AFRL-RH-WP-SR-2019-0006

BIOTHERMAL DECONTAMINATION OF BIOLOGICAL SPORES ON NYLON

Daniel P. Lorch
Angela M. G. Theys
METSS Corporation
300 Westdale Avenue
Westerville, OH 43082

William T. Greer
Air Force Research Laboratory
2215 First St., Bldg 33
Wright-Patterson AFB, OH 45433

November 2019

SPECIAL REPORT

AIR FORCE RESEARCH LABORATORY
711TH HUMAN PERFORMANCE WING
AIRMAN SYSTEMS DIRECTORATE
WRIGHT-PATTERSON AIR FORCE BASE OH 45433
AIR FORCE MATERIEL COMMAND
UNITED STATES AIR FORCE

NOTICE AND SIGNATURE PAGE

Using Government drawings, specifications, or other data included in this document for any purpose other than Government procurement does not in any way obligate the U.S. Government. The fact that the Government formulated or supplied the drawings, specifications, or other data does not license the holder or any other person or corporation; or convey any rights or permission to manufacture, use, or sell any patented invention that may relate to them.

Qualified requestors may obtain copies of this report from the Defense Technical Information Center (DTIC).

AFRL-RH-WP-SR-2019-0006 HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION IN ACCORDANCE WITH ASSIGNED DISTRIBUTION STATEMENT.

DISCLAIMER

This report has been prepared for the United States Government by METSS Corporation. In no event shall either the United States Government or METSS Corporation have any responsibility or liability for any consequences of use, misuse, inability to use, or reliance on the information contained in this report. The United States Government and METSS Corporation also do not warrant or otherwise represent the accuracy, adequacy, efficacy, or applicability of the contents of this report.

//signature//	//signature//
WILLIAM T. GREER, DR-04, WUM Aircraft CBRN Survivability Airman Systems Directorate 711th Human Performance Wing Air Force Research Laboratory	RICHARD D. SIMPSON, DR-IV, DAF Airman Bioengineering Division Airman Systems Directorate 711th Human Performance Wing Air Force Research Laboratory

This report is published in the interest of scientific and technical information exchange, and its publication does not constitute the Government's approval or disapproval of its ideas or findings.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
12-11-2019	Special Report	July 2019

4. TITLE AND SUBTITLE
Biothermal Decontamination (BTD) of Biological Spores on Nylon

5a. CONTRACT NUMBER
FA8650-19-D-6990

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Daniel P. Lorch[†]
Angela M. G. Theys[†]
William T. Greer[‡]

5d. PROJECT NUMBER

5e. TASK NUMBER
0001

5f. WORK UNIT NUMBER
H0P8

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
[†]METSS Corporation
300 Westdale Avenue
Westerville, OH 43082

8. PERFORMING ORGANIZATION REPORT NUMBER
G-18-6990 TO1

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
[‡]Air Force Materiel Command
Air Force Research Laboratory
711th Human Performance Wing
Airmen Systems Directorate
Airman Bioengineering Division
Cognitive Enhancement and Biodynamics Branch
Wright-Patterson AFB, OH 45433

10. SPONSOR/MONITOR'S ACRONYM(S)
AFRL/711 HPW/RHBNB

11. SPONSORING/MONITORING AGENCY REPORT NUMBER
AFRL-RH-WP-SR-2019-0006

12. DISTRIBUTION AVAILABILITY STATEMENT
Distribution A: Approved for public release.

13. SUPPLEMENTARY NOTES
88ABW-2020-0373, cleared on 4 February2020

14. ABSTRACT
This laboratory study was designed as a quick look into the dynamics of Biothermal Decontamination (170 °F and 90% relative humidity) on nylon. This study was not designed to be exhaustive, rather to provide quick, valuable insight into the delayed kinetics of decontamination on nylon webbing that were observed in prior studies. The limited results of this study indicate that the rate of decontamination of bacterial spores on nylon webbing was far slower than on non-porous nylon sheet stock, while the decay rates for nylon sheet were virtually the same as painted aluminum. This brief study indicates that decontamination efficiency is affected more by the texture/porosity of the substrate than the chemical composition. Further efficacy testing with a larger sample set is recommended to increase the statistical significance of the study results.

15. SUBJECT TERMS
Biothermal Decontamination (BTD); *Bacillus thuringiensis* variety kurstaki (Btk); *Bacillus anthracis*; biological warfare agent, D-value, Nylon, Nylon webbing, textiles

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
Unclassified					William T. Greer
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (*Include area code*)
U	U	U	SAR	20	N/A

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. 239.18

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1.0 EXECUTIVE SUMMARY

Biothermal decontamination (BTD) and hot air decontamination (HAD) have been demonstrated effective in inactivating spore-forming biological weapons (BW). BTD uses elevated temperature (160-190 ˚F) and high relative humidity (RH) (80-90%) and has been shown to be more efficient than HAD, which uses elevated temperature and low RH (<10%). In continued research of BTD and HAD with a range of contaminated substrate materials, nylon webbing is consistently difficult to decontaminate. However, it is not clear if the reduction in decontamination efficiency is caused by the porosity of the woven webbing material or an undefined chemical interaction (e.g., charge transfer) between the bacterial spores and nylon. If this effect can be attributed to the woven structure of the nylon material, then the nylon webbing may be viewed as a physical representative of other porous materials.

