0.22
			Painted Canvas		$6.26 \pm 0.65 \times 10^7$	0.00 ± 0.00	≥7.79 ± 0.04
			Archival Paper		$4.22 \pm 0.73 \times 10^7$	$8.65 \pm 5.42 \times 10^3$	3.78 ± 0.33
			Silk Fabric		$1.57 \pm 0.42 \times 10^7$	0.00 ± 0.00	≥7.18 ± 0.11
			Carbon Steel		$4.19 \pm 1.23 \times 10^7$	0.00 ± 0.00	≥7.61 ± 0.12
8	50/50	300/180 (with 24 Hour pre-humidification)	Glass	1.17×10^8	$4.96 \pm 0.80 \times 10^7$	$2.14 \pm 2.72 \times 10^2$	6.25 ± 1.20
			Bare Pine Wood		$2.59 \pm 0.81 \times 10^6$	$2.08 \pm 4.43 \times 10^1$	5.99 ± 0.79
			Painted Canvas		$8.11 \pm 0.57 \times 10^7$	0.00 ± 0.00	≥7.91 ± 0.02
			Archival Paper		$5.03 \pm 1.34 \times 10^7$	$4.20 \pm 4.90 \times 10^2$	6.01 ± 1.36
			Silk Fabric		$2.38 \pm 0.53 \times 10^7$	$3.00 \pm 4.70 \times 10^2$	5.53 ± 0.99
			Carbon Steel		$1.09 \pm 0.19 \times 10^8$	$4.41 \pm 2.94 \times 10^1$	7.67 ± 0.72
9	50/50	300/360	Glass	1.10×10^8	$5.25 \pm 0.67 \times 10^7$	0.00 ± 0.00	≥7.72 ± 0.05
			Bare Pine Wood		$6.37 \pm 1.54 \times 10^6$	0.00 ± 0.00	≥6.79 ± 0.11
			Painted Canvas		$9.58 \pm 0.76 \times 10^7$	0.00 ± 0.00	≥7.98 ± 0.03
			Archival Paper		$6.29 \pm 0.84 \times 10^7$	$2.10 \pm 2.33 \times 10^3$	4.68 ± 0.42
			Silk Fabric		$3.41 \pm 0.88 \times 10^7$	0.00 ± 0.00	≥7.52 ± 0.11
			Carbon Steel		$1.09 \pm 0.17 \times 10^8$	0.00 ± 0.00	≥8.03 ± 0.06
10	50/50	600/180	Glass	9.07×10^7	$4.62 \pm 0.52 \times 10^7$	0.00 ± 0.00	≥7.66 ± 0.04
			Bare Pine Wood		$8.16 \pm 1.14 \times 10^6$	0.00 ± 0.00	≥6.91 ± 0.05
			Painted Canvas		$8.51 \pm 0.92 \times 10^7$	0.00 ± 0.00	≥7.93 ± 0.04
			Archival Paper		$5.63 \pm 0.39x 10^7$	0.00 ± 0.00	≥7.75 ± 0.03
			Silk Fabric		$2.41 \pm 0.94 \times 10^7$	0.00 ± 0.00	≥7.36 ± 0.14
			Carbon Steel		$6.75 \pm 0.92 \times 10^7$	0.00 ± 0.00	≥7.83 ± 0.05

Table 5-1. EtO Efficacy Against *Bacillus anthracis* Spores[a] (Continued)

Test Number	Temp (°C) / RH (%)	Concentration (mg/L) / Contact Time (min)	Material	Inoculum (CFU/coupon)	Mean Recovered *B. anthracis* (CFU/coupon)		Decontamination Efficacy ± CI[d]
					Positive Control[b]	Test Coupon[c]	
11	50/60	300/180	Glass	1.08×10^8	$5.96 \pm 1.16 \times 10^7$	0.00 ± 0.00	≥7.77 ± 0.07
			Bare Pine Wood		$6.27 \pm 0.54 \times 10^6$	0.00 ± 0.00	≥6.80 ± 0.03
			Painted Canvas		$7.36 \pm 0.85 \times 10^7$	0.00 ± 0.00	≥7.86 ± 0.04
			Archival Paper		$5.03 \pm 0.53 \times 10^7$	0.00 ± 0.00	≥7.70 ± 0.04
			Silk Fabric		$2.69 \pm 0.65 \times 10^7$	0.00 ± 0.00	≥7.42 ± 0.11
			Carbon Steel		$4.74 \pm 0.48 \times 10^7$	0.00 ± 0.00	≥7.67 ± 0.04
12	50/60	600/360	Glass	1.35×10^8	$4.11 \pm 0.86 \times 10^7$	0.00 ± 0.00	≥7.61 ± 0.08
			Bare Pine Wood		$8.43 \pm 6.63 \times 10^6$	0.00 ± 0.00	≥6.85 ± 0.23
			Painted Canvas		$9.66 \pm 0.71 \times 10^7$	0.00 ± 0.00	≥7.98 ± 0.03
			Archival Paper		$4.23 \pm 0.90 \times 10^7$	0.00 ± 0.00	≥7.62 ± 0.08
			Silk Fabric		$2.21 \pm 0.72 \times 10^7$	0.00 ± 0.00	≥7.33 ± 0.12
			Carbon Steel		$2.85 \pm 1.12 \times 10^7$	0.00 ± 0.00	≥7.42 ± 0.17
13	50/75	150/180	Glass	9.73×10^7	$5.19 \pm 2.07 \times 10^7$	0.00 ± 0.00	≥7.69 ± 0.14
			Bare Pine Wood		$5.58 \pm 1.45 \times 10^6$	0.00 ± 0.00	≥6.73 ± 0.12
			Painted Canvas		$7.75 \pm 0.57 \times 10^7$	0.00 ± 0.00	≥7.89 ± 0.03
			Archival Paper		$4.09 \pm 0.36 \times 10^7$	0.00 ± 0.00	≥7.61 ± 0.03
			Silk Fabric		$1.80 \pm 0.67 \times 10^7$	0.00 ± 0.00	≥7.23 ± 0.14
			Carbon Steel		$7.38 \pm 1.18 \times 10^7$	0.00 ± 0.00	≥7.86 ± 0.06
14	50/75	300/45	Glass	8.33×10^7	$2.88 \pm 0.18 \times 10^7$	$4.03 \pm 5.43 \times 10^1$	6.43 ± 0.86
			Bare Pine Wood		$7.00 \pm 0.87 \times 10^6$	0.00 ± 0.00	≥6.84 ± 0.05
			Painted Canvas		$7.51 \pm 0.70 \times 10^7$	$7.46 \pm 14.4 \times 10^1$	7.57 ± 0.60
			Archival Paper		$4.40 \pm 2.23 \times 10^7$	0.00 ± 0.00	≥7.59 ± 0.24
			Silk Fabric		$2.02 \pm 0.88 \times 10^7$	0.00 ± 0.00	≥7.27 ± 0.16
			Carbon Steel		$3.63 \pm 0.53 \times 10^7$	0.00 ± 0.00	≥7.56 ± 0.05
15	50/75	300/90	Glass	1.09×10^8	$3.79 \pm 1.64 \times 10^7$	0.00 ± 0.00	≥7.54 ± 0.19
			Bare Pine Wood		$5.29 \pm 0.97 \times 10^6$	0.00 ± 0.00	≥6.72 ± 0.08
			Painted Canvas		$6.72 \pm 0.48 \times 10^7$	0.00 ± 0.00	≥7.83 ± 0.03
			Archival Paper		$4.54 \pm 0.45 \times 10^7$	0.00 ± 0.00	≥7.65 ± 0.04
			Silk Fabric		$3.11 \pm 1.02 \times 10^7$	0.00 ± 0.00	≥7.47 ± 0.16
			Carbon Steel		$1.31 \pm 0.10 \times 10^7$	0.00 ± 0.00	≥7.12 ± 0.03
16	50/75	300/180	Glass	8.13×10^7	$4.07 \pm 0.86 \times 10^7$	0.00 ± 0.00	≥7.60 ± 0.08
			Bare Pine Wood		$5.62 \pm 2.72 \times 10^6$	0.00 ± 0.00	≥6.71 ± 0.20
			Painted Canvas		$6.18 \pm 0.31 \times 10^7$	0.00 ± 0.00	≥7.79 ± 0.02
			Archival Paper		$3.81 \pm 0.35 \times 10^7$	0.00 ± 0.00	≥7.58 ± 0.04
			Silk Fabric		$1.90 \pm 0.65 \times 10^7$	0.00 ± 0.00	≥7.26 ± 0.12
			Carbon Steel		$2.99 \pm 0.31 \times 10^7$	0.00 ± 0.00	≥7.47 ± 0.04
17	37/75	300/45	Glass	1.02×10^8	$6.03 \pm 0.92 \times 10^7$	0.00 ± 0.00	≥7.78 ± 0.05
			Bare Pine Wood		$8.41 \pm 1.88 \times 10^6$	$3.27 \pm 6.57 \times 10^2$	5.55 ± 1.20
			Painted Canvas		$6.07 \pm 0.72 \times 10^7$	0.00 ± 0.00	≥7.78 ± 0.05
			Archival Paper		$4.87 \pm 1.16 \times 10^7$	0.00 ± 0.00	≥7.68 ± 0.10
			Silk Fabric		$3.12 \pm 0.47 \times 10^7$	0.00 ± 0.00	≥7.49 ± 0.06
			Carbon Steel		$7.33 \pm 0.69 \times 10^7$	$1.67 \pm 1.18 \times 10^2$	5.77 ± 0.36
18	37/75	300/90	Glass	1.10×10^8	$5.41 \pm 1.23 \times 10^7$	0.00 ± 0.00	≥7.72 ± 0.09
			Bare Pine Wood		$5.77 \pm 0.97 \times 10^6$	0.00 ± 0.00	≥6.76 ± 0.07
			Painted Canvas		$6.02 \pm 0.84 \times 10^7$	0.00 ± 0.00	≥7.78 ± 0.05
			Archival Paper		$3.35 \pm 0.52 \times 10^7$	0.00 ± 0.00	≥7.52 ± 0.06
			Silk Fabric		$2.47 \pm 0.87 \times 10^7$	0.00 ± 0.00	≥7.37 ± 0.14
			Carbon Steel		$4.24 \pm 0.58 \times 10^7$	0.00 ± 0.00	≥7.62 ± 0.06
19	37/75	300/180	Glass	1.18×10^8	$4.39 \pm 0.49 \times 10^7$	0.00 ± 0.00	≥7.64 ± 0.04
			Bare Pine Wood		$8.01 \pm 3.61 \times 10^6$	0.00 ± 0.00	≥6.87 ± 0.17
			Painted Canvas		$5.17 \pm 0.52 \times 10^7$	0.00 ± 0.00	≥7.71 ± 0.04
			Archival Paper		$2.46 \pm 0.68 \times 10^7$	0.00 ± 0.00	≥7.37 ± 0.12
			Silk Fabric		$1.29 \pm 0.27 \times 10^7$	0.00 ± 0.00	≥7.10 ± 0.08
			Carbon Steel		$3.04 \pm 0.48 \times 10^7$	0.00 ± 0.00	≥7.48 ± 0.06

