Thermal Destruction of *Bacillus Anthracis* Surrogates in a Pilot-Scale Incinerator

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ABSTRACT

The decontamination of a building following a biological warfare agent (such as *Bacillus anthracis*) release may result in a significant quantity of building decontamination residue (BDR). This BDR would consist of various materials such as ceiling tile, carpet, wallboard, concrete, and wood, which would be removed from the building either before or after decontamination efforts. Although the BDR is likely to have been decontaminated, the possibility exists for the presence of trace amounts of biological agent. Although a likely disposal technique for the BDR is high temperature incineration, complete destruction of microbiological organisms in an incinerator environment is not a certainty, due to heat transfer limitations and matrix effects. This paper describes experiments that were performed in a pilot-scale rotary kiln incinerator to evaluate the thermal destruction of *B. anthracis* surrogates (bacterial spores) present within bundles of carpeting and ceiling tile. Another purpose of the experiments was to evaluate sampling and analytical techniques to measure spores in the exhaust gas and material bundles. No spores were detected via any of the three sampling trains in the exhaust gas for the carpet burn tests conducted in July and August 2005, although the detection limit was determined to be 22 spores/dry standard ft^3. Combustion of the nylon-6 carpet resulted in increased nitrogen oxide (NO_x) emissions, with short-term spikes in emissions levels from approximately 30 ppm baseline to roughly 150 ppm with each carpet bundle charged. The use of biological indicator strips to quantify thermal destruction of spores embedded within carpet and ceiling tile bundles was successful. Spores embedded within wet ceiling tile bundles took at least 35 minutes to completely destroy, possibly exceeding typical incinerator solid waste residence times. The charging of ceiling tile bundles had minimal impact on the air pollutant emissions studied.

INTRODUCTION

After a building has gone through decontamination activities following a terrorist attack with a biological warfare (BW) agent such as *Bacillus anthracis (B.A.)*, there will be a significant amount of residual material and waste to be disposed. This material is termed "building decontamination residue" (BDR). Although it is likely that the BDR will have already been decontaminated, the possibility exists for remaining *B.A.* spores to be

present in absorbent and/or porous material such as carpet, fabric, ceiling tiles, office partitions, furniture, and personal protective equipment (PPE) and other materials used during cleanup activities. It is likely that much of this material will be disposed of in high-temperature thermal incineration facilities, such as medical/pathological waste incinerators, municipal waste combustors, and hazardous waste combustors.[1]

Although pathogens such as *B.A.* present in BDR are killed at typical incineration temperatures (> 800 °C [1472 °F]), gas-phase residence times (> 2 s), and solid-phase residence times (> 30 min), it is possible for some of the pathogens to escape the incinerator due to bypassing the flame zones, cold spots, and incomplete penetration of heat through the bed.[1] In the early 1990's, the US EPA performed testing of commercial hospital waste incinerators[2] by inputting large quantities of *Geobacillus stearothermophilus* (an anthrax surrogate) spores into the combustors and measuring the number leaving in the stack emissions and in the incinerator bottom ash, in terms of Log reduction in spore concentration. It was determined that, in certain cases, only a 3-Log_{10} reduction in spore destruction was found, in spite of acceptably high operating temperatures and sufficiently long residence times.

As a result of the 2001 anthrax attacks, the EPA instituted an experimental and theoretical research program to investigate issues related to the thermal destruction of contaminated BDR[3] initially including carpeting, ceiling tile, and wallboard. Tests are being performed at bench- and pilot-scale. This paper describes experiments that were performed in a pilot-scale rotary kiln incinerator to evaluate the thermal destruction of *B. A.* surrogates (bacterial spores) present within bundles of carpeting and ceiling tile. Another purpose of the experiments was to evaluate three novel sampling and analytical techniques to measure bacterial spores in the exhaust gas. Thermal destruction of spores in the ceiling tile was determined by quantification of spore strips. Emissions of NO_x, carbon monoxide (CO), total hydrocarbons (THC), and other pollutants were measured on a continual basis throughout the testing.

The results described in this paper may be of use to incinerator owners and operators that choose to combust BDR, by providing some technical background and guidance regarding what might be required to ensure complete destruction of biological agents and the potential impacts on air emissions.