It is important to understand how the delayed decontamination kinetics on nylon webbing relate to other materials, so informed decisions can be made relative to how complex systems, like aircraft, are prepared for decontamination. This laboratory study was designed as a quick look into the kinetics of the BTD process (170 °F and 90% RH) on nylon. The study was not designed to be exhaustive, rather to provide quick, valuable insight into a topic that may or may not warrant further investigation. Specifically, METSS evaluated BTD on two types of nylon materials – non-porous sheet stock and woven webbing – and compared the results to painted aluminum coupons, which have been used as the standard substrate for decontamination efficiency tests over the years. Having the same basic chemical composition, the main difference in the two nylon materials is the texture/porosity; therefore, a difference in decontamination efficiency could be more definitively tied to the morphology rather than the chemical composition.

The limited results of this study indicate that the rate of decontamination of bacterial spores on nylon webbing was far slower than on nylon sheet. The decay rates were virtually the same on nylon sheet and painted aluminum, with D-values of 12.1 and 12.3 hours, respectively, and required an estimated exposure of 3 days to achieve a 6-log reduction. The decay rate (D-value) on nylon webbing, however, was much slower at 27.9 hours and required an estimated 7 days to achieve a 6-log reduction.

This brief study indicates that decontamination efficiency is affected more by the texture/porosity of the nylon webbing than the chemical composition. Further efficacy testing with a larger sample set is recommended to increase the statistical significance of the study results. It is also recommended that testing be performed with a variety on woven materials versus a variety of *B. anthracis* surrogates to understand how these results relate to other textiles and/or porous materials as well as different strains of bacterial spores.

2.0 INTRODUCTION

Over the past decade, Biothermal Decontamination, BTD, has been investigated as viable method for aircraft decontamination. Specifically, BTD utilizes hot air and high humidity to inactivate microorganisms, including biological warfare agents (BWAs) like *Bacillus anthracis*. BTD was first demonstrated effective for neutralization of biological agent simulants in laboratory tests conducted in 2007 and 2008.[1] This research was initiated by Air Force Research Laboratory (AFRL) at Wright-Patterson Air Force Base and performed by Battelle Memorial Institute, Columbus, Ohio. This led to Joint Biological Aircraft Decontamination System (JBADS) Tier I testing, wherein three independent government labs, Edgewood Chemical Biological Center,[2] Naval Surface Warfare Center Dahlgren Laboratory (NSWCDD),[3] and AFRL at Brooks City-Base,[4] evaluated BTD with various simulants and spore preparations, and at least one strain of virulent *Bacillus anthracis*, Ba. Decontamination times varied according to the exact humidity and spore strains tested, but between the three laboratories, BTD conditions were shown effective at reducing at least a 6-log challenge for several strains of *Bacillus* spores.

AFRL (Wright-Patterson) conducted multiple field demonstrations of BTD[1,5,6,7] with support from Air Mobility Command, which culminated in a Joint Capability Technology Demonstration (JCTD) of the JBADS.[8,9,10] Meanwhile, NSWCDD continued fundamental research of BTD with support from the Defense Threat Reduction Agency (DTRA). The research included Tier II testing to establish models for the efficacy of BTD with varying combinations of temperature and humidity.[11,12] This study also included multiple substrates, representative of aircraft materials. The research showed that BTD of nylon webbing was considerably slower than on Aircraft Performance Coating (APC), anti-skid patches, wiring insulation, and polypropylene plastic, particularly at lower RH. The authors suggested that the hydrophobicity or electrostatic nature of the substrates may have impacted the decontamination efficiency. While this may be true, nylon webbing was also the only porous material included in the study, making it difficult to resolve the contribution of texture versus the material composition.

Similar findings appeared in the NSWCDD Tier III studies for JBADS, wherein the same five aircraft materials were inoculated with clean or dirty spores.[13,14] Although the decontamination rates were affected by the inclusion of foreign matter, the longest decontamination times were again observed for nylon webbing. Around this time, there was also a growing interest in the utility of HAD, which is similar to BTD but uses extremely low humidity instead of extremely high humidity. NSWCDD evaluated HAD against four strains of *Bacillus* spores on four different aircraft materials – wiring insulation, APC, plastic, and nylon webbing – and once again, the longest decontamination times were required for nylon webbing.[15] However, in this study, decontamination on the nylon webbing required weeks longer than the other materials, as opposed to a differences of a few days noted in BTD studies.

Obviously, nylon webbing is a difficult material to decontaminate, leading experts to recommend removal and safe disposal of all nylon webbing from aircraft (cargo netting, seat belts, etc.) prior to decontamination with BTD or HAD. However, it is not clear how this knowledge can be used to inform decisions about other materials on the aircraft during decontamination. If the root cause is a physical interaction that impacts the destruction of the spores, then other materials may also require special consideration in the decontamination process. If the root cause is the texture or porosity of the material, other textiles and porous materials (seat covers, wire bundles, wood, etc.) should be more carefully considered in the overall decontamination operation.