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).
[b] Positive Controls = inoculated, not decontaminated coupons.
[c] Test Coupons = inoculated, decontaminated coupons.
[d] Laboratory Blank = not inoculated, not decontaminated coupon.
[e] Procedural Blank = not inoculated, decontaminated coupon.
[f] CI = confidence interval (± 1.96 × SE).
[g] "-" Not Applicable.

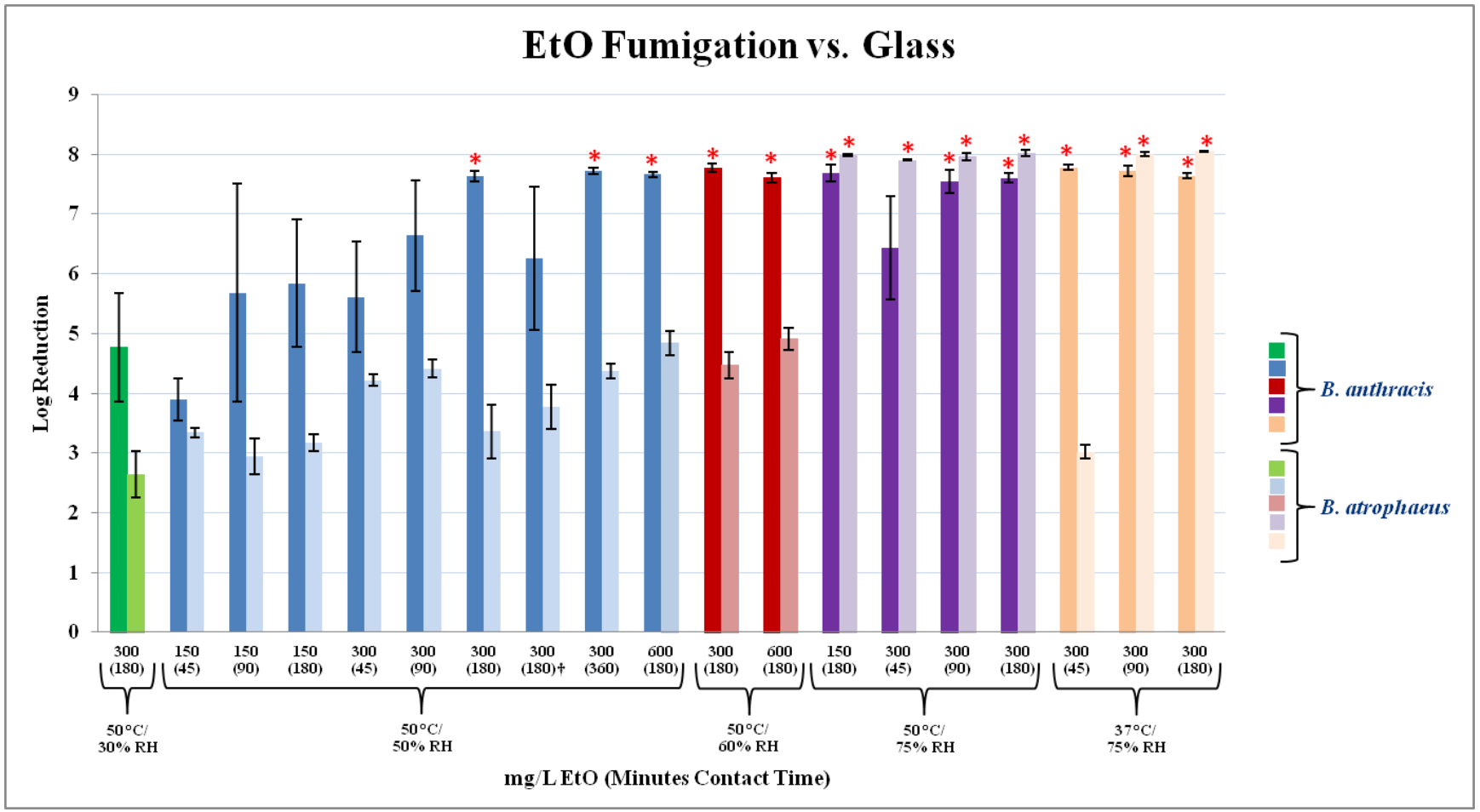
Table 5-2. EtO Efficacy Against *Bacillus atrophaeus* Spores[a]

