Pilot-scale Experimental and Theoretical Investigations into the Thermal Destruction of a *Bacillus anthracis* Surrogate Embedded in Building Decontamination Residue Bundles

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Abstract

Bacillus anthracis (*B. anthracis*) spores were released through the US mail system in 2001, highlighting the need to develop efficacious methods of decontaminating and disposing of materials contaminated with biological agents. Incineration of building decontamination residue is a disposal option of such material, although the complete inactivation of bacterial spores via this technique is not a certainty. Tests revealed that

under some circumstances, *Geobacillus stearothermophilus* (*G. stearothermophilus;* a surrogate for *B. anthracis*) spores embedded in building materials remained active after 35 minutes in a pilot-scale incinerator, and survived with internal material bundle temperatures reaching over 500 °C. A model was also developed to predict survival of a bacterial spore population undergoing thermal treatment in an incinerator, using the thermal destruction kinetic parameters obtained in a laboratory setting. The results of the pilot-scale incinerator experiments are compared to model predictions to assess the accuracy of the model.

Introduction

Incineration of Spore-Containing Material. In the fall of 2001, *B. anthracis* spores were sent through the US Postal Service to various locations in Florida, New Jersey, New York, and Washington, D.C. Twenty-two cases of anthrax infection (or suspected infection) resulted in five deaths (*1*). The sites contaminated by the *B. anthracis* bacterium underwent decontamination activities to inactivate any residual live spores.

After decontamination activities following an attack with a biological warfare (BW) agent such as *B. anthracis*, 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 viable *B. anthracis* spores to be present on porous materials such as carpet, wallboard, ceiling tiles, and many other materials used during cleanup activities. Indeed,

viable *B. anthracis* spores may be dispersed over wide areas within a building and escape detection (and thus possibly escape decontamination) due to incomplete sampling (*2*) or limitations in sampling methods. Further, clean up levels for BW agents are lacking or are controversial and may allow spores to be present, as infectious dose levels for BW agents are not well understood (3). Regardless, it is likely that much of this BDR will be disposed of in high-temperature incineration facilities, such as medical/pathological waste incinerators, municipal waste combustors, and hazardous waste combustors (*4*).

Although pathogens such as *B. anthracis* spores present in BDR are most likely inactivated at typical incineration temperatures (> 800 °C [1472 °F]), gas-phase residence times (> 2 s), and solid-phase residence times (> 30 minutes), it is possible for some of the pathogens to escape destruction in the incinerator due to bypassing the flame zones, the presence of cold spots, and incomplete penetration of heat through the bed into packed or bundled materials. In fact, in the early 1990's, the US EPA performed testing of hospital waste incinerators by inputting large quantities of *G. stearothermophilus* spores along with the waste into the combustors and measuring the number leaving in the stack emissions and in the incinerator bottom ash. It was observed that in certain cases, only a 3 $\log_{10}$ reduction (LR) in spores was found, in spite of acceptably high operating temperatures and sufficiently long residence times (*5*).

As a result of the 2001 *B. anthracis* incident, the US EPA began an experimental and theoretical research program to investigate issues related to the thermal destruction of contaminated BDR, initially including carpet, ceiling tile, and wallboard as model

materials. Tests are being performed at bench- and pilot-scale, and are being used to model the behavior of bundles of these materials in full-scale incinerators (*6*). This paper describes experiments (primarily performed in a pilot-scale rotary kiln incinerator simulator [RKIS]) to examine the impact that bundle (wet and dry) material, exposure time, incinerator temperature, and internal bundle temperature have on the destruction of *G. stearothermophilus* biological indicator (BI) spore strips, and in particular, characterize the worst-case conditions (i.e., the longest exposure periods in the incinerator, or the highest temperatures) in which spores may survive in an incinerator environment. Another facet of this study was to determine whether an empirically-based mathematical model, widely used in controlled sterilization processes, could be applied to accurately predict the thermal destruction of the BIs in an incinerator environment.

The results described in this paper will be of use to regulatory authorities, incinerator owners/operators, and other decision makers who choose to combust BDR, by providing some technical background and guidance regarding what might be required to ensure/demonstrate complete destruction of BW agents. Understandably, this is a critically important issue for owners and operators of incinerators, due to concern about contamination of their business assets and potential liability (*4*). The work presented here should also make it easier for regulatory authorities to authorize and possibly indemnify such facilities processing BDR. The results from this paper will also be of use for the personnel performing the removal, sizing, and packaging of the BDR at the contaminated site. Finally, although there are currently no US federal standards regarding the destruction of pathogenic bacteria in medical waste incinerators, there are guidelines and

consensus based standards under development at the state level. The work presented herein could be used as additional technical background in further developing these guidelines and standards.

Modeling the Thermal Destruction Kinetics of Spore Populations. Thermal destruction studies of microorganisms may be carried out at the molecular level, or may be conducted on microbial populations under various thermal treatment conditions. For the latter type of study, which is the subject of this research, either a mechanistic or an empirical approach may be used to predict the thermal destruction kinetics of a microbial population. Empirical approaches are more typically used, in which experimental data are gathered under a small set of controlled conditions, and then a mathematical or statistical model is developed from these data and used to predict inactivation under other conditions (*7*).