This laboratory study was designed as a quick look into the dynamics of BTD (170 °F and 90% RH) on nylon. This study was not designed to be exhaustive, rather to provide quick, valuable insight into a topic that may or may not warrant further study. Specifically, METSS evaluated BTD on two types of nylon – non-porous sheet stock and woven webbing – and compared the results to painted aluminum coupons, which have been used as the standard substrate for decontamination efficiency tests over the years. Having the same basic chemical composition, the main difference in the two nylon materials is their texture/porosity; therefore, a difference in decontamination efficiency could be more definitively tied to the morphology rather than the chemical composition.

Coupons of nylon webbing and nylon sheeting were inoculated with >7-log spores of *Bacillus thuringiensis* variety kurstaki (Btk), allowed to dry, and then exposed to BTD conditions of 170 °F at 90% RH in an environmental chamber. For comparison, coupons of painted aluminum (MIL-PRF-85285 topcoat) were also inoculated in the same manner and exposed to the same conditions as a control. Painted coupons were used extensively in the development of BTD; therefore, there is a wealth of historical data by which to compare the painted coupons, as well as the nylon coupons. Replicate coupons were assayed periodically over a 5-day period to generate a kill curve (expressed as log spores recovered versus time). The values in the range of linear decay were then used to determine the D-value, defined as the time at which a decrease of 90% or 1-log of the initial population is observed under constant conditions.

3.0 METHODS

The methods used to conduct the efficacy tests were adapted from previous bench-scale BTD studies including spore preparation, coupon inoculation, BTD treatment, coupon analysis, and data interpretation. The methodologies were used to assess the rate of decay of Btk on two nylon substrates including webbing (Figure 1) and smooth sheeting. For comparative purposes METSS also included aluminum coupons that were primed (according to MIL-PRF-23377) and painted (MIL-PRF-85285). The three substrates are shown side-by-side in Figure 2.

Figure 1. Nylon Webbing

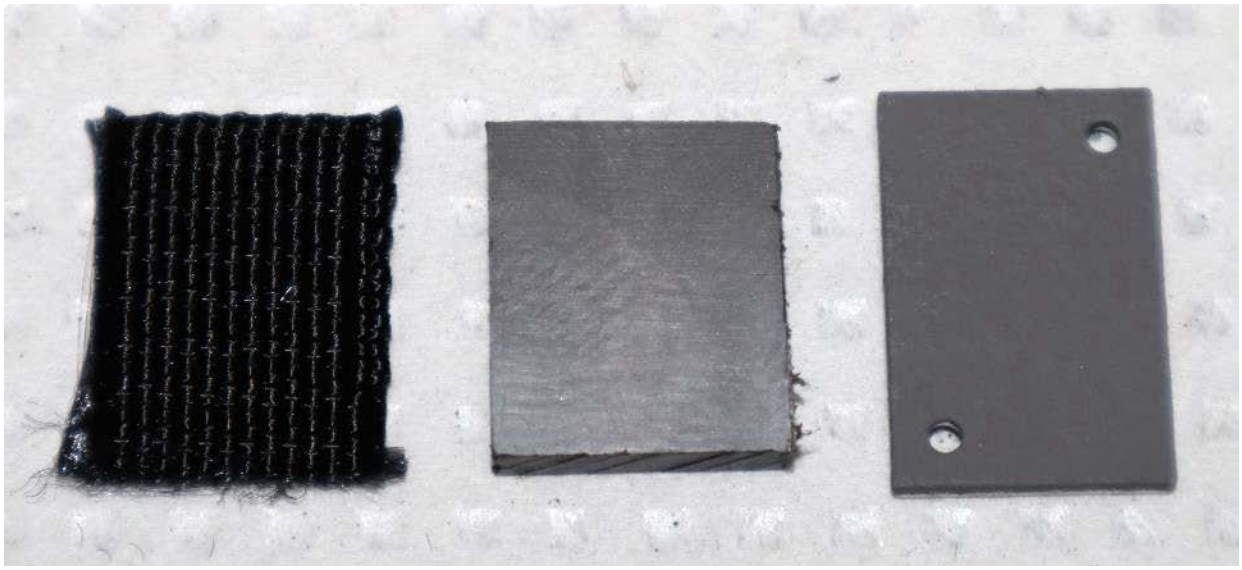


Figure 2. Test substrates (left to right: nylon webbing, nylon sheet, painted aluminum)

3.1 Coupon Preparation

Nylon webbing coupons were cut from a bulk role of webbing material (Grainger, Item 14A774). Nylon sheet coupons (approximately ½" x ¾") were prepared from a 0.125" thick sheet of Nylon 6/6 (Grainger; Item 2XNT8). Frayed material from the edges was removed prior to test. Aluminum 2024 T3 coupons were conversion coated, primed (MIL-PRF-23377), and painted with polyurethane topcoat (MIL-PRF-85285). The coupons were laid side-by-side on foil, wrapped, and then sterilized by autoclaving for 15 minutes at 121 °C. Once cool, the coupons were placed in a biosafety cabinet, unwrapped from the foil, and transferred aseptically using sterile forceps into multiple, sterile empty Petri dishes until they were inoculated. Autoclaving did not alter the visual appearance of the substrates.