Test Number	Temp (°C) / RH (%)	Concentration (mg/L) / Contact Time (min)	Material	Inoculum (CFU/coupon)	Mean Recovered *B. anthracis* (CFU/coupon)		Decontamination Efficacy ± CI[d]
					Positive Control[b]	Test Coupon[c]	
1	50/30	300/180	Glass	1.27×10^8	$1.05 \pm 0.08 \times 10^8$	$3.23 \pm 2.28 \times 10^5$	2.65 ± 0.39
			Bare Pine Wood		$5.04 \pm 1.58 \times 10^6$	$3.55 \pm 3.61 \times 10^3$	3.28 ± 0.36
			Painted Canvas		$7.47 \pm 1.00 \times 10^7$	$5.45 \pm 0.30 \times 10^4$	2.13 ± 0.05
			Archival Paper		$6.69 \pm 1.26 \times 10^7$	$4.07 \pm 4.43 \times 10^2$	5.40 ± 0.40
			Silk Fabric		$2.09 \pm 2.07 \times 10^7$	$1.51 \pm 0.99 \times 10^3$	4.12 ± 0.40
			Carbon Steel		$2.47 \pm 0.90 \times 10^7$	$2.32 \pm 3.67 \times 10^5$	2.44 ± 0.63
2	50/50	150/45	Glass	1.04×10^8	$1.00 \pm 0.12 \times 10^8$	$4.63 \pm 0.84 \times 10^4$	3.34 ± 0.079
			Bare Pine Wood		$4.53 \pm 1.30 \times 10^6$	$1.47 \pm 1.52 \times 10^2$	4.67 ± 0.42
			Painted Canvas		$6.63 \pm 1.12 \times 10^7$	$1.04 \pm 0.23 \times 10^5$	2..81 ± 0.11
			Archival Paper		$3.82 \pm 2.06 \times 10^7$	$1.54 \pm 0.41 \times 10^3$	4.35 ± 0.25
			Silk Fabric		$1.04 \pm 0.18 \times 10^7$	$6.05 \pm 6.80 \times 10^1$	5.84 ± 0.95
			Carbon Steel		$3.45 \pm 2.31 \times 10^7$	$1.36 \pm 0.45 \times 10^4$	3.34 ± 0.31
3	50/50	150/90	Glass	1.21×10^8	$9.95 \pm 1.71 \times 10^6$	$1.41 \pm 1.06 \times 10^5$	2.94 ± 0.30
			Bare Pine Wood		$7.88 \pm 6.21 \times 10^6$	0.00 ± 0.00	≥6.82 ± 0.23
			Painted Canvas		$6.27 \pm 1.40 \times 10^7$	$7.33 \pm 2.50 \times 10^5$	1.95 ± 0.17
			Archival Paper		$5.15 \pm 0.38 \times 10^7$	$4.08 \pm 8.90 \times 10^1$	7.25 ± 0.90
			Silk Fabric		$1.13 \pm 0.38 \times 10^7$	$1.15 \pm 2.18 \times 10^3$	5.31 ± 1.47
			Carbon Steel		$1.11 \pm 0.11 \times 10^7$	$2.03 \pm 1.20 \times 10^4$	2.79 ± 0.22
4	50/50	150/180	Glass	1.25×10^8	$8.86 \pm 1.13 \times 10^7$	$6.25 \pm 1.83 \times 10^4$	3.17 ± 0.14
			Bare Pine Wood		$9.53 \pm 13.7 \times 10^6$	$7.35 \pm 7.21 \times 10^1$	5.25 ± 0.87
			Painted Canvas		$7.10 \pm 0.68 \times 10^7$	$6.67 \pm 2.08 \times 10^4$	3.04 ± 0.12
			Archival Paper		$5.84 \pm 1.68 \times 10^7$	$8.53 \pm 4.22 \times 10^2$	4.86 ± 0.23
			Silk Fabric		$1.39 \pm 0.89 \times 10^7$	$1.93 \pm 1.67 \times 10^2$	4.93 ± 0.37
			Carbon Steel		$8.85 \pm 1.35 \times 10^6$	$2.53 \pm 1.53 \times 10^4$	2.60 ± 0.21
5	50/50	300/45	Glass	1.22×10^8	$9.86 \pm 1.65 \times 10^7$	$6.03 \pm 1.23 \times 10^3$	4.22 ± 0.10
			Bare Pine Wood		$9.39 \pm 4.65 \times 10^6$	$1.80 \pm 1.86 \times 10^2$	5.17 ± 0.96
			Painted Canvas		$7.92 \pm 0.57 \times 10^7$	$7.09 \pm 2.80 \times 10^4$	3.08 ± 0.16
			Archival Paper		$5.26 \pm 0.44 \times 10^7$	$2.89 \pm 1.23 \times 10^4$	4.29 ± 0.16
			Silk Fabric		$1.27 \pm 0.33 \times 10^7$	$7.53 \pm 3.33 \times 10^2$	4.26 ± 0.23
			Carbon Steel		$1.49 \pm 0.44 \times 10^6$	$6.69 \pm 2.56 \times 10^3$	3.36 ± 0.20
6	50/50	300/90	Glass	1.10×10^8	$1.12 \pm 0.05 \times 10^8$	$4.65 \pm 1.75 \times 10^3$	4.41 ± 0.15
			Bare Pine Wood		$8.13 \pm 1.47 \times 10^6$	$2.54 \pm 2.72 \times 10^2$	5.36 ± 1.25
			Painted Canvas		$7.15 \pm 1.39 \times 10^7$	$5.89 \pm 1.82 \times 10^4$	3.09 ± 0.14
			Archival Paper		$5.02 \pm 0.97 \times 10^7$	7.46 ± 14.4 x 10	7.39 ± 0.60
			Silk Fabric		$1.45 \pm 0.96 \times 10^7$	$1.45 \pm 2.43 \times 10^3$	4.38 ± 0.64
			Carbon Steel		$4.21 \pm 2.03 \times 10^7$	$4.54 \pm 2.66 \times 10^3$	3.98 ± 0.30
7	50/50	300/180	Glass	1.14×10^8	$9.11 \pm 0.77 \times 10^7$	$6.06 \pm 5.46 \times 10^4$	3.36 ± 0.45
			Bare Pine Wood		$5.39 \pm 1.60 \times 10^6$	$3.20 \pm 3.41 \times 10^2$	4.53 ± 0.59
			Painted Canvas		$5.89 \pm 0.55 \times 10^7$	$4.66 \pm 1.00 \times 10^4$	3.11 ± 0.10
			Archival Paper		$4.25 \pm 0.52 \times 10^7$	$3.53 \pm 1.98 \times 10^2$	5.14 ± 0.24
			Silk Fabric		$1.65 \pm 0.44 \times 10^7$	$8.68 \pm 8.00 \times 10^1$	5.65 ± 0.81
			Carbon Steel		$1.94 \pm 0.93 \times 10^7$	$6.07 \pm 2.43 \times 10^3$	3.49 ± 0.23
8	50/50	300/180 (with 24 Hour pre-humidification)	Glass	1.39×10^8	$1.04 \pm 0.09 \times 10^8$	$2.46 \pm 2.22 \times 10^4$	3.78 ± 0.37
			Bare Pine Wood		$2.07 \pm 1.16 \times 10^6$	7.40 ± 14.31	5.96 ± 0.63
			Painted Canvas		$1.88 \pm 0.52 \times 10^7$	$3.35 \pm 0.64 \times 10^5$	1.74 ± 0.11
			Archival Paper		$1.37 \pm 0.20 \times 10^7$	0.00 ± 0.00	≥7.13 ± 0.06
			Silk Fabric		$4.97 \pm 2.23 \times 10^6$	$3.42 \pm 7.42 \times 10^1$	6.20 ± 0.90
			Carbon Steel		$2.45 \pm 1.54 \times 10^5$	$8.89 \pm 8.26 \times 10^4$	0.64 ± 0.61
9	50/50	300/360	Glass	1.08×10^8	$1.19 \pm 0.25 \times 10^8$	$5.12 \pm 1.42 \times 10^3$	4.37 ± 0.13
			Bare Pine Wood		$1.27 \pm 0.48 \times 10^7$	$1.07 \pm 1.44 \times 10^2$	5.82 ± 1.06
			Painted Canvas		$5.23 \pm 0.78 \times 10^7$	$1.61 \pm 0.61 \times 10^4$	3.53 ± 0.13
			Archival Paper		$4.29 \pm 0.45 \times 10^7$	$4.33 \pm 2.57 \times 10^2$	5.08 ± 0.31
			Silk Fabric		$9.35 \pm 4.50 \times 10^6$	$1.00 \pm 0.97 \times 10^2$	5.07 ± 0.39
			Carbon Steel		$1.01 \pm 0.38 \times 10^7$	$4.82 \pm 2.05 \times 10^3$	3.33 ± 0.21
10	50/50	600/180	Glass	1.08×10^8	$1.04 \pm 0.16 \times 10^8$	$1.62 \pm 0.63 \times 10^3$	4.84 ± 0.20
			Bare Pine Wood		$6.31 \pm 1.78 \times 10^6$	$3.37 \pm 4.04 \times 10^1$	5.78 ± 0.83
			Painted Canvas		$6.99 \pm 0.92 \times 10^7$	$4.11 \pm 0.44 \times 10^4$	3.23 ± 0.06
			Archival Paper		$4.31 \pm 0.94 \times 10^7$	0.00 ± 0.00	≥7.63 ± 0.08
			Silk Fabric		$1.77 \pm 0.89 \times 10^7$	0.00 ± 0.00	≥7.20 ± 0.20
			Carbon Steel		$2.63 \pm 0.94 \times 10^7$	$1.13 \pm 0.57 \times 10^4$	3.38 ± 0.23