EXPERIMENTAL

Facility Description

Tests were performed from July 2005, through December 2005, using EPA's Rotary Kiln Incinerator Simulator (RKIS), which is located at EPA's campus in Research Triangle Park, North Carolina. The RKIS consists of a primary and secondary combustion chamber (SCC), each rated at 73 kW (250,000 BTU/hr). The RKIS can burn a variety of fuels, including surrogate hazardous waste, although for the tests described herein, only natural gas was burned in the primary chamber. The RKIS is equipped with continuous emissions monitors (CEMs) for measuring oxygen (O_2), carbon dioxide (CO_2), water vapor (H_2O), CO, NO_x, sulfur dioxide (SO_2), and THC. However, for the tests described

herein, only data for O_2, CO_2, CO, NO_x were logged for all of the tests, with THC levels measured for some of the testing, for various technical reasons. Emissions were monitored in the exhaust duct, where sufficient mixing and cooling had taken place. Temperature readings are taken throughout the kiln system, although only kiln exit temperatures are reported here. Figure 1 is a diagram of the RKIS (note surrogate wastes were not used in the tests described herein); further details of the RKIS can be found elsewhere.[1] Some general operating characteristics of the kiln during the tests are summarized in Table 1. An overview of the test program reported here is found in Table 2.

Figure 1. US EPA's Rotary Kiln Incinerator Simulator [4]

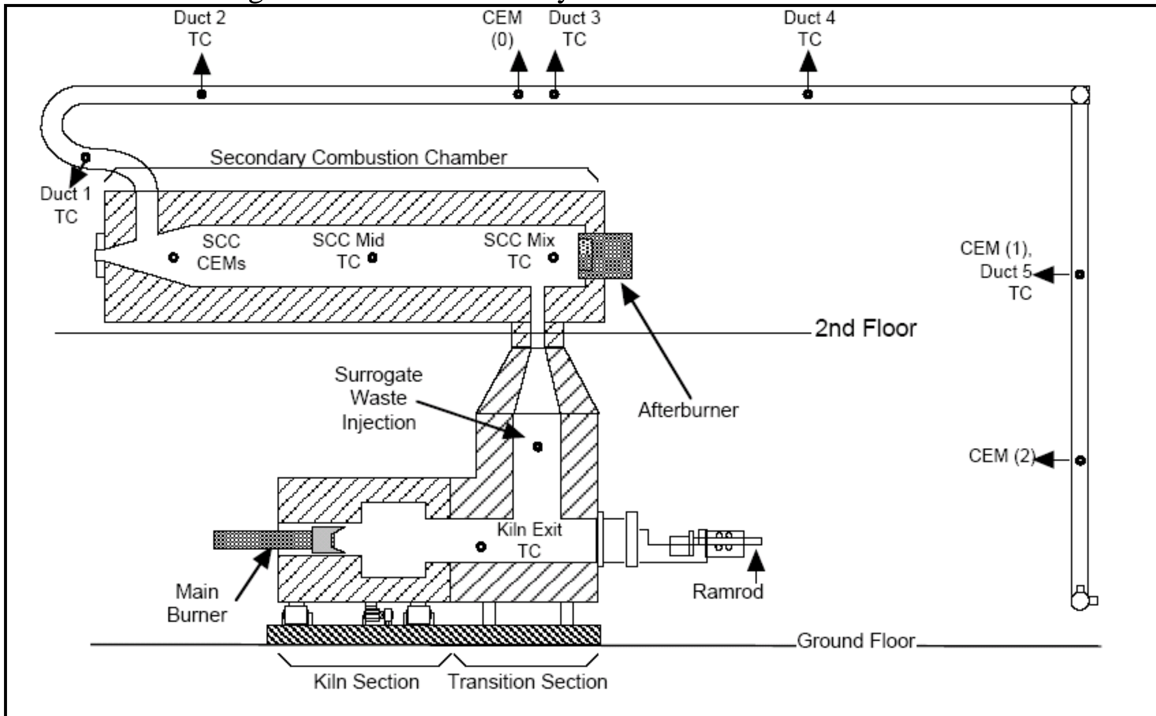


Table 1. Summary of RKIS Baseline Operating Parameter Values

RKIS Operating Parameter	Range or approximate value for tests
Primary combustion chamber (PCC) air flow	83 – 89 standard cubic meters per hour (2920 – 3145 standard cubic feet per hour; SCFH)
Natural gas flow PCC	~ 5.7 SCM per hour (200 SCFH)
Natural gas flow SCC	0 SCFH
Air flow SCC	~ 20 SCM per hour (700 SCFH)
Draft PCC	~ 0.05 inches water
Kiln rotation speed	0.5 rpm for the tests conducted in July and August. Kiln was not rotating for the October and December tests.
Kiln exhaust gas oxygen level	~ 15.5%