3.2 Spore Preparation

Just prior to coupon inoculation, a suspension of Btk spores was prepared directly from DiPel Technical Powder (Valent BioSciences Corporation; lot number 256-890-V9; strain ABTS-351). This lot was quantified to contain 1.0×10^{11} spores per gram. A 0.19-g aliquot of DiPel was resuspended with 9.5 mL sterile dH_2O to make a suspension of approximately 2×10^9 colony forming units (cfu)/ milliliter (mL). To achieve a homogenous suspension of individual spores, the suspension was vortexed vigorously for 1 minute, probe-sonicated for 20 seconds at a setting of 40% amp, and then vortexed vigorously again for 1 minute. A 4-mL aliquot of this suspension was then added to 16 mL of sterile dH_2O in a 50-mL conical tube; representing the Coupon Challenge Suspension at a concentration of approximately 4×10^8 cfu/mL. After vortexing this suspension for 1 minute, a 2.5-ml aliquot was transferred to a 50 mL conical tube and used to measure the concentration following standard spread plating methods using phosphate buffered saline supplemented with 0.05% Tween 20 (PBST) as the diluent and Tryptic Soy Agar (TSA) as the growth medium. The remainder of the suspension was used to inoculate coupons as described in Section 3.3.

3.3 Coupon Inoculation

One day prior to efficacy testing, thirty of each type coupon were inoculated with $>10^7$ spores. This number of coupons allowed triplicate control coupons (inoculated but not BTD treated) and triplicate BTD treated coupons to be assayed for up to five time points including T_0. The coupons were inoculated as follows: The Coupon Challenge Suspension was vortexed briefly, after which three coupons were promptly inoculated individually with a 100 µL droplet using a micropipette. The next set of three coupons were inoculated in the same manner until all coupons were inoculated. Since spores tend to settle out of suspension quickly when prepared at relative high concentration such as 10^8 cfu/ml, only three coupons were inoculated at a time before re-vortexing the spore suspension and inoculating the next set of three coupons. The appearance of the coupons immediately following inoculation and after the inoculum had dried are shown in Figure 3. Once all coupons were inoculated, coupons were left in the Biosafety Cabinet (BSC) undisturbed with the Petri lids remaining off to allow the inoculum to dry overnight. The spore inoculum rapidly absorbed into the webbing but did not do so on nylon sheeting or painted aluminum coupons. After drying, the spore smear could be clearly seen on the painted aluminum and nylon sheeting, but no visible dried inoculum could be seen on the nylon webbing.

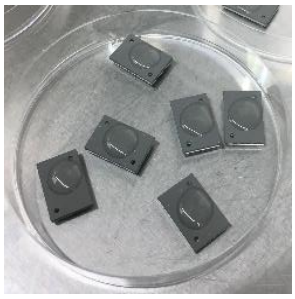
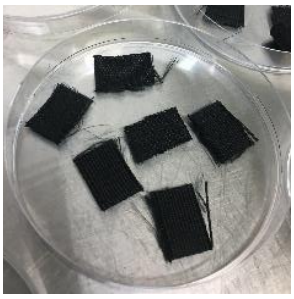
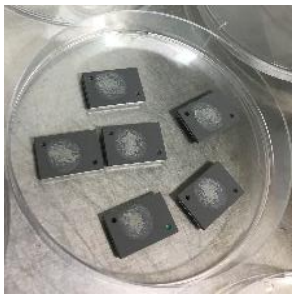
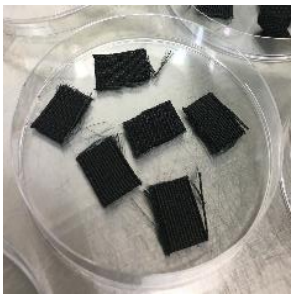
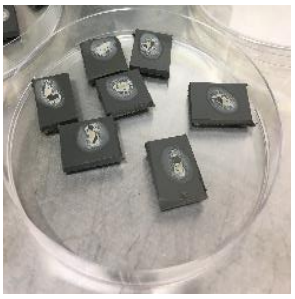
Wet Inoculum			
Dry Inoculum			
	Painted Aluminum	Nylon Webbing	Nylon Sheet

Figure 3. Coupons after inoculation with Btk spores (pre and post-drying)

3.4 BTD Treatment

A Thermotron environmental chamber was used to expose the spores to BTD conditions. Just prior to the test, the chamber was set to 170 °F (76.7 °C) and 90% RH and allowed to equilibrate to these conditions. Once the conditions were stable, fifteen inoculated coupons of each substrate were placed in the chamber with the Petri dish lids removed and chamber door closed (representing T_0). The rate at which the BTD process rendered bacterial spores inactive (i.e., non-culturable or non-viable) was determined by processing (extracting and enumerating) three replicate coupons per substrate periodically over the course of several days. The intent was to acquire several data points during the period of linear decay from which the decay rates could be calculated. Prior BTD decontamination tests that were conducted at 170 °F and 90% RH, resulted in up to a 7.0-log reduction in Btk in 96 hours (4 days), so it was anticipated linear decay would occur during the first few days. The intent of the test was not necessary to carry out sample analysis until complete inactivation was achieved, rather to establish the overall rate of decontamination. Triplicate inoculated control coupons, incubated at 20-25 °C and ambient relative humidity, were also processed at each time point to demonstrate spore stability at ambient conditions. The chamber temperature and relative humidity were monitored and recorded manually throughout the test.