Table 5-2. EtO Efficacy Against *Bacillus atrophaeus* Spores[a] (Continued)

Test Number	Temp (°C) / RH (%)	Concentration (mg/L) / Contact Time (min)	Material	Inoculum (CFU/coupon)	Mean Recovered *B. anthracis* (CFU/coupon)		Decontamination Efficacy ± CI[d]
					Positive Control[b]	Test Coupon[c]	
11	50/60	300/180	Glass	1.19×10^8	$1.05 \pm 0.12 \times 10^8$	$4.05 \pm 2.64 \times 10^3$	4.47 ± 0.22
			Bare Pine Wood		$4.48 \pm 5.13 \times 10^6$	$4.72 \pm 6.43 \times 10^1$	5.64 ± 1.06
			Painted Canvas		$4.26 \pm 0.79 \times 10^7$	$8.01 \pm 0.39 \times 10^4$	2.72 ± 0.07
			Archival Paper		$2.51 \pm 0.69 \times 10^7$	0.00 ± 0.00	≥7.38 ± 0.11
			Silk Fabric		$1.77 \pm 0.68 \times 10^7$	$8.01 \pm 8.97 \times 10^1$	5.71 ± 0.80
			Carbon Steel		$1.41 \pm 0.62 \times 10^7$	$5.29 \pm 3.03 \times 10^3$	3.49 ± 0.40
12	50/60	600/360	Glass	1.18×10^8	$1.02 \pm 0.24 \times 10^8$	$1.31 \pm 0.56 \times 10^3$	4.91 ± 0.18
			Bare Pine Wood		$4.43 \pm 2.27 \times 10^6$	0.00 ± 0.00	≥6.60 ± 0.19
			Painted Canvas		$3.74 \pm 0.41 \times 10^7$	$9.91 \pm 3.84 \times 10^3$	3.60 ± 0.16
			Archival Paper		$1.54 \pm 0.93 \times 10^7$	$1.20 \pm 1.01 \times 10^2$	5.47 ± 0.89
			Silk Fabric		$5.52 \pm 1.91 \times 10^6$	$4.04 \pm 4.30 \times 10^1$	5.65 ± 0.88
			Carbon Steel		$2.44 \pm 0.89 \times 10^7$	$2.11 \pm 1.68 \times 10^3$	3.16 ± 0.39
13	50/75	150/180	Glass	1.17×10^8	$9.70 \pm 0.47 \times 10^7$	0.00 ± 0.00	≥7.99 ± 0.02
			Bare Pine Wood		$1.40 \pm 0.42 \times 10^6$	0.00 ± 0.00	≥6.13 ± 0.12
			Painted Canvas		$4.70 \pm 2.02 \times 10^7$	0.00 ± 0.00	≥7.63 ± 0.19
			Archival Paper		$2.09 \pm 0.80 \times 10^7$	0.00 ± 0.00	≥7.30 ± 0.14
			Silk Fabric		$1.09 \pm 0.49 \times 10^7$	0.00 ± 0.00	≥7.01 ± 0.14
			Carbon Steel		$1.08 \pm 0.22 \times 10^7$	0.00 ± 0.00	≥7.03 ± 0.08
14	50/75	300/45	Glass	1.08×10^8	$8.03 \pm 0.20 \times 10^7$	0.00 ± 0.00	≥7.90 ± 0.01
			Bare Pine Wood		$6.19 \pm 1.43 \times 10^6$	0.00 ± 0.00	≥6.78 ± 0.09
			Painted Canvas		$8.14 \pm 0.46 \times 10^7$	0.00 ± 0.00	≥7.91 ± 0.02
			Archival Paper		$5.32 \pm 1.14 \times 10^7$	0.00 ± 0.00	≥7.72 ± 0.09
			Silk Fabric		$1.31 \pm 0.45 \times 10^7$	0.00 ± 0.00	≥7.09 ± 0.14
			Carbon Steel		$1.61 \pm 0.69 \times 10^7$	0.00 ± 0.00	≥7.18 ± 0.16
15	50/75	300/90	Glass	1.15×10^8	$9.21 \pm 1.46 \times 10^7$	0.00 ± 0.00	≥7.96 ± 0.06
			Bare Pine Wood		$6.78 \pm 5.39 \times 10^6$	0.00 ± 0.00	≥6.75 ± 0.24
			Painted Canvas		$4.26 \pm 0.73 \times 10^7$	0.00 ± 0.00	≥7.62 ± 0.07
			Archival Paper		$1.01 \pm 0.52 \times 10^7$	0.00 ± 0.00	≥6.96 ± 0.19
			Silk Fabric		$1.12 \pm 0.18 \times 10^7$	0.00 ± 0.00	≥7.05 ± 0.07
			Carbon Steel		$1.28 \pm 0.75 \times 10^7$	0.00 ± 0.00	≥7.02 ± 0.29
16	50/75	300/180	Glass	1.16×10^8	$1.05 \pm 0.15 \times 10^7$	0.00 ± 0.00	≥8.02 ± 0.05
			Bare Pine Wood		$3.49 \pm 1.07 \times 10^6$	0.00 ± 0.00	≥6.52 ± 0.13
			Painted Canvas		$4.92 \pm 0.57 \times 10^7$	0.00 ± 0.00	≥7.69 ± 0.04
			Archival Paper		$1.17 \pm 0.25 \times 10^7$	0.00 ± 0.00	≥7.06 ± 0.08
			Silk Fabric		$6.69 \pm 2.17 \times 10^6$	0.00 ± 0.00	≥6.81 ± 0.11
			Carbon Steel		$4.94 \pm 2.39 \times 10^7$	0.00 ± 0.00	≥6.66 ± 0.15
17	37/75	300/45	Glass	1.40×10^8	$7.40 \pm 1.42 \times 10^7$	$7.07 \pm 1.60 \times 10^4$	3.02 ± 0.12
			Bare Pine Wood		$6.95 \pm 2.50 \times 10^6$	$2.66 \pm 2.06 \times 10^2$	4.54 ± 0.43
			Painted Canvas		$8.17 \pm 1.09 \times 10^7$	$2.37 \pm 1.39 \times 10^4$	3.64 ± 0.34
			Archival Paper		$4.84 \pm 0.79 \times 10^7$	$9.21 \pm 2.19 \times 10^3$	3.73 ± 0.13
			Silk Fabric		$1.07 \pm 0.32 \times 10^7$	$1.01 \pm 0.27 \times 10^3$	4.02 ± 0.15
			Carbon Steel		$2.33 \pm 1.62 \times 10^7$	$1.50 \pm 0.22 \times 10^4$	3.12 ± 0.26
18	37/75	300/90	Glass	1.12×10^8	$1.01 \pm 0.07 \times 10^8$	0.00 ± 0.00	≥8.00 ± 0.03
			Bare Pine Wood		$5.63 \pm 3.90 \times 10^6$	0.00 ± 0.00	≥6.66 ± 0.27
			Painted Canvas		$5.56 \pm 0.94 \times 10^7$	0.00 ± 0.00	≥7.74 ± 0.06
			Archival Paper		$7.91 \pm 4.28 \times 10^6$	0.00 ± 0.00	≥6.86 ± 0.17
			Silk Fabric		$1.03 \pm 0.44 \times 10^7$	0.00 ± 0.00	≥6.98 ± 0.17
			Carbon Steel		$9.07 \pm 2.91 \times 10^6$	0.00 ± 0.00	≥6.94 ± 0.13
19	37/75	300/180	Glass	1.23×10^8	$1.12 \pm 0.02 \times 10^7$	0.00 ± 0.00	≥8.05 ± 0.01
			Bare Pine Wood		$3.60 \pm 0.53 \times 10^6$	0.00 ± 0.00	≥6.55 ± 0.06
			Painted Canvas		$7.39 \pm 1.52 \times 10^7$	0.00 ± 0.00	≥7.86 ± 0.08
			Archival Paper		$1.80 \pm 0.96 \times 10^7$	0.00 ± 0.00	≥7.22 ± 0.17
			Silk Fabric		$5.33 \pm 2.48 \times 10^7$	0.00 ± 0.00	≥7.67 ± 0.24
			Carbon Steel		$1.46 \pm 0.37 \times 10^7$	0.00 ± 0.00	≥7.15 ± 0.10