Numerous mathematical models have been used to describe the thermal destruction of bacterial populations, although the logarithmic or first order function and the Arrhenius model have been the predominant approaches used for disinfection and sterilization applications the past century (*8*). Pflug et al. (*9*) and Joslyn (10) provide excellent overviews of the literature on the theories and models of microbial death and the factors affecting the thermal resistance of bacteria. Generally the Arrhenius approach is used if it is assumed thermal destruction behaves like a chemical reaction. A form of the Arrhenius model, and the model most commonly used today and required by various international standards organizations, is called the Bigelow or z-value model (*9*). This

116 model utilizes the concept of the D-value, F-value, and the z-value. The D-value is
117 decimal reduction time, which is the time required at a given temperature (and other
118 specified conditions) to reduce a microbial population to one tenth of its original
119 population, i.e., to achieve a 90% reduction, or a LR of 1. The F-value is the thermal
120 death time, which is the time required to completely destroy the microbial population at a
121 given temperature (and other specified conditions).
122
123 Thus the F-value for a given temperature is related to the D-value as follows:

$$\text{F-value} = \text{LP}*\text{D-value} \quad (1)$$

125
126 Where LP is the $\log_{10}$ number of the microbial population. The United States
127 Pharmacopeia (USP) requirements for the labeling of BIs require the use of the following
128 equation when determining the F-value of the BI, referred to as "kill time" in the BI
129 industry (*11*).
130

$$\text{F-value} = (\text{labeled D-value}) * (\text{LP of BI} + 4) \quad (2)$$

132
133 The z-value is the temperature change required for the D- or F-value to change by a
134 factor of 10. In the Supporting Information (SI) section of this paper, a figure is provided
135 to illustrate how the D- and z-values are related (12).
136
137 From the relationships described above, thermal destruction processes operated at
138 different temperatures can be compared to each other using the z-value, as follows (*8*):

$$F_1\text{-value} = F_0\text{-value} / 10^{(T_1 - T_0)/z\text{-value}} \quad (3)$$

Where:

F_0-value = thermal death time at temperature T_0 (e.g., the F-value reported by the BI manufacturer)

F_1-value = thermal death time at temperature T_1

Note the F_0-value in Equation 3 may be replaced with any time interval to calculate the equivalent thermal treatment time at a different temperature T_i; see Equation 4.

$$t_i = t_0 / 10^{(T_i - T_0)/z\text{-value}} \quad (4)$$

Where:

t_0 = time interval at temperature T_0

t_i = equivalent thermal treatment time at temperature T_i

Equation 4 may be used to analyze thermal treatment processes with *variable* temperature profiles by calculating t_i for each time step (with t_0 in Equation 4 set equal to the interval of the time step) of the thermal process, and summing these (Equation 5) to obtain an equivalent F-value (F_p) for the overall process (13).

$$\sum_{i=1}^{n} t_i = F_p \quad (5)$$

Where:

n = number of time steps in the treatment process

F_p = equivalent thermal processing time had the process been held constant at T_0

This integration approach allows one to calculate an equivalent exposure period of the BI, referenced to the same constant temperature (T_0) that the BIs were subjected to under laboratory conditions. In other words, the thermobacteriological kinetic data (D_0-, F_0-, and z-values) gathered from controlled laboratory experiments performed at a constant temperature may be used to predict survivability of a microbial population exposed to a variable time/temperature profile such as the interior of a bundle of BDR in an incinerator.

Biological Indicator Thermal Destruction Kinetic Data. For the RKIS experiments described in this paper, two different batches of BIs with spores of *G. stearothermophilus* (surrogate for *B. anthracis*) were used. The population data, as well as the D-, z-, and F-values for the BIs used in the RKIS tests, may be found in the SI. A third batch of *G. stearothermophilus* BIs were tested separately under dry heat conditions at 175 °C to obtain the kinetic parameters used in the modeling. For these BIs, the F-value was 3.15 minutes and the z-value was 63.3 ºC; refer also to the SI.

Mechanisms for the Thermal Destruction of Bacterial Spores. The mechanisms associated with the thermal destruction of bacterial spores depend on whether the heat is "wet" (air is saturated with water vapor, i.e., steam sterilization) or "dry" (relative humidity of the air is less than 100%). A discussion and review of literature (*10, 14 - 20*)

184 on the dry and wet thermal destruction mechanisms of bacterial spore populations are
185 found in the SI.
186
187 **Experimental**
188
189 **Apparatus.** The pilot-scale tests described herein were performed using US EPA's
190 RKIS, which is located at EPA's campus in Research Triangle Park, North Carolina.
191 Further details of the RKIS and its operation during testing are found elsewhere (21) and
192 in the SI. Table 1provides an overview of the experimental test program.
193
194 **Table 1. Overview of Pilot-Scale Test Program**

BI Spore Specie and Material Bundle Tested	**Number of Wet Bundle Runs**	**Number of Dry Bundle Runs**
G. stearothermophilus in carpet	13	22
G. stearothermophilus in ceiling tile, mid-range kiln gas temperature	8	13
G. stearothermophilus in ceiling tile, high kiln gas temperature	20	16
G. stearothermophilus in wallboard	4	5

195
196 **Methods: Tests with *G. stearothermophilus* BIs Embedded in Various Simulated**
197 **BDR Bundles** The bundles consisted of pieces of building material that were 7.62 cm (3
198 inches) wide by 7.62 cm (3 inches) high and approximately 1.91 cm (¾ inch) thick, that

were stacked and held together using a titanium or stainless steel cage, such that the length of the bundle was 27.9 cm (11 inches) long. (No information is available regarding the actual BDR bundle sizes that resulted from the decontamination of the buildings that were contaminated following the 2001 "anthrax" attacks. However, it is expected that actual bundle sizes would have been larger than the size that was chosen for this study, in an attempt to minimize handling of such material.) A small metal pipe was embedded inside the bundle, and 2 *G. stearothermophilus* BI spore strips were placed inside the metal pipe; a Type K thermocouple (to measure internal bundle temperature) was also inserted into the pipe. The internal pipe and kiln gas temperatures, along with other kiln operating variables, were recorded by a data acquisition system approximately once every second. Additional details of this technique for measuring the internal BDR temperature are described elsewhere (*22*). Some of the bundles were soaked in water and then fed to the kiln (wet bundles in Table 1), and others were fed dry; in either case, each bundle was weighed prior to feeding to the kiln.