3.5 Coupon Analysis

To extract the spores from the surface of the coupons, treated and untreated coupons were placed in the BSC and aseptically transferred to 50-mL conical tubes each containing 10-mL of PBST. To minimize the potential for cross-contamination of treated samples from untreated samples, all treated samples were processed first. The tubes were vortexed for 20 seconds, probe sonicated for

20 seconds, and then vortexed again for 30 seconds. Between samples, the sonicating probe was disinfected with 0.5% sodium hypochlorite (sprayed and then wiped with a Wypall), followed by a rinse with sterile water (to remove residual bleach), and then disinfected again with 70% isopropyl alcohol (IPA) (spray and wipe) and allowed to dry. Extracted samples were then enumerated using standard spread plating and/or membrane filtration techniques, depending on the expected concentration of culturable spores remaining in the sample. The extracts were then serially diluted (1:10; 0.5 mL of sample into 4.5 mL of PBST diluent) to a dilution specified by the Principal Investigator. In general, triplicate 100 µL aliquots of three dilutions were spread plated onto TSA plates in order to obtain plates that contained 25 to 250 colonies per plates. As the decay was observed throughout the cycle, lower dilutions were plated. As near complete kill was anticipated, three 100-µL aliquots and five 200-µL aliquots of undiluted extract were spread plated and the remainder of the extract was filtered through a membrane (Thermo Fisher Scientific; part no. 130-4020; 0.2 µm CN filter) that was later applied directly to a growth plate. All plates were incubated for 3 days at 30-35 °C and observed daily. If no Btk colonies were observed after 1 day of incubation, the plates were incubated another two days (observed daily). The number of colonies per plate were counted and recorded. Spread plates having 25-250 colonies were used to calculate cfu/mL using the following equation:

*Mean cfu/plate * Corresponding Serial Dilution Factor * 10 (plating dilution factor) = cfu/mL*

If spread plates counts were low (<25 plate), the counts from the membrane filter were used to calculate cfu/mL and total cfu recovered/coupon. The total cfu recovered/coupon was, in turn, converted to $\log_{10}$ cfu recovered/coupon and plotted as kill curves depicting the quantity of active spores recovered versus time.

Triplicate positive control coupons (untreated inoculated coupons) were processed with each set of triplicate BTD-treated coupons in the same manner as described above except that dilutions all coupons were plated at $10^{-5, -6, \text{and } -7}$ at each time point. These controls were analyzed to demonstrate spore stability at room temperature and to demonstrate consistency and accuracy of the extraction and analysis procedure. To avoid cross contamination, the positive controls were processed after the BTD-treated coupons were processed. Two negative control coupons (sterile, uninoculated coupons) were also assayed with each group of BTD-treated and positive control coupons to demonstrate that aseptic practices were used in processing the samples and that cross-contamination had not occurred. The negative controls were assayed before and after all other samples in the group were processed. Reagent controls were also included with each group of coupon samples including one non-inoculated TSA plate and one TSA plate inoculated with 100 µL PBST.

3.6 Data Analysis

The concentration of spores recovered versus time was plotted to generate a kill curve and subsequently used to calculate the decay rate and decimal reduction time or D-value. The D-value (D) is defined as the time at a constant condition (170 °F and 90% RH for this study) in which a decrease of 90% or 1-log of the initial population was observed. The D-value was determined by measuring the slope of the kill curves where linear decay was observed and then calculated as the negative inverse of the slope as shown in formula below.[16]

$$D = [-1 / \text{slope}]$$

The extraction efficiency of recovering the spores from the coupons was determined by comparing the quantity of spores recovered from control coupons to the quantity of spores inoculated onto the coupons (as determined based on the enumeration of the inoculation suspension).

4.0 RESULTS AND DISCUSSION

4.1 Efficacy

The kill curves for the treated coupons are shown in Figure 4. Samples were analyzed at 0, 12, 24, 34.5, 49, and 75 hours. The results illustrate the rate of decay on the nylon webbing was far slower than on the nylon sheet. The reason for the difference decay rates between the nylon materials cannot be speculated other than the obvious differences in material construction. While the webbing is far more porous, the recovery efficiencies (as shown later in section 4.4) between the two nylon materials were very comparable; therefore, the methodology used to extract/recover the spores is unlikely the cause.