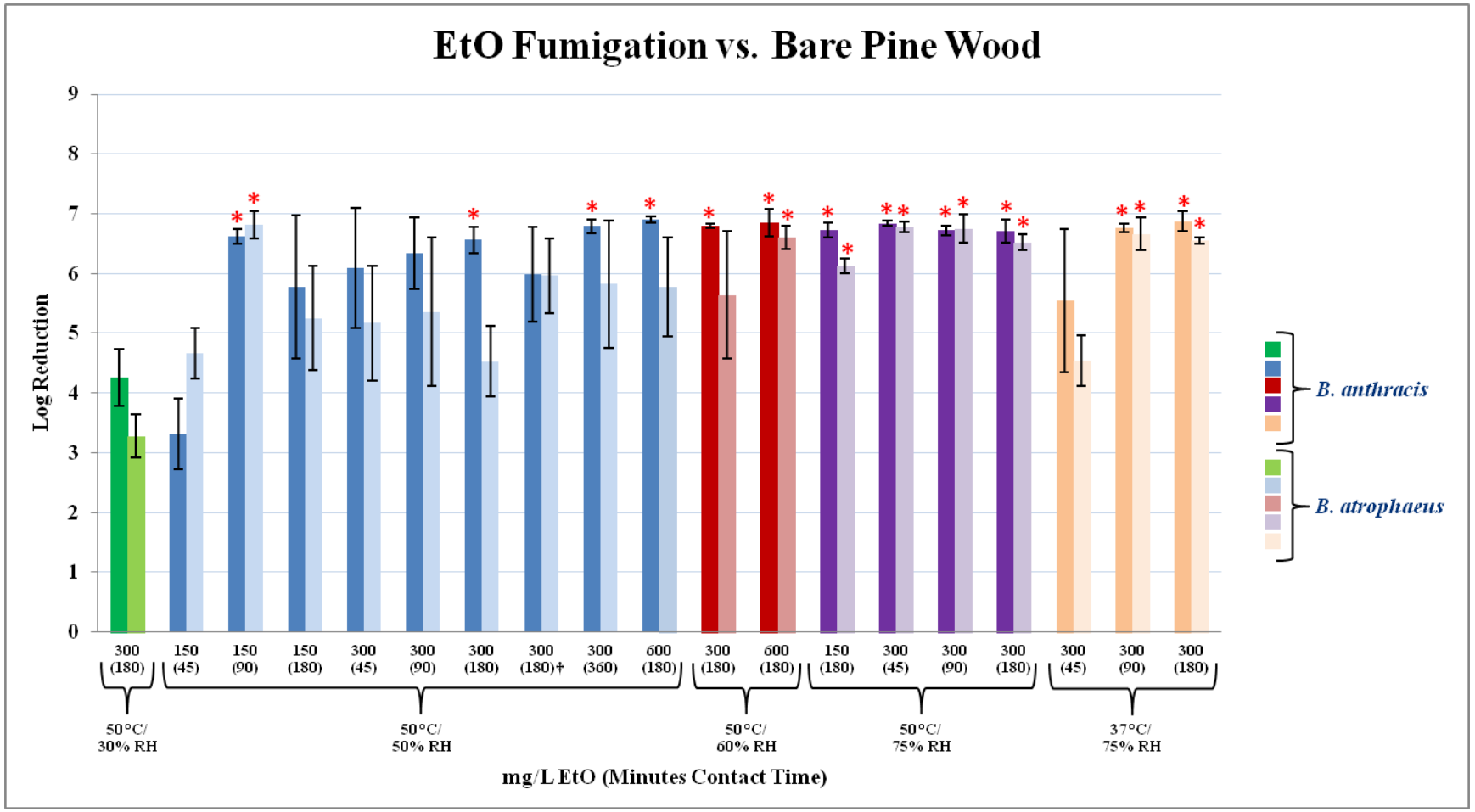
[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).
[b] Positive Controls = inoculated, not decontaminated coupons.
[c] Test Coupons = inoculated, decontaminated coupons.
[d] CI = confidence interval (± 1.96 × SE).



*Complete inactivation achieved.

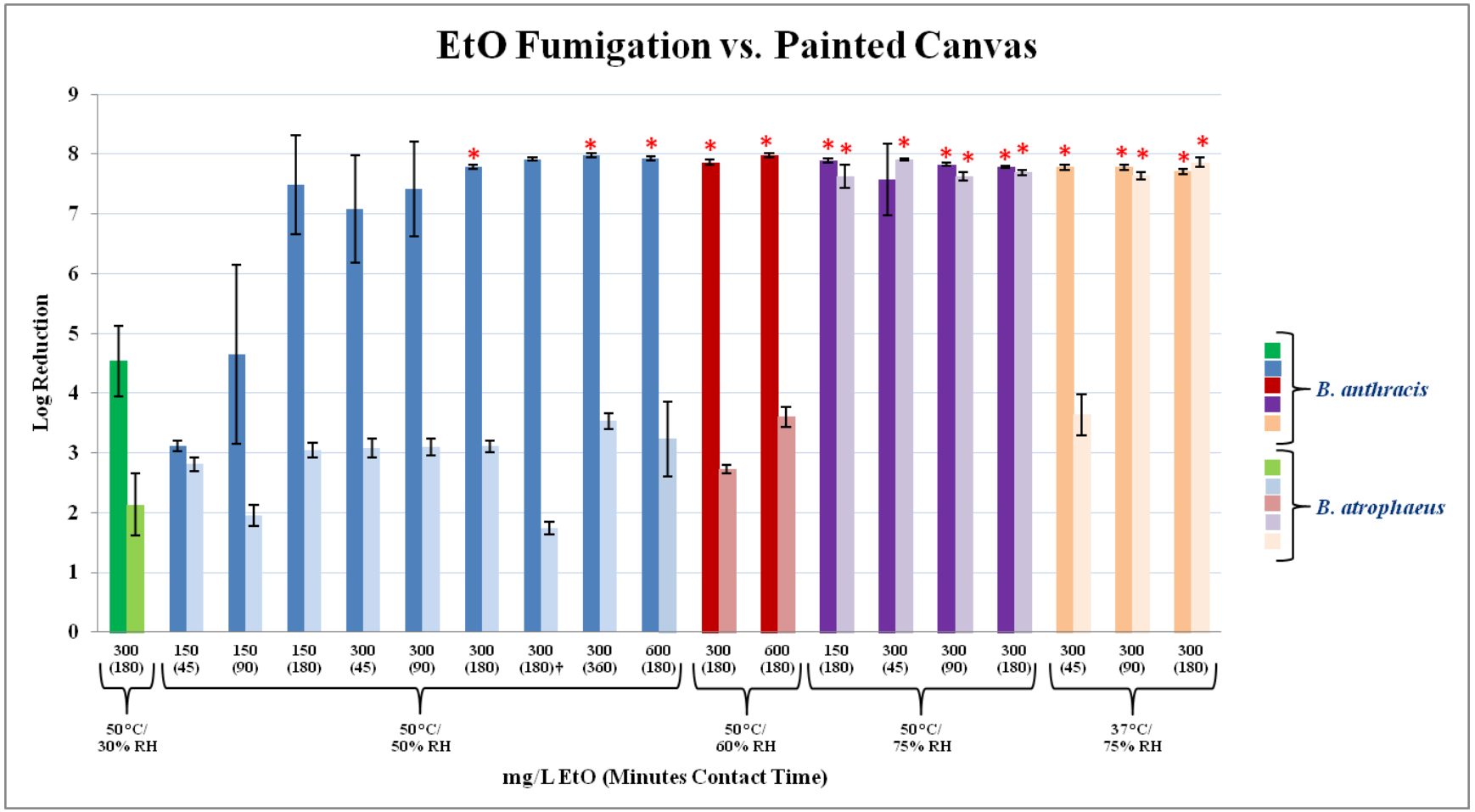
† Samples pre-humidified @ 50% RH for 24 hours prior to fumigation