Table 2. Summary of RKIS Tests

Date of Test	Building Material	Bacterial spore surrogate/form	Purpose of test	Comments
7/20, 7/21, 7/27, and 8/17/05	Carpet in sterile water	Geobacillus Stearothermophilus soaked in the water	Determine emissions of spores; Evaluate spore emissions sampling trains	Kiln rotating
10/6, 10/7, & 10/13/05	Carpet soaked in water	2 BI strips in sealed pipe with thermocouple (T/C)	Thermal destruction quantification using spore strip technique.	First strip used to determine if living or dead; second strip to quantify. On 10/6, T/C not working correctly
12/8-12/9/05	Ceiling tile	No spores; just temperature profiles	Temperature profiles	Bundles wet and dry
12/13, 12/16, 12/20, and 12/29/05	Ceiling tile	2 BI strips in sealed pipe with T/C	Thermal destruction quantification using spore strip technique.	Both wet and dry bundles tested

Building Material Experiments

Tests performed in July and August 2005, were with carpet bundles soaked in a solution of sterile water containing *Geobacillus stearothermophilus* at a level of approximately 10^8 spores per gallon (2.6 x 10^7 per liter). The carpet bundles were made from Nylon-6 material, weighed approximately 1 pound dry, and weighed on average about 2.1 pounds (0.95 kg) wet. The wetted bundles were approximately 3 inches wide by 3 inches high by 11 inches (7.6 cm by 7.6 cm by 28 cm) long, and sealed in plastic bags. For each day of testing, a total of six carpet bundles were fed to the kiln, with one bundle fed every 10 minutes. The primary purpose of these tests was to determine spore emissions levels in the exhaust gas while evaluating the bacterial spore emissions sampling trains themselves as well. Sampling for bacterial spores in the exhaust gas began with the first bundle fed; sample gas extraction occurred approximately in the area of CEM (2) as noted in Figure 1. The three spore emissions sampling trains are discussed in more detail below.

Tests performed in October 2005 were with the same carpet type and size described above, and all samples were wetted (to simulate carpeting that may have become wetted

during the decontamination process). However, for this series of tests, the carpet samples were bundled together using a titanium cage, and a small sealed metal pipe was embedded into the carpet bundle. Inside the metal tube were two biological indicator (BI) spore strips and a Type K thermocouple (to measure charge probe temperature). Additional details of this pipe – BI – thermocouple arrangement can be found elsewhere.[1] For each of the three days of testing, a total of five carpet bundles were fed to the kiln. The primary purpose of these carpet tests was to evaluate thermal destruction of spores as a function of time the bundle remained in the kiln. The carpet bundle was thrown into the kiln at the end opposite of the burner (the kiln was not rotating), and removed after the set time period using a gaffe to hook and remove the titanium cage and remaining carpet bundle. A secondary purpose for this series of tests was to evaluate the method used to determine thermal destruction efficiency, i.e., through the use of spore strips inside a sealed pipe. This approach to quantifying spore destruction using BI strips is described in more detail below.

Tests in December 2005 were performed with ceiling tile bundles. The bundles consisted of 14 pieces; each individual piece was 7.62 cm x 7.62 cm x 1.9 cm (3 inches by 3 inches, and ¾ inch) thick. The ceiling tile pieces were bound together in a cage made from titanium, and the total length of the bundle was about 11 inches (28 cm). The purposes of these ceiling tiles tests were to evaluate thermal destruction of spores as a function of time and gas temperature, and any potential air pollution emissions impacts. (Initial tests with the ceiling tiles were conducted without bacterial spores, to characterize temperature profiles.) Subsequent tests for spore thermal destruction analysis were conducted using BI strips encased in a metal pipe, which was embedded into the ceiling tile bundle - similar to what was done for the carpet bundle BI tests. Tests were conducted with both wetted and dry bundles. The mass of the dry bundles was on average approximately 500 grams; while the mass of bundles that were soaked in water averaged about 1519 grams (includes mass of titanium cage).