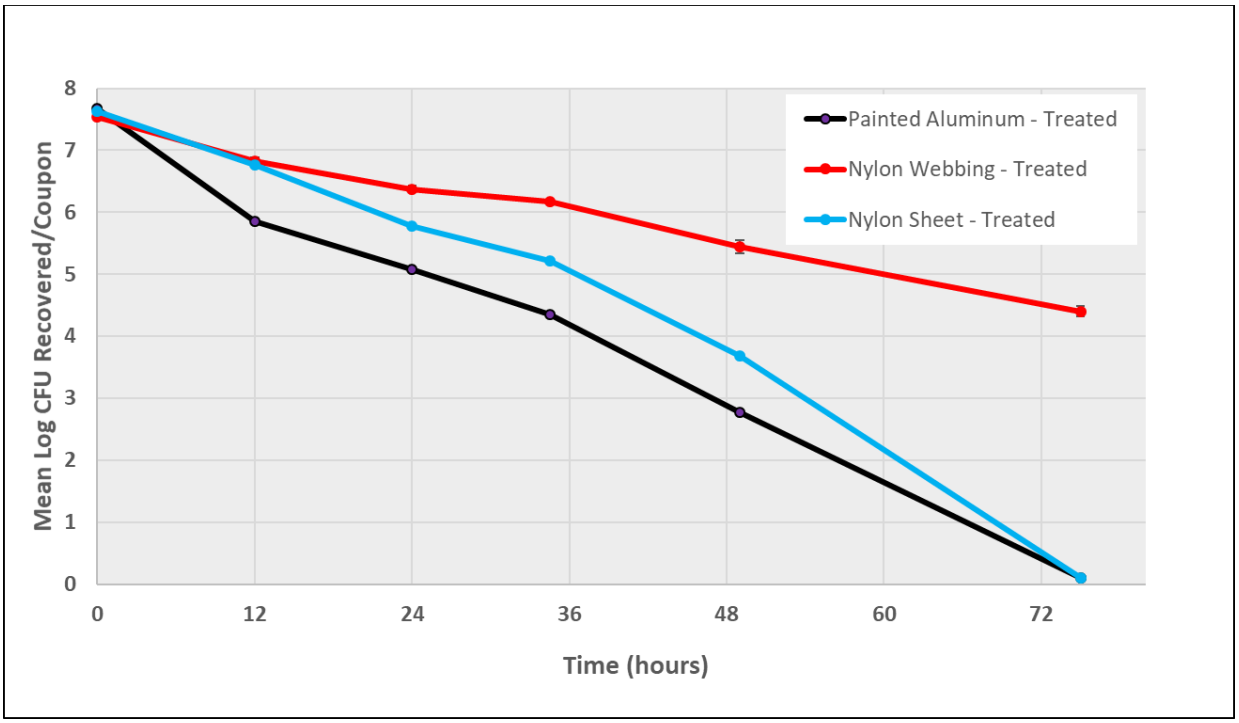


Figure 4. Kill curves for Btk spore inoculated coated alloy, nylon webbing, and nylon sheet coupons and treated to BTD (170 °F & 90% RH)

The results for the untreated controls are shown in Figure 5. These results illustrate the spores were stable throughout the duration of the test and that the analysis method was consistent as standard deviations between triplicate samples were minimal.

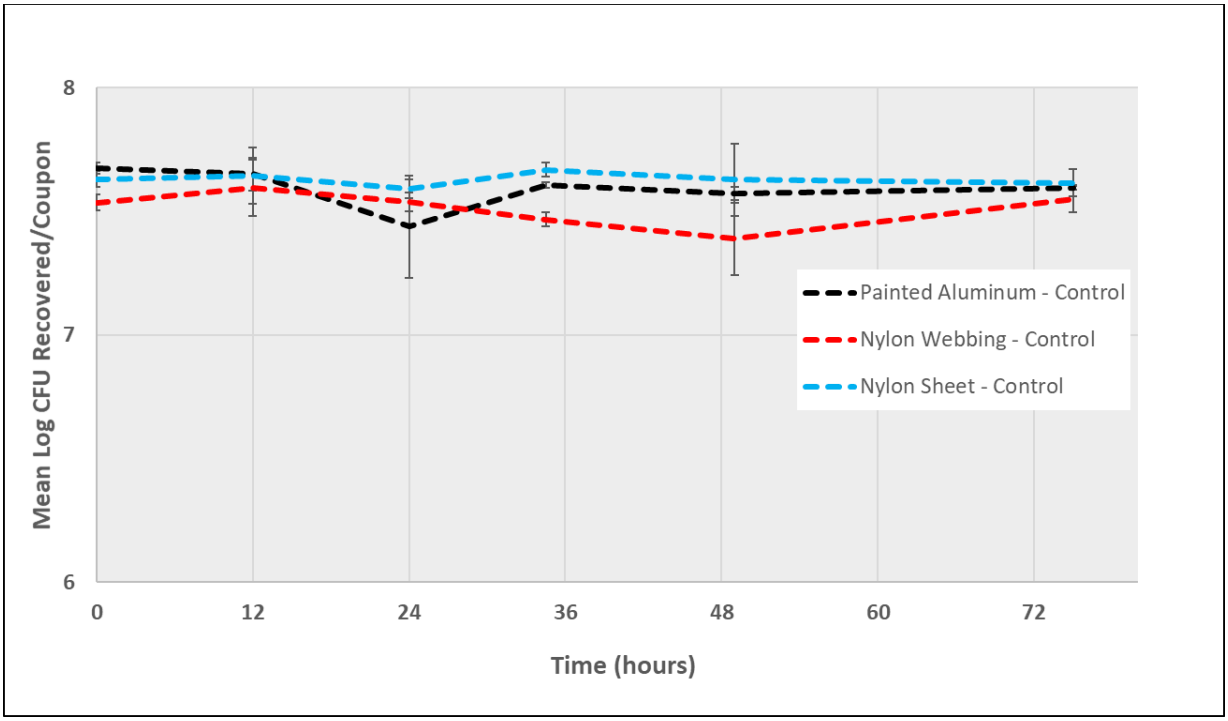


Figure 5. Recovery of Btk spores from non-BTD treated control coupons

4.2 Decay Rates

Decay rates were calculated from data within the linear range of decay which was between 12 to 49 hours. These segments of the decay curves are shown in Figure 6 along with the linear regression equations for each.