The majority of the tests for all the materials were conducted at mid-range kiln gas exit temperatures (approximately 824 °C), but a second set of tests for the ceiling tile bundles was conducted at a higher temperature (approximately 1093 °C). The bundles were 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 cage/bundle. After removal from the kiln, the bundles were quenched in water, and the pipes were removed. The BIs were then removed from the pipes aseptically and analyzed using the microbiological techniques described in more detail below.

Methods: Spore viability. For these tests, two *G. stearothermophilus* (ATCC 7953) BI spore strips (Raven Biological Laboratories, Inc, Omaha, NE) were placed in the pipe enclosure.

Qualitative tests: After completion of each test run, *G. stearothermophilus* 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) (mechanical convection incubator) for 7 days as suggested by the manufacturer. Development of turbidity during the 7-day incubation period was scored as positive; absence of growth (no turbidity) was scored negative. These procedures were recommended by the BI manufacturer.

Quantitative tests: The spore population of the second BI was quantified by placing the spore strip in a sterile bag with 99ml of sterile water to prepare a 1/100 dilution (w/v) and homogenized in a Nasco masticator blender at 10 beats per second. The homogenate was then diluted as needed to achieve reliable counts/plate (30 – 300 colonies/plate). Dilutions were plated in triplicates on trypticase soy agar (TSA) plates and incubated in a mechanical convection incubator (Precision –Model 6LM) at 55°C (131 °F) ± 2 for 24 hours. Positive controls (*G. stearothermophilus* BI not subject to thermal treatment) were analyzed quantitatively, as previously described, for 12 of the 18 days of testing; geometric mean population = 2.0 E6. The spore population for both the heat-treated spore strip and the positive control was determined by colony-forming units (CFU) (23).

$$LR = \log_{10}(c) - \log_{10}(t) \quad (6)$$

where

(c) = geometric mean of the populations for the two batches of *G. stearothermophilus* BIs used in testing

= 2.5 E6

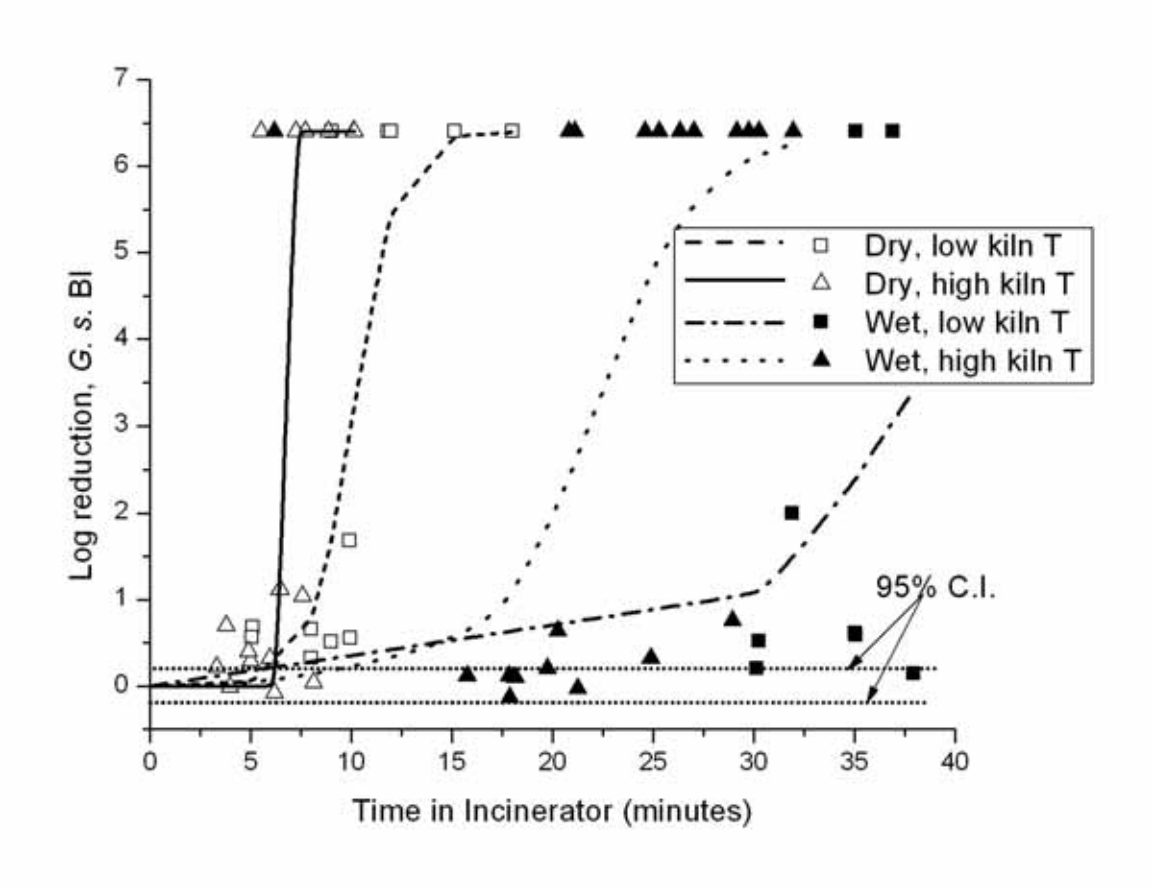
t = CFU of heat-treated spore strip

Modeling Approach. The model's accuracy was assessed by comparing the F-value provided by the BI manufacturer to the calculated F_p (see Equation 5) for that test run. A calculated F_p that is greater than F-value provided by the manufacturer would imply that the spores on the BI should not survive that particular test run. This prediction of BI survival was then compared to the actual BI survival result.