Spore Emission Test Methods

During our carpet burn tests 3 sampling trains were co-located to pull from the exhaust at a location defined by a straight run. Each train was defined by an ice-chilled impinger train with gas flow controlled by an EPA Method 5 box. Each impinger train consisted of two Greenberg-Smith impingers filled with a sterile, pH 7.4 phosphate buffer solution, 1 empty impinger, and a silica gel impinger to protect the gas meter. The 3 trains differed in the approach to sample cooling prior to the impinger train. These were: cooled sample probe, dilution sample probe, and virtual impactor probe.

The “cooled probe” technique uses a pH 7.4 phosphate buffer solution for the collection of spores. The gas stream is cooled to less than 38 °C (100 °F) using a cooled probe prior to impinger collection. The cooled probe consisted of an Allihn condenser with ice water pumped through the jacket.

The “virtual impactor” technique uses a virtual impactor to concentrate the larger biological particles and separate them from the fine particulate prior to impinger collection, with a cutpoint of 0.5 microns.

The “dilution probe” technique uses a pH 7.4 phosphate buffer solution for the collection of spores. The gas stream will be cooled to less than 38 °C (100 ° F) using a dilution

manifold prior to impinger collection. The sample stream is mixed with HEPA filtered air to provide this dilution.
After sampling, each impinger solution was analyzed separately. Upon receipt, samples were centrifuged to concentrate all particulate, including spores, into a single solid "plug" that was recovered, washed, and diluted to 1 mL with sterile buffer solution. Analysis consisted of spreading 0.1 ml of this concentrated sample onto 3 replicate trypticase soy agar plates (TSA) plates, incubating at 60 ºC (140 °F) over night, and manual counting. A quantifiable limit of 30 CFU (colony forming units) per plate is used. Data from our bio laboratory have shown a typical RSD (relative standard deviation) of 30 % for samples above this quantifiable limit. Plates over 300 CFU are rejected as "too numerous to count" and repeated at a greater dilution factor. Based on the above described analysis, this results in an impinger detection limit of 300 CFU, or 1,200 CFU per gas sample (based on 4 impingers in the train).
Using a sample flow rate of 0.91 dry, standard (20 º C, or 68 °F) ft^3/minute, a sample time of 1 hour, this corresponds to a detection limit of about 22 spores/dry standard ft^3, or about 20 spores per standard cubic foot, wet basis. Note that this quantifiable limit determination assumes that no spores are lost in the sampling probe.

Bacteriological Methods

As mentioned previously, *Geobacillus stearothermophilus* (ATCC 7953) spore strips - 2.0 x 10^6 spores/strip (Raven Biological Laboratories, Inc.) were used as biological indicators. Two spore strips were used per test; both were placed in a metal pipe and embedded inside the material sample.

After completion of the test, spore survivability was qualitatively analyzed by placing one of the heat-treated spore strips in 25 ml of sterile nutrient broth (NB) and incubated at 55°C ± 2 (131 °F) for 7 days. A positive control consisting of a spore strip of *G. stearothermophilus* (non-heated) in 25 ml of NB was incubated along with the heat-treated spore strip. Development of turbidity during the 7-day incubation period was scored as positive. Absence of growth (no turbidity) was scored negative.
To quantify the spore population (survivability) after the test, a 1/100 dilution of the 2nd heat-treated spore strip (w/v) was homogenized in a Nasco masticator blender – 10 beats/sec. Dilutions were plated in triplicates on TSA plates and incubated at 55°C (131 °F) ± 2 for 24 hours. Concomitantly, a positive control consisting of a 1/100 dilution of a *G. stearothermophilus* non-heated spore strip was homogenized, plated and incubated at 55°C (131 °F) ± 2 for 24 hours to determine the initial spore population prior to heating. The spore population for both the heat-treated spore strip and the positive control was determined by colony-forming units (CFU)[5].
Spore survivability was determined by Log_{10} Reduction (LR)[6]:

$$LR = \log_{10}(c) - \log_{10}(t) \quad (1)$$

where
c = CFU of positive control
t = CFU of heat-treated spore strip

RESULTS AND DISCUSSION

Spore Emission Test Results

No spores were detected via any of the three sampling trains in the exhaust gas for the carpet burn tests conducted in July and August 2005. The possibility exists that spores were indeed present in the exhaust gas, i.e., not destroyed in the kiln or in the exhaust gas, but were below the level of detection. With the average amount of water soaked into the carpet bundle being around 1.1 pounds (0.5 kg), and the spore density in the water at 1×10^8 spores/gallon (2.6×10^7 per liter), this corresponds to approximately 1.32×10^7 spores per bundle, or 7.92×10^7 total spores charged to the incinerator during the test. With gas flow determined at the point of sampling to be on average 100.1 standard ft^3/minute, the maximum number of spores that could be present in the exhaust during the 1-hour test, but still escape detection, is 120,000 CFU. Therefore the minimum destruction efficiency is calculated to be 2.8 LR (see equation 1).

For all of these tests, average kiln exit temperatures prior to the feeding of the carpet ranged from about 804 – 827 °C (1480 – 1520 ºF). With the feeding of each bundle every 10 minutes, kiln exit temperature rose between 38 – 149 °C (100 - 300 ºF), but then backed down and stabilized at around 815 °C (1500 ºF), until another bundle was fed.

Consistent with work done previously to characterize the impact combustion of carpet has on air pollutant emissions[7], NO_x emissions spiked temporarily (referred to as a transient puff) from a baseline level of about 30 ppm to a peak of about 150 ppm, then back to the baseline level. This puff occurred over the course of about 10 minutes, the time interval that each carpet bundle was charged to the kiln. In general, CO emissions increased as well, with baseline levels of about 20 – 30 ppm increasing by about 5-10 ppm with the introduction of the carpet bundles. Correspondingly, exhaust gas oxygen baseline levels were about 15.5 %, were reduced to between 11-12% with the feeding of each bundle, but then rose back to baseline levels prior to the introduction of another bundle.

Carpet – Spore Thermal Destruction Results

As mentioned above, these tests were conducted in October 2005 to quantify bacterial spore thermal destruction efficiency using BI spore strips, as a function of time the bundle was left in the kiln. The results of these tests are shown in Tables 3 through 5. During these experiments, Raven Spore Strips were used. The manufacturer states the organism, *Geobacillus stearothermophilus*, is present on the strip at a concentration of 2.0×10^6. Our analysis of spore strips which were not subjected to any thermal treatment (i.e., a positive control) resulted in a level of 1.9×10^6, confirming the manufacturer's stated level. As can be seen in the tables below, in general, thermal destruction (surviving spores $< 1.00 \times 10^6$) didn't begin until approximately 7 minutes (see runs D and E of 10/7 test, and run F of 10/13 test). Not coincidentally, the tests resulting in the greatest destruction of spores also indicate the spore strips were subjected to the highest temperatures (maximum charge probe temperature). In general, spore strips left in the kiln longer were exposed to higher temperatures, although there are exceptions, which may be due to various reasons, including variable kiln gas exit temperature and variable placement within the kiln bed. Lastly, a negative control (TSA with no known spores

was poured into plates or into the tubes used for the spore strips and examined for growth) was used once per test, and all were found to have no growth.

Table 3. Carpet/Spore strip chamber: Surviving Number of Spores for Tests Conducted 10/6/05

Run designation	A	C	D	E	F	Negative Control
Number of spores surviving	1.35 x 10^6	1.25 x 10^6	1.53 x 10^6	2.08 x 10^6	1.24 x 10^6	0
Time in kiln (minutes)	2:04	4:53	4:53	6:10	6:10	0:00
Average Kiln Exit Temperature ° C (°F)	Not available	803 (1477)	807 (1485)	809 (1489)	818 (1504)	
Maximum Charge Probe Temperature ° C (°F)	56 (133)	112 (233)	101 (214)	120 (248)	103 (218)	

Table 4. Carpet/Spore strip chamber: Surviving Number of Spores for Tests Conducted 10/7/05