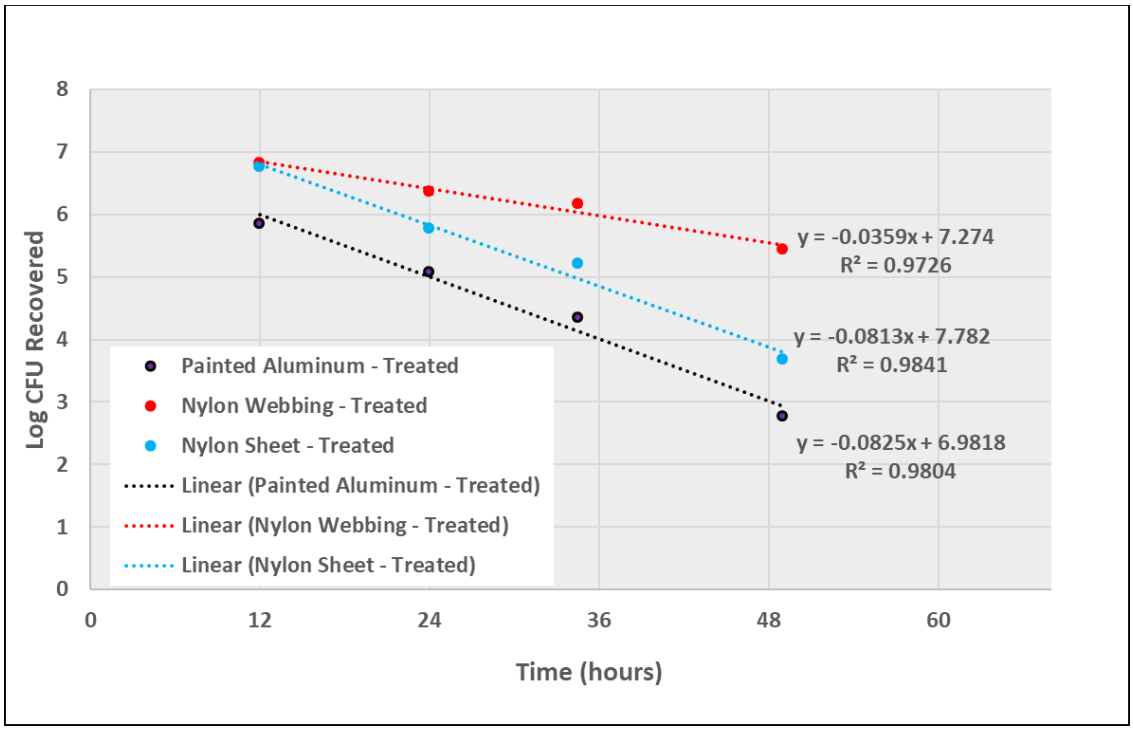


Figure 6. D-value determination from BTD treated coupons

The calculated slope, D-value, and time required to achieve 6-log reduction are shown in Table 1. The decay rates were virtually the same on nylon sheet and painted aluminum with D-values of 12.1 and 12.3 hours, respectively and requiring an estimated exposure of 3 days to achieve a 6-log reduction. The decay rate on nylon webbing, however, was much slower at 27.9 hours requiring an estimated 7 days to achieve a 6-log reduction.

Table 1. Btk D-values from BTD Treated Alloy and Nylon Coupons

Substrate	Slope of Coupon Kill Curve	D-value (hours)	Time to Achieve 6-log Reduction (days*)
Painted Aluminum	-0.0825	12.1	3.0
Nylon Webbing	-0.0359	27.9	7.0
Nylon Sheet	-0.0813	12.3	3.1

*D-value multiplied by 6 then divided by 24 hours.

4.3 Quality Controls

All negative media and buffer controls tested negative for growth on each day of testing. Positive media controls tested positive (i.e., supported growth of Btk), as expected. All uninoculated control coupons assayed between treated and untreated sample sets tested negative.

4.4 Extraction Efficiency

The inoculation suspension was measured to be $6.3x10^8$ cfu/mL. Since the inoculation volume was 0.1 mL, the measured inoculation concentration was 6.3×10^7 cfu/coupon or 7.8 log cfu/coupon. Based on the inoculation concentration and the mean log cfu recovered for the untreated controls for each substrate, the extraction efficiency from substrates were all above 50% (Table 2) and deemed acceptable for the purpose of this study.

Table 2. Extraction Efficiencies

Substrate	Mean CFU Recovered*	Percent Recovery
Painted Aluminum	3.3×10^7	52%
Nylon Webbing	3.3×10^7	53%
Nylon Sheet	4.3×10^7	68%

*Mean recovery of controls from all time points.

5.0 CONCLUSIONS

This study was conducted to compare the decay rate of a *B. anthracis* spore surrogate inoculated onto porous and non-porous nylon materials when treated to BTD conditions. The decay rate was approximately 2.3 times slower on nylon webbing compared to both nylon sheeting and painted aluminum. The porous nature of the webbing construction is believed to contribute to the adverse effect on the otherwise efficacious properties of BTD.