Figure 5-1. Summary of decontamination efficacies for EtO fumigation testing on glass.



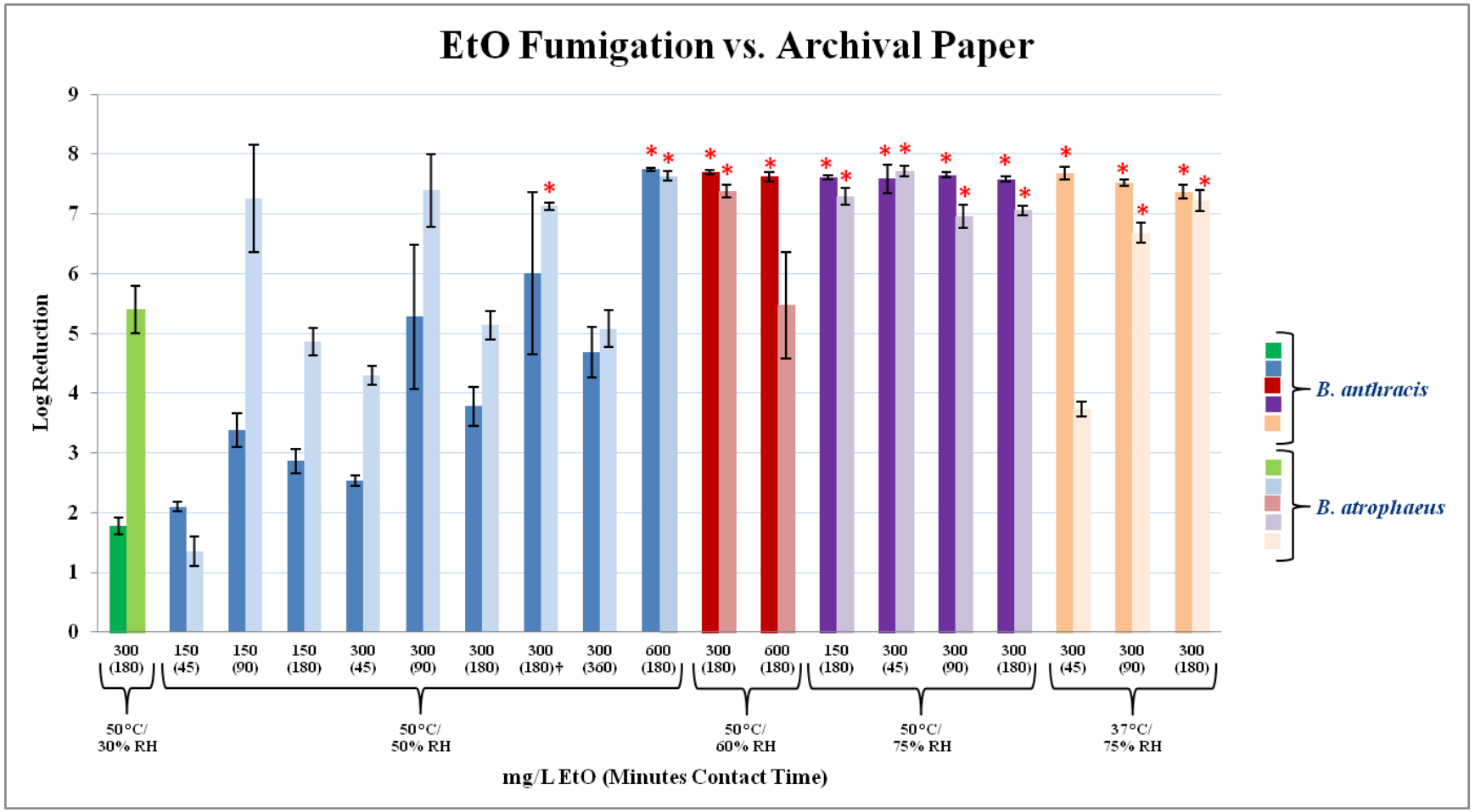
*Complete inactivation achieved.
† Samples pre-humidified @ 50% RH for 24 hours prior to fumigation

Figure 5-2. Summary of decontamination efficacies for EtO fumigation testing on bare pine wood.



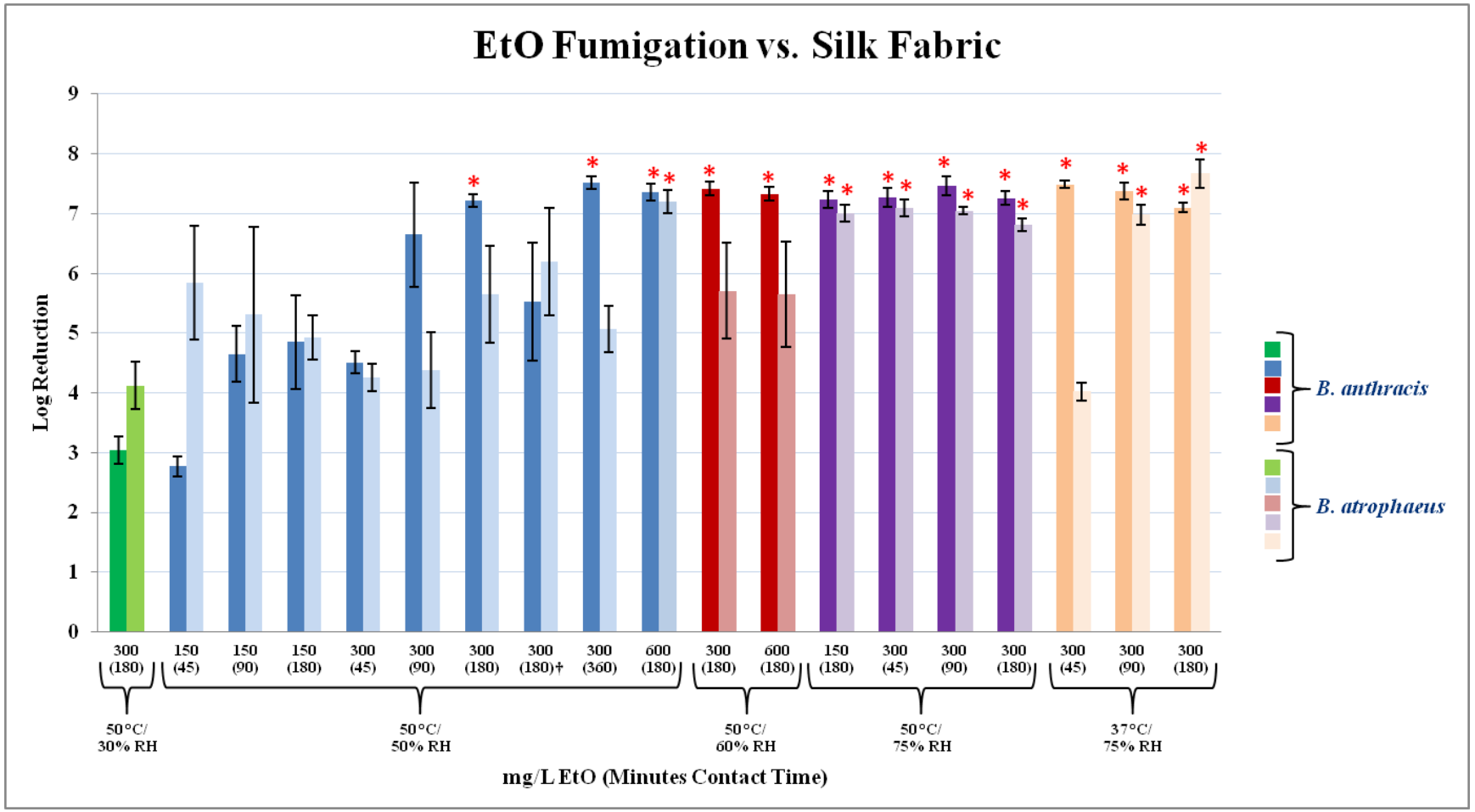
*Complete inactivation achieved.
† Samples pre-humidified @ 50% RH for 24 hours prior to fumigation