Run Designation	A	C	D	E	F	Negative Control
Number of Spores Surviving	1.77 x 10^6	1.38 x 10^6	9.7 x 10^5	0 (NG)	1.50 x 10^6	0
Time in kiln (minutes)	5:53	5:57	7:05	6:46	5:07	0:00
Average Kiln Exit Temperature ° C (°F)	831 (1527)	833 (1532)	833 (1532)	843 (1549)	842 (1547)	
Maximum Charge Probe Temperature ° C (°F)	218 (425)	138 (280)	264 (507)	552 (1026)	142 (287)	

NG= No growth

Table 5. Carpet/Spore strip chamber: Surviving Number of Spores for Tests Conducted 10/13/05

Run Designation	A	C	D	E	F	Negative Control
Number of Spores Surviving	1.42 x 10^6	1.24 x 10^6	1.57 x 10^6	1.52 x 10^6	2.4 x 10^5	0
Time in kiln (minutes)	4:02	4:02	5:00	6:00	6:51	0:00
Average Kiln Exit Temperature ° C (°F)	783 (1441)	772 (1421)	788 (1451)	786 (1447)	792 (1457)	
Maximum Charge Probe Temperature ° C (°F)	Not functioning	84 (184)	137 (278)	292 (557)	261 (501)	

Ceiling Tile - Temperature Profiles and Spore Thermal Destruction Results

The thermal destruction test results for the spores in ceiling tile bundles are found in Tables 6 through 9, and Figures 2-3. As with the carpet bundles, the initial number of spores on the strips was 2.0 x 10^6. A positive control test was conducted on 12/19/05, Run D, and the post-run spore count was 1.3 x 10^6. For the dry ceiling tile bundles, a 1 to 2 $\log_{10}$ reduction in the number of spores occurs sometime between 5 to 10 minutes, and complete destruction (6 $\log_{10}$ reduction) occurring after 12 minutes. For the wet ceiling tile bundles, although the results are somewhat variable, reduction in spores (from a 1-2 $\log_{10}$ reduction up to complete destruction) occurs between 35 – 38 minutes.

However, as shown in Figure 3, spore survival is dependent on internal bundle temperature, regardless of whether the bundle is wet or dry.

Baseline (prior to the ceiling tile charge) NO_x emissions were between approximately 20-25 ppm, and temporarily increased by a few ppm with the introduction of each ceiling tile bundle.

Table 6. Surviving Number of Spores for Tests Conducted 12/13/05

Run Designation	A	B	C	D	Negative Control
Number of Spores Surviving	5.3×10^5	6.9×10^5	7.0×10^5	5.3×10^4	0
Time in kiln (minutes)	5:07	5:05	9:58	9:55	0:00
Wet or dry bundle	dry	dry	dry	dry	
Average Kiln Exit Temperature ° C (°F)	779 (1435)	779 (1435)	779 (1435)	777 (1431)	
Maximum Charge Probe Temperature ° C (°F)	206 (402)	108 (227)	212 (414)	499 (930)	

Table 7. Surviving Number of Spores for Tests Conducted 12/16/05

Run Designation	A	B	C	D	E	F	G	Negative Control
Number of Spores Surviving	TFTC	TFTC	TFTC	TFTC	TFTC	7.3×10^5	TFTC	0
Time in kiln (minutes)	11:50	11:58	15:09	15:08	18:00	30:15	35:04	0:00
Wet or dry bundle	dry	dry	dry	dry	dry	wet	wet	
Average Kiln Exit Temperature ° C (°F)	803 (1477)	804 (1480)	806 (1483)	802 (1475)	802 (1475)	798 (1469)	801 (1473)	
Maximum Charge Probe Temperature ° C (°F)	335 (635)	398 (748)	562 (1044)	643 (1189)	464 (868)	Not working	357 (675)	