6.0 LIST OF ABBREVIATIONS

AFRL .....Air Force Research Laboratory
APC.....Aircraft Performance Coating
BSC.....Biosafety Cabinet
BTD.....Biothermal Decontamination
Btk.....Bacillus thuringiensis variety kurstaki
BW .....Biological Weapons
cfu .....colony forming units
HAD.....Hot Air Decontamination
JBADS .....Joint Biological Agent Decontamination System
JCTD.....Joint Capability Technology Demonstration
mL .....milliliter
NSWCDD .....Naval Surface Warfare Center Dahlgren Laboratory
PBST.....Phosphate Buffered Saline with Tween 20
RH.....Relative Humidity
TSA.....Tryptic Soy Agar

7.0 REFERENCES

[1] T. Provens, R. Cahall, M. Shaw, M. Arthur, K. Heater, A. Theys, M. Robertson. *Thermal Inactivation of Bacillus Thuringiensis: Final Report Detailing Results for Thermal Inactivation.* ADB356943. AFRL-RH-WP-TR-2010-0010. November 2009.

[2] V.K. Rastogi, L. Wallace, L.S. Smith, S.S. Shah, and R. Foster. *Laboratory-Scale Demonstration of Hot Moist Air as a Bio-Decon Technology for Large-Frame Aircraft Interior Surfaces*. ADB3665681. ECBC-TR-831. January 2011.

[3] T. Buhr, A.A. Young, D. McPherson, C. Hooban, Z. Minter, D. Shegogue, D. Kota, V. Rozanski, M. Hammon. *Sporicidal Efficacy Testing with Hot Humid Air to Evaluate the JBADS Decon System*. NSWCDL Final Report: Spore Preparations, Environmental Chambers, and Heat Kill of Bacillus Spores (B. anthracis Ames, B. anthracis ΔSterne, B. cereus ATCC 4342, B. thuringiensis Al Hakam). April 07, 2010.

[4] M. Sloan, A. Tijerina, M. Woitaske, I. Rosas, J. Kiel, A. De Los Santos. *Validation of Joint Biological Agent Decontamination System (JBADS) Program*. ADB3618081. AFRL-RH-BR-TR-2010-0053. April 2010.

[5] T. Provens, K. Heater, A. Theys, M. Arthur, and D. Lorch. *Demonstration of C-130 Biological Thermal Decontamination at Little Rock Air Force Base*. ADB379627. AFRL-RH-WP-TR-2012-0039. October 2011.

[6] T. Provens, D. Lorch, A. Theys, K. Heater. *Bio-Thermal Inactivation Add-on Laboratory Study*. ADB382453. AFRL-RH-WP-TR-2012-0090. April 2012.

[7] R. Cahall and K. Heater. *Thermal Inactivation of Bacillus Thuringiensis (Bt) in Water*. ADB397654. AFRL-RH-WP-TR-2013-0122. November 2013.

[8] A. Theys and W. Greer. *Analytical Test Report for the Joint Biological Agent Decontamination System (JBADS) Joint Capability Technology Demonstration (JCTD) Operational Utility Assessment (OUA)*. USAF Contract No. FA8650-13-D-6405, 0002. ADB412326. AFRL-RH-WP-TR-2015-0020. August 2015.

[9] T.L. Buhr, A.A. Young, M. Bensman, Z.A. Minter, N.L. Kennihan, C.A. Johnson, M.D. Bohmke, E. Borgers-Klonkowski, E.B. Osborn, S.D. Avila, A.M.G. Theys, P.J. Jackson. *Hot, humid air decontamination of a C-130 aircraft contaminated with spores of two acrystalliferous Bacillus thuringiensis strains, surrogates for Bacillus anthracis.* J. Appl. Microbiology. Volume 120, Issue 4. January 2016.

[10] A. Theys and W. Greer. *Supplemental Data for the Joint Biological Agent Decontamination System (JBADS) Aerosol Test*. USAF Contract No. FA8650-13-D-6405, 0002. ADB410973. AFRL-RH-WP-TN-2015-0001. June 2015.

[11] E.J. Prokop, J.R. Crigler, C.M. Wells, A.A. Young, T.L. Buhr. Response surface modeling for hot, humid air decontamination of materials contaminated with Bacillus anthracis ΔSterne and Bacillus thuringiensis Al Hakam spores. AMB Express 4:21, 2014.

[12] T. Buhr, et al. *Sporicidal Efficacy Testing with Hot, Humid Air to Evaluate the Joint Biological Air Decontamination System, Tier II*. NSWCDD/TR-14/147. April 2014.

[13] T.L. Buhr, A.A. Young, Z.A. Minter, C.M. Wells, D.C. McPherson, C.L. Hooban, C.A. Johnson, E.J. Prokop, and J.R. Crigler. *Test method development to evaluate hot, humid air decontamination of materials contaminated with Bacillus anthracis ΔSterne and B. thuringiensis Al Hakam spores*. J. Appl. Microbiology. ISSN 1364-5072. 2012.

[14] T. Buhr, et al. *Sporicidal Efficacy Testing with Hot, Humid Air to Evaluate the Joint Biological Air Decontamination System, Tier III*. NSWCDD/TR-15/72. September 2014.

[15] T. Buhr, et al. *Bacillus Spore Decontamination with Hot, Dry Air*. NSWCDD/TR-15/324. June 2015.

[16] Mazzola, P. G, T. Penna, A. Martins. *Determination of decimal reduction time (D value) of chemical agents used in hospitals for disinfection purposes*. BMC Infectious Diseases. October 2003. 3 (24): 1471-2334.