Using Equation 4 and a spreadsheet, t_i was calculated for each time step (t_0 =15 seconds), using the *G. stearothermophilus* BI dry heat kinetic destruction data: T_0 = 175 °C and z = 63.3 °C. (This time interval was chosen after sensitivity analyses indicated a 1-second time increment had minimal (0.3 % difference) impact on the model result.) The variable T_1 was the average temperature reading of the thermocouple inside the pipe enclosure for each 15-second time step. The F_p of each test run was then determined by summing up

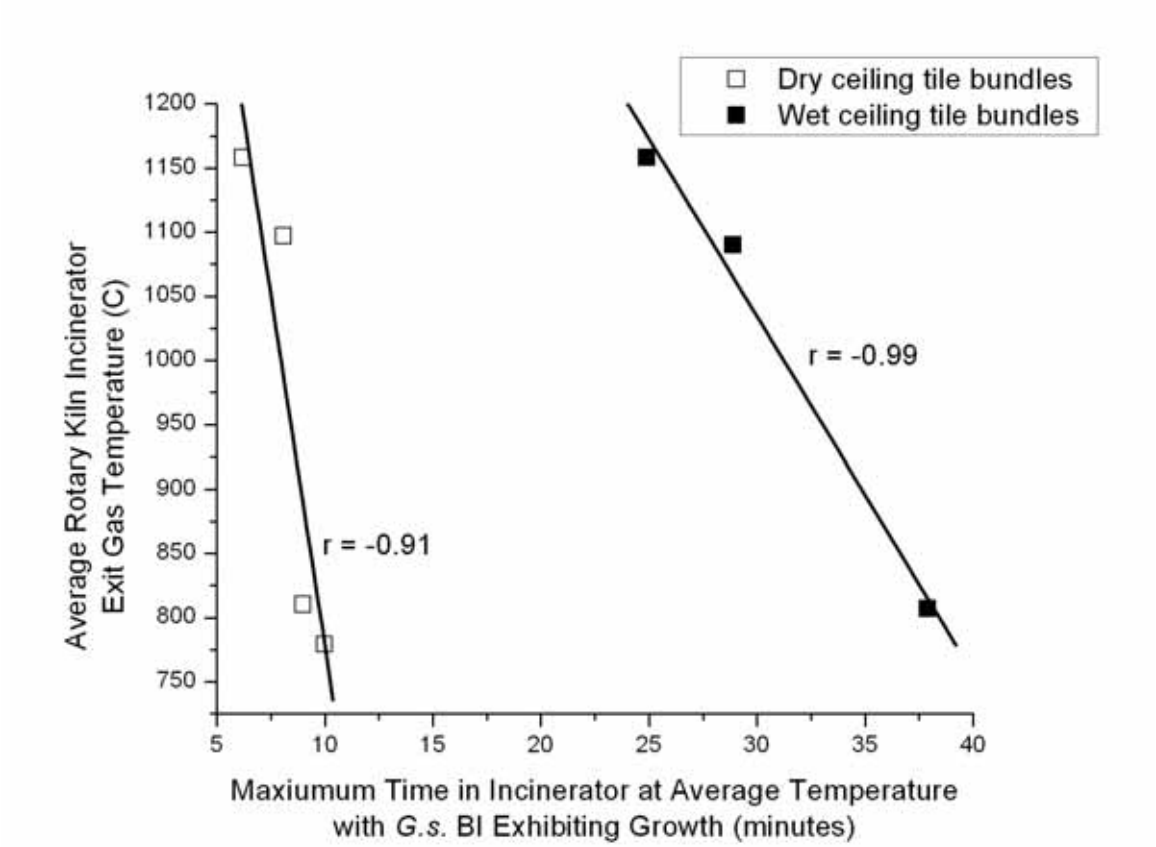
267 the individual t_i values calculated for each time step for that test run. Some example
268 model calculations are found in the SI.
269
270 **Results and Discussion**
271
272 **Effects of Experimental Parameters on the Thermal Resistance of *G.***
273 ***stearothermophilus.*** The results of the RKIS experiments, illustrating the impact of the
274 dry and wet building material bundle, and the exposure time in the RKIS, on the LR of
275 the *G.s* BI embedded in the bundle, are shown in Figures 1 – 3 and in the SI. For the BIs
276 that exhibited no growth following thermal treatment, they were assigned a LR value of
277 6.4 (using Equation 6) and assuming a value of 1 for the variable "t". The 95%
278 confidence intervals for the positive control BI data, plotted in terms of LR with the mean
279 at zero, are also shown in these figures. Also in Figures 1 and 3-4, the data are fitted with
280 sigmoidal curve functions to assess trends; further details on this analysis and results are
281 found in the SI.
282
283 The LR data for the ceiling tile tests are shown in Figure 1, and these data are reduced in
284 Figure 2 to indicate the worst-case conditions (maximum exposure period for a given
285 incinerator temperature) in which the BIs are still not completely inactivated. In one test
286 with a wet bundle, the spores survived a 38 minute exposure in the RKIS. It is noted that
287 the solid-phase residence time for some incinerators may be less than 30 minutes, thus
288 these data show the possibility that in some circumstances, bacterial spores may survive
289 in an incinerator environment. The wet ceiling tiles offer the most thermal resistance of

290 all the conditions tested, due to the refractory materials used to produce the tiles, as well
291 as the large amounts of water the bundles can hold. As expected and as shown in Figures
292 1 and 2, BIs embedded in wet bundles and exposed to lower furnace temperatures require
293 longer exposure periods (approximately 30 additional minutes, compared to the dry
294 bundles at high furnace temperatures) for complete inactivation.



295

296 **Figure 1. Log reduction of *G. stearothermophilus* BIs in ceiling tile bundles (wet or**
297 **dry) vs. time in RKIS, with sigmoidal curve fit of data, and 95% confidence interval**
298 **of the positive controls centered around LR = 0 . High kiln temperatures were**
299 **above 1093 ºC; low kiln temperatures < 824 ºC.**



300

301 **Figure 2. Maximum time in RKIS in which *G. stearothermophilus* BI survived, as a**
302 **function of average furnace gas temperature, for wet and dry ceiling tile bundles,**
303 **with linear fit of the data**

304

305 As shown in Figure 3, the longest exposure period in which spores survived in the
306 wallboard bundles (wet) was 27 minutes, and 3 out of the 5 bundles exposed longer than
307 25 minutes had BIs that were not inactivated. A similar plot of the carpet bundle LR data
308 is found in the SI, where it can be seen that carpet bundles offer the least thermal
309 resistance, in which the maximum exposure time that a BI was not completely inactivated
310 was only 9 minutes.