TFTC= too few to count

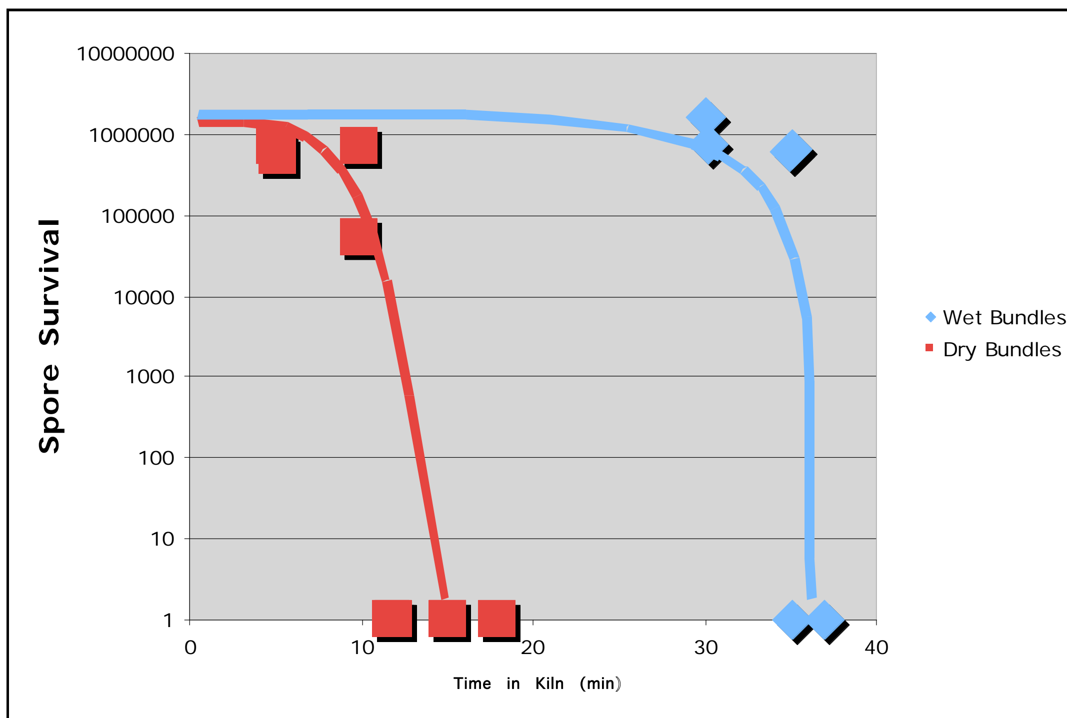
Table 8. Surviving Number of Spores for Tests Conducted 12/20/05

Run Designation	A	B	C	E	Negative Control
Number of Spores Surviving	6.5×10^5	1.6×10^6	6.2×10^5	TFTC	0
Time in kiln (minutes)	35:03	30:08	35:01	36:55	0:00
Wet or dry bundle	wet	wet	wet	wet	
Average Kiln Exit Temperature ° C (°F)	801 (1473)	806 (1482)	808 (1486)	810 (1491)	
Maximum Charge Probe Temperature ° C (°F)	300 (572)	128 (263)	144 (292)	438 (821)	

Table 9. Surviving Number of Spores for Tests Conducted 12/29/05

Run Designation	A	B	C	D (positive control)	E	F	H	Negative Control
Number of Spores Surviving	1.2×10^6	5.5×10^5	TFTC	1.3×10^6	7.8×10^5	2.5×10^4	1.8×10^6	0
Time in kiln (minutes)	8:01	8:01	9:03	0:00	9:00	31:55	37:55	0:00
Wet or dry bundle	dry	dry	dry		dry	wet	wet	
Average Kiln Exit Temperature °C (°F)	824 (1516)	822 (1512)	821 (1510)	Not applicable	818 (1505)	819 (1507)	821 (1509)	
Maximum Charge Probe Temperature °C (°F)	165 (329)	162 (323)	265 (509)	Not applicable	568 (1055)	Not functioning	275 (527)	