Figure 5-3. Summary of decontamination efficacies for EtO fumigation testing on painted canvas.



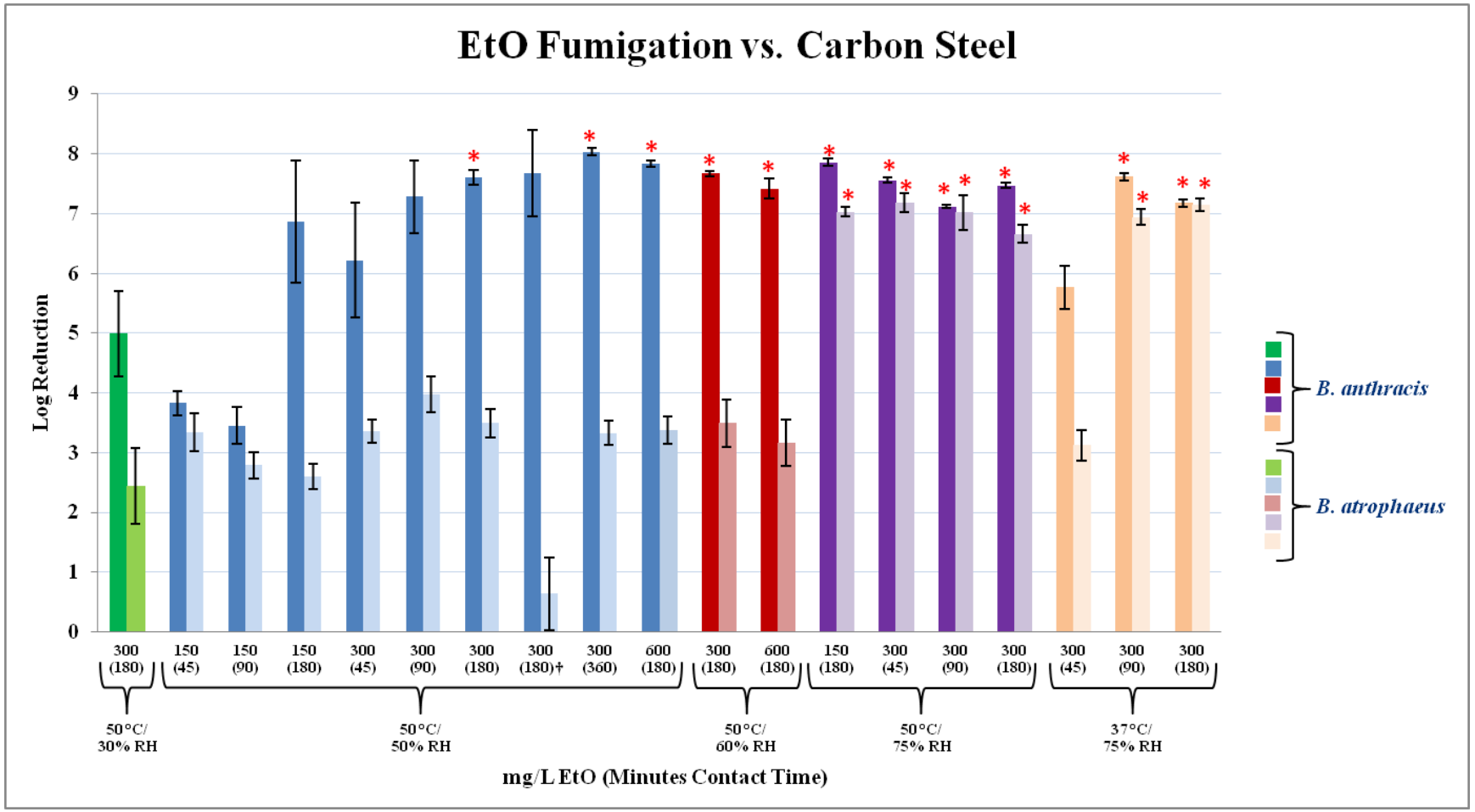
*Complete inactivation achieved.
† Samples pre-humidified @ 50% RH for 24 hours prior to fumigation

Figure 5-4. Summary of decontamination efficacies for EtO fumigation testing on archival paper.



*Complete inactivation achieved.
† Samples pre-humidified @ 50% RH for 24 hours prior to fumigation

Figure 5-5. Summary of decontamination efficacies for EtO fumigation testing on silk fabric.



*Complete inactivation achieved.
† Samples pre-humidified @ 50% RH for 24 hours prior to fumigation

Figure 5-6. Summary of decontamination efficacies for EtO fumigation testing on carbon steel.

Table 5-3. EtO Efficacy against *Bacillus atrophaeus* Spores[a] on Archival Paper

Test Number	Concentration (mg/L) / Contact Time (min)	Temp (°C) / RH (%)	Material	Inoculation Type	Inoculum (CFU/coupon)	Mean Recovered *B. atrophaeus* (CFU/coupon)		Decontamination Efficacy ± CI[d]	
						Positive Control[b]	Test Coupon[c]		
20	150/45	50/50	Archival Paper	Liquid	1.15×10^5	$1.62 \pm 0.21 \times 10^4$	$3.95 \pm 1.50 \times 10^2$	1.64 ± 0.15	
				Dry	-- [†]	$7.51 \pm 1.18 \times 10^4$	$3.58 \pm 3.14 \times 10^5$*	1.97 ± 0.59	2.44 ± 0.74*
					-- [‡]	$6.41 \pm 0.77 \times 10^5$	$1.67 \pm 1.72 \times 10^3$	2.90 ± 0.59	

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on six individual coupons, the mean percent recovery on those six coupons, and decontamination efficacy (log reduction).
[b] Positive Controls = inoculated, not decontaminated coupons.
[c] Test Coupons = inoculated, decontaminated coupons.
[d] CI = confidence interval (± 1.96 × SE).
[†] Samples (N=3) immediately processed, not placed in control chamber. Inoculation concentration is unknown.
[‡] Samples (N=3) added to control chamber for the 45 minute contact time. Inoculation concentration is unknown.
* Average of all six coupons.

5.2 Surface Damage to Materials

At the end of each decontamination test, the procedural blanks were visually compared to the laboratory blanks, and test coupons were visually compared to positive controls, to assess any impact EtO and/or the test conditions 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 five of the six material surfaces after being exposed to EtO. A noticeable change was observed on carbon steel after inoculation with *B. atrophaeus* (Figure 6-7). The inoculation spots appear to have oxidized and this apparent oxidation was observed with and without the presence of EtO. Due to the noticeable difference in both test and positive control coupons (the apparent oxidation was not observed on blank coupons), the apparent oxidation was due to the inoculation material rather than due to contact with the EtO fumigant. The carrier buffer of the *B. atrophaeus* spores (PBSTx) and the high RH may have contributed to the oxidation of the coupon.