311

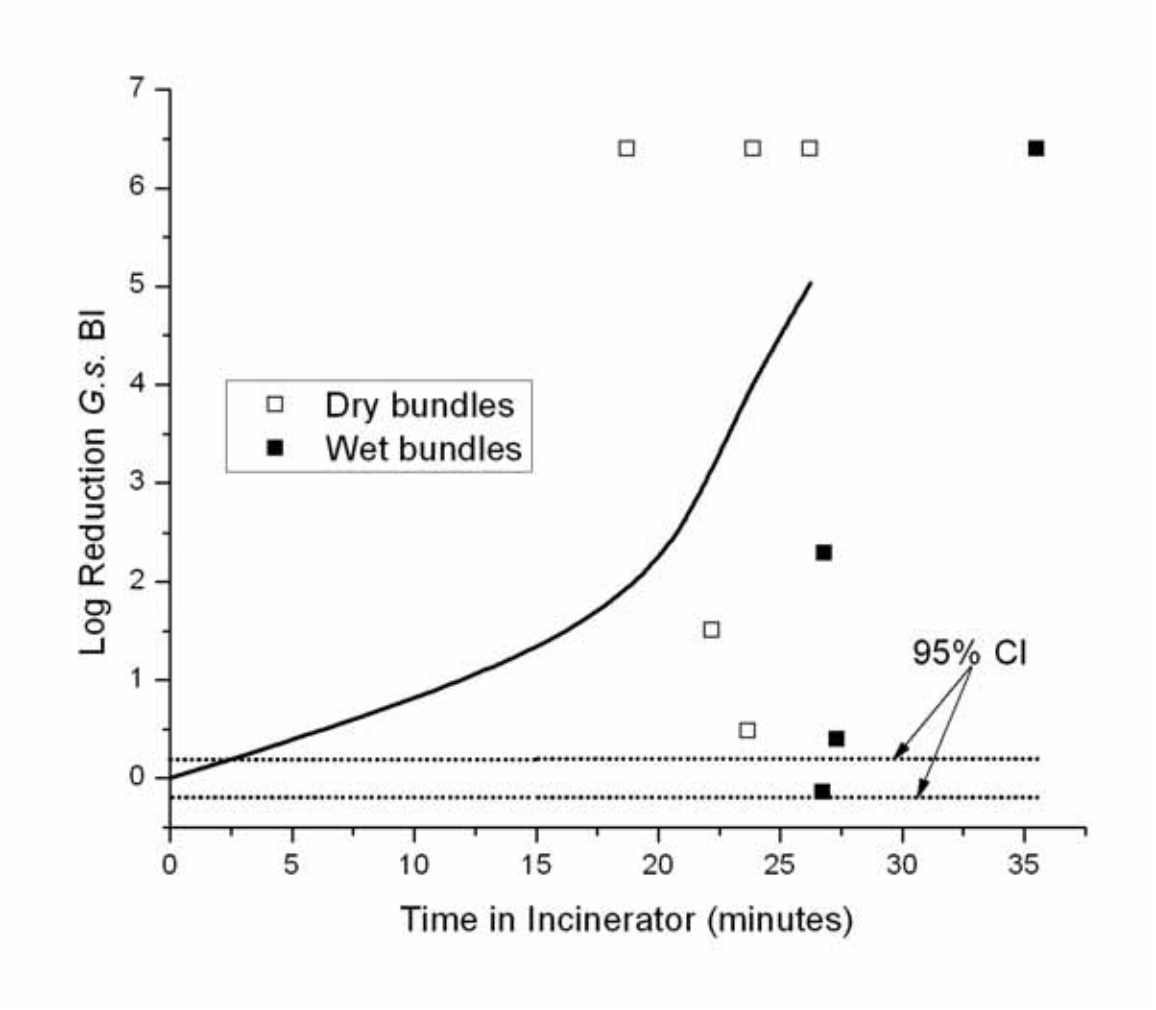


Figure 3. Log reduction of G. stearothermophilus BIs in Wallboard Bundles vs. Time in Incinerator (Average kiln gas temperatures ranged from 773 to 864 °C [1423 to 1588 °F]), with sigmoidal curve fit of combined wet and dry data, and 95% confidence interval of the positive controls centered on a LR = 0

Another approach to statistically characterize or visualize these results is with a histogram, indicating the percent of BIs surviving as a function of exposure period; see the SI for a histogram of the carpet LR results as an example. Trends may also be assessed using the x-half statistic, which was determined with the sigmoidal curve fits,

and represents the time (see Figures 1 and 3) or internal bundle temperature (Figure 4) corresponding to half the full y-axis value, i.e., a LR = 3.2. These x-half data are summarized in the SI.

Additionally, although the maximum exposure periods in which BIs remain active has been discussed, it is worth noting as well the minimum exposure periods in which the BIs were inactivated. For the BIs embedded in wet ceiling tile bundles, the minimum exposure period in which a BI was inactivated was 6 minutes. This relatively wide range in exposure periods in which the BIs become inactivated is not unexpected, and is most likely due the variability in the kiln gas temperature (refer to Figure 2 and the SI regarding kiln temperatures). However, other possible reasons include the inherent thermal resistance variability of the *G. stearothermophilus* BI and environmental factors such as the variability of gas temperature within the kiln (the bundles were not all placed at the exact same location within the kiln), variable bundle moisture content, and variable physical characteristics of the bundle affecting heat transfer to the BI. These latter three variables impact the internal bundle temperature (the actual temperature the BI is exposed to), which is discussed below.