Figure 2. Ceiling Tile Bundle Spore Survival as Function of Time in Kiln

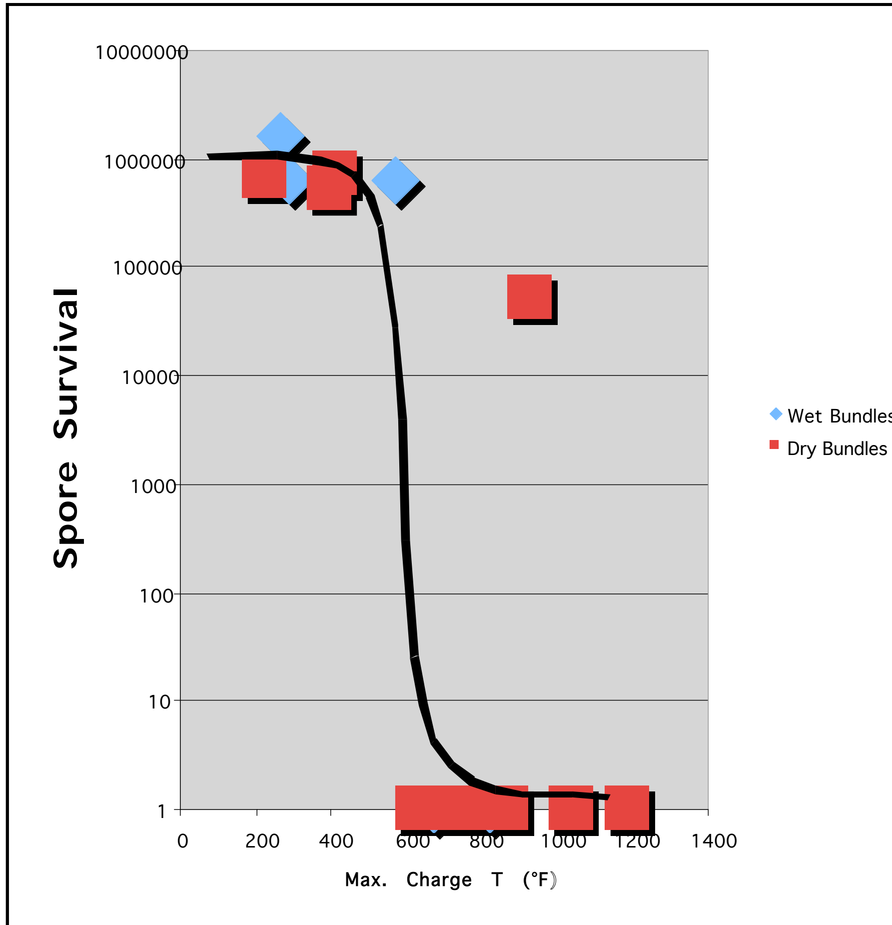


CONCLUSIONS/SUMMARY

A series of tests was performed at a pilot scale incinerator with the following objectives: determine thermal destruction of *B. Anthracis* surrogate spores in carpet bundles by measuring their levels in the exhaust gas; evaluate the bacterial spore emissions sampling

trains used for this measurement; evaluate thermal destruction of spores in carpet and ceiling tile bundles as a function of time and temperature, using BI strips; and lastly, evaluate any potential air pollution impacts associated with the thermal treatment of the building materials. The results described herein mostly have implications for incinerator owners and operators that may have to deal with BDR, by providing some technical

Figure 3. Ceiling Tile Bundle Spore Survival as a Function of Internal Temperature



background and guidance regarding what might be required to ensure complete destruction of biological agents and the potential impacts on air emissions.

No spores were detected via any of the three sampling trains in the exhaust gas for the carpet burn tests conducted in July and August 2005. The detection limit was determined to be 22 spores/dry standard ft^3, corresponding to a maximum potential number of spores emitted equal to 120,000, and a minimum destruction efficiency of 2.8 LR. Additional research is needed to lower the detection limit for the three sampling trains. Combustion of the nylon-6 carpet resulted in increased NO_x emissions, with short-term spikes in emissions from approximately 30 ppm baseline to roughly 150 ppm with each carpet bundle charged.

The use of BI strips to quantify thermal destruction of spores embedded within carpet and ceiling tile bundles was successful. BI strips are normally only used in a qualitative manner, i.e., to indicate whether complete destruction of spores was obtained or not. But this novel, quantitative approach was useful in determining the time window when thermal destruction begins and when complete destruction is obtained, as a function of the building material, and whether the material is wet or dry. Spores embedded within wet ceiling tile bundles took at least 35 minutes to completely destroy, possibly exceeding typical incinerator solid waste residence times. The charging of ceiling tile bundles had minimal impact on the air pollutant emissions studied.

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