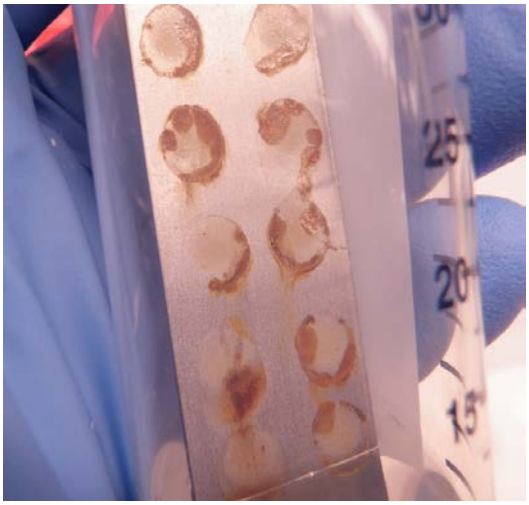


Figure 5-7. Oxidation on carbon steel coupons inoculated with *B. atrophaeus*.

6.0 Discussions and Conclusions

6.1 Effects of Temperature and Relative Humidity on Efficacy

The tests performed under this evaluation highlight the importance of achieving a proper RH and temperature to achieve the maximum efficacy of EtO. A single test was conducted at 30% RH and low LRs were achieved. Using these parameters (50 °C, 30% RH, 300 mg/L EtO for 180 minutes), LRs of 1.78 (archival paper) to 4.99 (carbon steel) were observed for *B. anthracis* and LRs of 2.13 (painted canvas) to 5.40 (archival paper) were observed for *B. atrophaeus*. These LRs are below the target of LR ≥ 6 to demonstrate a process as a sporicidal decontaminant based upon quantitative testing; the conditions described above were not suitable to achieve sporicidal decontaminant requirements.

The effects of 50 °C and 50% RH were assessed next. At least a 6 LR was achieved against *B. anthracis* on all materials at 50 °C and 50% RH using 600 mg/L EtO for a contact time of 180 minutes; further complete inactivation (no viable spores recovered on test coupons) was achieved. This efficacy was also demonstrated at 300 mg/L EtO for 180 and 360 minutes against all materials types except archival paper. In contrast, EtO was not efficacious against *B. atrophaeus* at 50 °C and 50% RH for up to 600 mg/L EtO and 360 minutes (the highest combination of concentration and time tested), with the exception of archival paper and silk fabric where a few instances of LRs ≥6.20 were observed. These data suggest that *B. atrophaeus* is more difficult to inactive than *B. anthracis* when testing with EtO under these conditions.

When the RH was raised to 60%, at least a 6 LR (and complete inactivation) was achieved against *B. anthracis* at both conditions tested (300 mg/L EtO for 180 minutes and 600 mg/L EtO for 360 minutes) and for all six coupon types with LRs ranging from ≥6.80 to ≥7.98. However, LRs ranged from 2.72 (painted canvas) to ≥7.38 (archival paper) at 300 mg/L EtO for 180 minutes and 3.16 (carbon steel) to ≥6.60 (bare pine wood) at 600 mg/L EtO and 360 minutes against *B. atrophaeus*. These data suggest that EtO is a less efficacious decontaminant against *B. atrophaeus* at these test conditions.

At 50 °C and 75% RH, EtO was effective against both *B. anthracis* and *B. atrophaeus*, even when using only 150 mg/L EtO for a contact time of 180 minutes (the lowest combination tested). A least a 6 LR was achieved on all materials at all parameters tested. Further, complete inactivation was achieved on all materials with the exception of of glass (6.43 LR) and painted canvas (7.57 LR) at 300 mg/L EtO for 45 minutes against *B. anthracis*. Although complete inactivation was not achieved on these two materials, LRs were still >6 LR, suggesting a high efficacy.

In general, lowering the temperature from 50 °C to 37 °C resulted in decreased efficacy for *B. atrophaeus* on all materials at 45 min. Lower EtO efficacy against *B. anthracis* at 45 min with the same temperature decrease was observed for only bare pine wood and carbon steel.

EtO is an effective decontaminant against *B. anthracis* under optimal combinations of concentration, contact time, temperature, and RH. At a minimum, the following combinations of parameters should be achieved for EtO to be effective against glass, bare pine wood, painted canvas, archival paper, silk fabric and carbon steel:

- 50 °C, 50% RH, ≥600 mg/L EtO for ≥180 minutes
- 50 °C, 60% RH, ≥300 mg/L EtO for ≥180 minutes
- 50 °C, 75% RH, ≥150 mg/L EtO for ≥180 minutes
- 37 °C, 75% RH, ≥300 mg/L EtO for ≥90 minutes

Additionally, although less effective against *B. atrophaeus*, a greater than 6 LR was achieved on all six coupon types under the following conditions:

- 50 °C, 75% RH, ≥150 mg/L EtO for ≥180 minutes
- 37 °C, 75% RH, ≥300 mg/L EtO for ≥90 minutes

In general, as the RH increases, so does efficacy. The amount of EtO and the contact time may decrease as the RH increases and still be efficacious. *B. atrophaeus* should be considered a suitable surrogate for *B. anthracis* when testing at ≥37 °C and ≥75% RH.

6.2 Effects of Material Type on Efficacy

In general, *B. anthracis* was the most resistant to EtO decontamination when the *B. anthracis* was inoculated on archival paper. LRs ranged from 1.78 to 6.01 on this coupon type in instances where complete inactivation was not achieved (Tests 1-9). *B. anthracis* on glass and bare pine wood was the least resistant to EtO decontamination as these coupon types exhibited higher LRs than the other four coupon types. LRs ranged from 3.89 to 6.64 and 3.31 to 6.09 for glass and bare pine wood, respectively, when complete inactivation was not achieved (Tests 1-6, 8).

In contrast, archival paper (3.73 to 7.39 LR) and bare pine wood (3.28-5.96 LR) were the least resistant to *B. atrophaeus* decontamination, while painted canvas (1.74 to 3.63 LR) and carbon steel (0.64 to 3.98 LR) were the most resistant to *B. atrophaeus* decontamination using EtO.

6.3 Effects of Inoculation Method on Efficacy

Although not significant, when comparing inoculation methods on archival paper, a dry inoculation method was easier to decontaminate with EtO than a liquid inoculation method (2.44 vs 1.64 LR). Several factors could contribute to this difference and more testing at different parameters and with different materials should be completed in order to fully understand the differences, if any.

Fewer organisms were recovered from the three dry-inoculated positive controls that were processed immediately than the three held in the control chamber for the 45 minute contact time (Table 6-3). This difference in recovery is interesting to note and may be due to the RH in the chamber slightly re-hydrating the spores, promoting higher recovery rates. More testing would need to be completed to assess the effect of RH on this recovery fully.

7.0 References

1. USEPA, Determining the Efficacy of Liquids and Fumigants in Systematic Decontamination Studies for *Bacillus anthracis* Using Multiple Test Methods. US EPA Report 600/R-10/088, December 2010.

2. Dorland WAN. Dorland's illustrated medical dictionary (standard version). In: Dorland WE, editor, 29th ed., Philadelphia, W.B. Saunders & Co, 2088, 2000.

3. Test/Quality Assurance Plan for The Evaluation of Ethylene Oxide for the Inactivation of *Bacillus anthracis*, Version 1; April 2012 and Amendment 1, October, 2012. (Available upon request by contacting the EPA Project Leader).

4. Anderson Products, Inc., website accessed August 28, 2013, www.anpro.com.

5. Calfee, M.W., S.D. Lee, S.P. Ryan, "*A rapid and repeatable method to deposit bioaerosols on material surfaces,* Journal of Microbiological Methods, 92, 375-380, 2013.

6. *Standard Practice for Maintaining Constant Relative Humidity by Means of Aqueous Solutions.* ASTM International, October, 2006.

7. 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.

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