The *G. stearothermophilus* BI log reduction results data, plotted as a function of the maximum internal bundle temperature reached during each test, are shown in Figure 4. This analysis shows that except for three ceiling tile tests, no *G. stearothermophilus* spores survive beyond 315 °C (600 °F), regardless of bundle material or exposure time in incinerator. After closer inspection of the data, no technical reasons could be found to

disregard the three *G. stearothermophilus* BIs surviving up to between 425 and 565 °C (800 and 1050 °F). These results point to the probabilistic nature of the BIs, i.e., that although 116 out of 119 (97%) BIs did not survive internal bundle temperatures beyond 315 °C, 0.8 % of the BIs survived exposure up to approximately 540 °C. The x-half value for the data in Figure 4 is 246 ºC. For further analysis and visualization of the results, a histogram of these data is found in the SI.

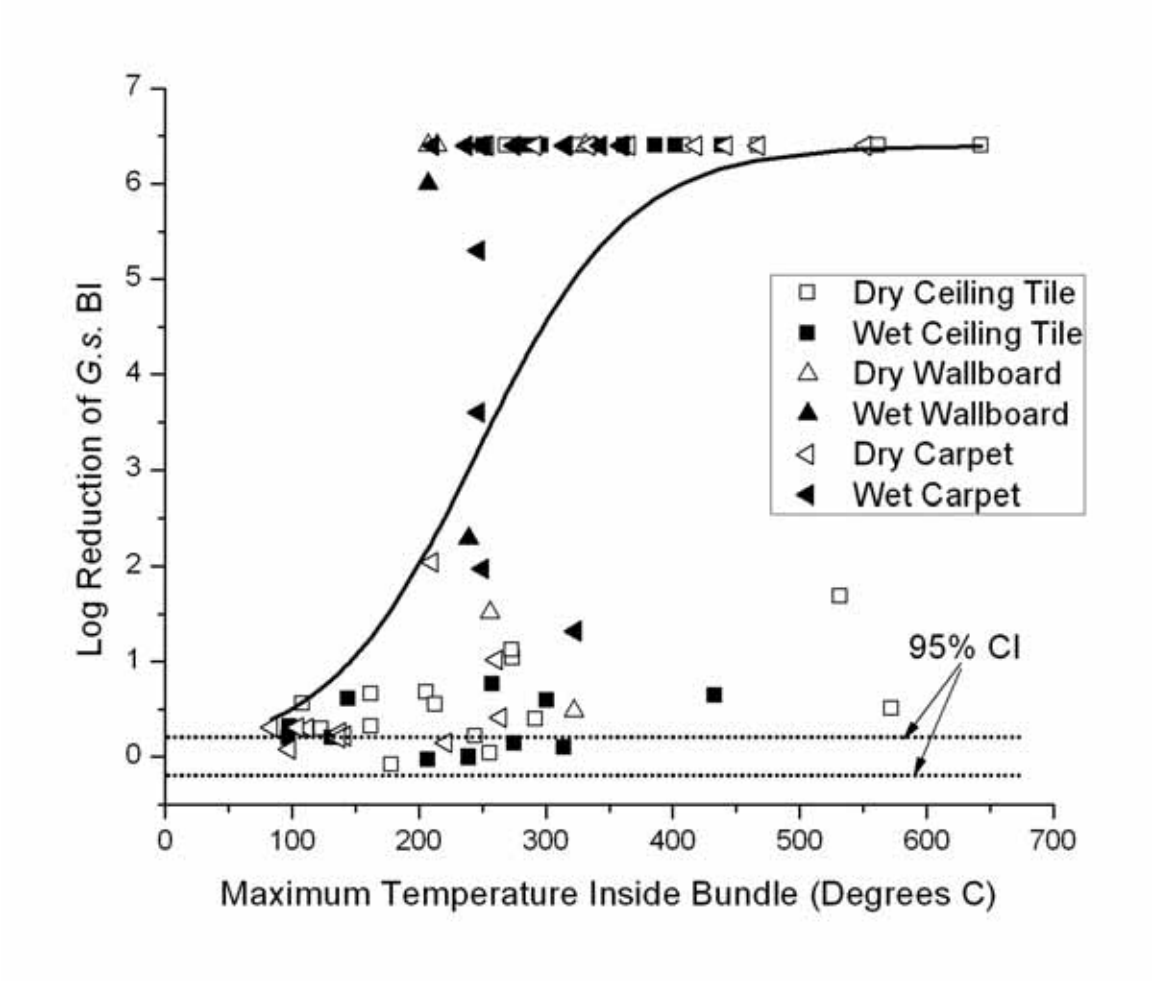
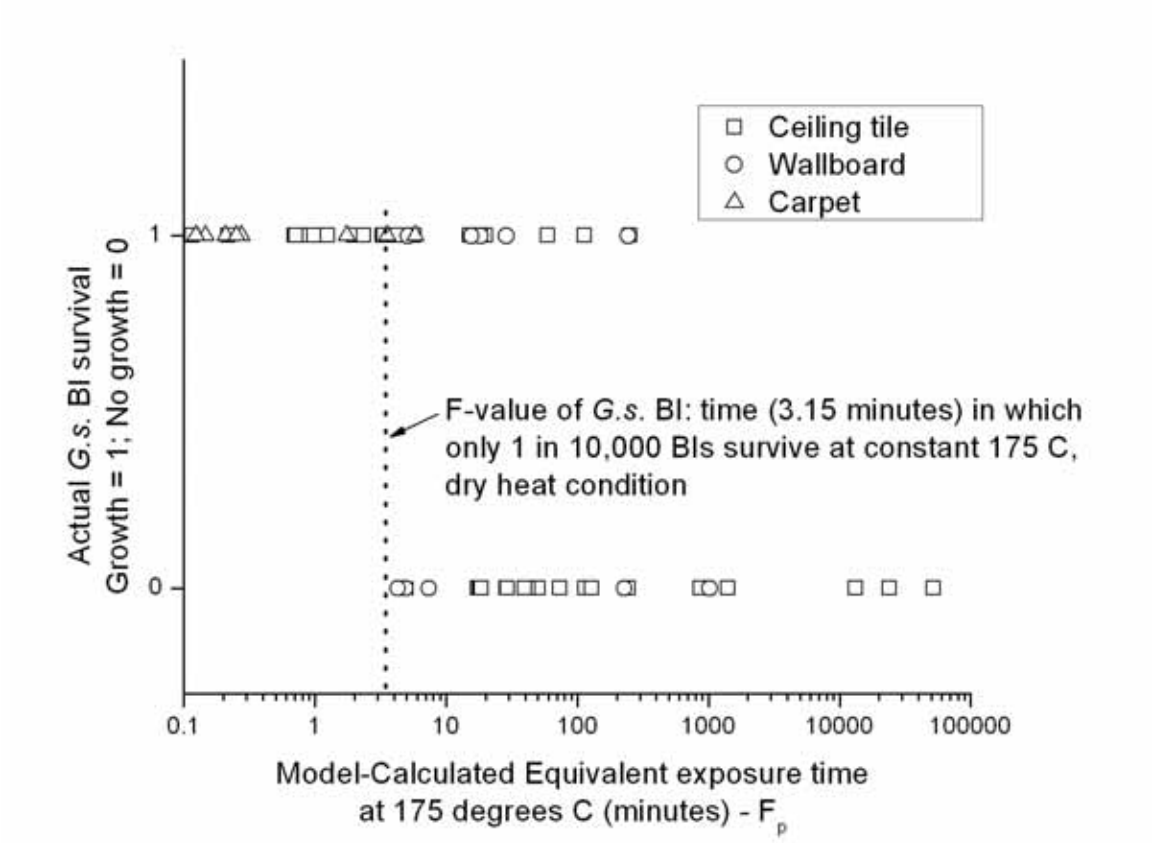


Figure 4. Log reduction of *G. stearothermophilus* BI vs. maximum temperature in material bundle, with sigmoidal curve fit of the combined data, and 95% confidence interval of the positive controls centered around LR = 0.

Comparison of Model-Predicted and Actual BI Survival. In Figure 5, the *G. stearothermophilus* BI results are plotted qualitatively ("growth" or "no growth") versus the model-determined, calculated F_p for that test run. Note that in all cases where the calculated F_p for a particular test run was less than the actual F-value (for the *G. stearothermophilus* BIs used, the F-value, or F_0, was 3.15 minutes at dry heat conditions), the model predicts that the BI would show growth, and in fact, all the *G. stearothermophilus* BIs did show growth. However, there were numerous (44 %) test runs in which the calculated F_p was greater than the actual F-value (i.e., model predicts BI would not show growth), but the BI in fact did show growth. In other words, the *G. stearothermophilus* BIs are more thermally resistant than what would be predicted by this theoretical approach. In a final observation, none of the *G. stearothermophilus* BIs showed growth when subjected to a thermal treatment process with a calculated F_p greater than 300 minutes.



371

372 **Figure 5. Actual *G. stearothermophilus* BI survival vs. equivalent exposure time**

373 **(model-calculated F_p) in RKIS.**

374

375 Because the BIs are more thermally resistant than predicted by the model, this implies

376 that a much larger z-value is needed to fit the data, or a z-value that increases with an

377 increase in temperature. The Bigelow model used in this study assumes a constant z-

378 value, while a z-value that increases with temperature is an Arrhenius model (*9*). This

379 coincides with Denison et al., who have noted that although the kinetics represented by

380 the z-value approach closely match the kinetics represented by an Arrhenius-type

381 approach at the temperatures where the D and z-value were determined, the two

382 approaches deviate at temperatures in excess of 200 °C, so it is possible that the kinetics

383 of microbe destruction at higher temperatures may be better represented by an Arrhenius
384 approach (*6*). The temperatures the BIs were exposed to in this study were variable and
385 indeed exceeded the temperature range in which the z-value was determined.
386 Bliem et al. corroborate that the z-value may vary with temperature, and in particular,
387 have presented data from the literature showing the z-values for *G. stearothermophilus* to
388 increase with increasing temperature (*8*). In contrast, however, Pflug et al. presented data
389 indicating a decrease in the z-value with increasing temperature (*9*). Further research is
390 needed to investigate the use of BI z-values that increase with increasing temperature,
391 and to develop an Arrhenius model to fit the empirical data. Probabilistic modeling
392 approaches may also be warranted.
393
394 Overall, it appears that *G. stearothermophilus* spore populations may survive longer in
395 incineration environments (in one case, 38 minutes), and at higher temperatures (in a few
396 cases, over 500 ºC), than is expected based on a standard kinetic model used in
397 sterilization applications. Additionally, the use of thermobacteriological concepts to
398 assess incinerator performance while processing building materials containing embedded
399 spores is a promising technique, but so far it has been more valuable at identifying
400 necessary conditions to achieve spore destruction, as opposed to proving sufficient
401 conditions to assure spore destruction. This experimental and theoretical effort provides
402 useful information to support decision-making activity concerning the disposal of wastes
403 resulting from cleanup of a facility contaminated with biological agents.
404
405 **Acknowledgments**

406 The authors would like to thank Richie Perry, Jared Novak, and Chris Pressley for their
407 professional work and technical support in the laboratories used in this study.
408

409 **Supporting Information Available**

410 Figure illustrating how the D- and z-value are related; example using Equation 3; details
411 on the RKIS and its operation during testing; BI kinetic data; review of spore thermal
412 destruction mechanisms; example histograms for some of the results; LR results for
413 carpet bundles; sigmoidal curve fits and associated coefficients of resulting curves;
414 example model parameter data and outputs for one test run. This information is available
415 free of charge via the Internet at http://pubs.acs.org.
416

417 **Literature Cited**

418 1. Inglesby, T. V.; O'Toole, T.; Henderson, D.A.; Bartlett, J. G.; Ascher, M. S.;
419 Eitzen E.; Friedlander, A. M.; Gerberding, J.; Hauer, J.; Hughes, J., et al. Anthrax
420 as a biological weapon. *Journal of the American Medical Association* **2002**, 287
421 (17), 2236-2252.
422 2. National Research Council of the National Academies, Committee on Standards
423 and Policies for Decontaminating Public Facilities. *Reopening public facilities*
424 *after a biological attack: a decision making framework.* The National
425 Academies Press, Washington, D.C. 2005.
426 3. Fitch, J. P., Raber, E., Imbro, D.R. Technology challenges in responding to
427 biological or chemical attacks in the civilian sector. *Science* **2003,** 302, 1350-
428 1354.

4. Wilhelmi, J., Kremer, F., and Gallardo, V. Report on the homeland security workshop on transport and disposal of wastes from facilities contaminated with chemical or biological agents. United States Environmental Protection Agency Report 600/R-04/065. November, 2003.
5. Wood, J., Lemieux, P., and Lee, C.W. Destruction efficiency of microbiological organisms in medical waste incinerators: a review of available data. *Proceedings from the International Conference on Incineration and Thermal Treatment Technologies*, Phoenix, AZ, May 10-14, 2004.
6. Denison, M., Montgomery, C., Zhao, W., Bockelie, M., Sarofim, A. and Lemieux, P. Advanced modeling of incineration of building decontamination residue. *Proceedings from the Air and Waste Management Association's 98th Annual Conference & Exhibition*, Minneapolis, MN, June 21-24, 2005.
7. Weavers, L. K. and Wickramanayake, G. B. Kinetics of the inactivation of microorganisms. In *Disinfection, Sterilization, and Preservation,* 5th Edition; Block, S., Ed.; Lippincott Williams & Wilkins: Philadelphia, PA, 2000; 65 - 78.
8. Bliem, R.F., and Nowak, W.G. Assessment of the inaccuracy inherent in the F_0 and A_0 concepts of microbial inactivation. *Pharmazeutische Industrie* **2005**, 67 (7), 819-822.
9. Pflug, I. J., Holcomb, R. G., and Gomez, M. M. Principles of the thermal destruction of microorganisms. In *Disinfection, Sterilization, and Preservation,* 5th Edition; Block, S., Ed.; Lippincott Williams & Wilkins: Philadelphia, PA, 2000, 79 - 124.

10. Joslyn, L. J. Sterilization by heat. In *Disinfection, Sterilization, and Preservation,* 5th Edition; Block, S., Ed.; Lippincott Williams & Wilkins: Philadelphia, PA, 2000, 695 - 728.
11. United States Pharmacopeia. Biological indicator for dry-heat sterilization, paper carrier. USP 29 Biological / Official Monographs, 280-281. Available at http://www.usp.org.
12. Raven Biological Laboratories, Inc. Certificate of analysis for biological indicators, *G. stearothermophilus* spore strips, Lot 3167961, Batch 796S. May 1, 2006.
13. Earle, R.L., and Earle, M.D. In *Fundamentals of Food Reaction Technology*. Leatherhead Publishing: Surrey, UK, 2003, 73-95.
14. Russell, A. D. Principles of antimicrobial activity and resistance. In *Disinfection, Sterilization, and Preservation,* 5th Edition; Block, S., Ed.; Lippincott Williams & Wilkins: Philadelphia, PA, 2000, 31 - 55.
15. Rank, E. and Pflug, I. J. Dry heat destruction of spores on metal surfaces and on potatoes during baking. *Journal of Food Protection* **1977,** 40 (9), 608 – 613.
16. Brannen, J. P. and Garst, D. M. Dry heat inactivation of *Bacillus subtilis* var. *niger* spores as a function of relative humidity. *Applied Microbiology* **1972**, 23 (6), 1125 – 1130.
17. Peeler, J. T., Reyes, A. L., Crawford, R. G., Wehby, A. J., and Campbell, J. E. Thermal resistance of *Bacillus subtilis* var. *niger* in a closed system. *Applied and Environmental Microbiology* **1977**, 33 (1), 52-58.

18. Raven Biological Laboratories, Inc. Report for dry heat D-value and z-value determination on *G. stearothermophilus* spore strips. September 6, 2006.
19. United States Pharmacopeia. Biological indicators – resistance performance tests. USP 29, Microbiological Tests / (55), 2501 - 2502. Available at http://www.usp.org.
20. Electric Power Research Institute. *Technical Assistance Manual: State regulatory oversight of medical waste treatment technologies: A report of the State and Territorial Association on Alternative Treatment Technologies (STAATT).* EPRI, Palo Alto, CA: 1998. TR-112222.
21. Lemieux, P., Stuart, E.S., and Ryan, J. V. Pilot-scale studies on the effect of bromine addition on the emissions of chlorinated organic combustion by-products. *Waste Management* **2002**, 22, 381–389.
22. Lemieux, P. Pilot-scale combustion of building decontamination residue. *Proceedings from the Air and Waste Management Association 98th Annual Meeting,* Minneapolis, MN. June 21-24, 2005.
23. Koch, A.L. Growth measurements. In *Methods for General and Molecular Bacteriology;* Gerhardt, P., Murray, R.G.E., Wood, W.A., Krieg, N.R., editors. Washington, D.C. 1994. ASM P248-277.
24. DeVries, T.A. and Hamilton, M. A. Estimating the microbial log reduction. Part 1. Quantitative assays. Quantitative Microbiology **1999**, 1, 29 – 45.

Table of Contents Brief:

Under certain conditions, *G. stearothermophilus* bacterial spore populations may survive longer in incineration environments, and at higher temperatures, than expected.