Study of Thermal Destruction of *Bacillus anthracis'* Surrogates Spiked on Building Materials

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ABSTRACT

A significant amount of contaminated material may need to be disposed of after a bio-terrorism attack. The efficacy of disposal of building materials contaminated with biological agents by incineration is complicated by matrix effects associated with the contaminant and the material it is on. It is important to know the relative difficulty of destroying these biological agents bound to different materials. This project examines the thermal destruction of surrogate biological agents that are present on several common building materials including ceiling tiles and wallboard. A laboratory-scale thermal reactor was constructed to examine the effect of building material, heating temperature and residence time on the destruction of *Bacillus anthracis'* surrogates. Results of the study showed that the destruction of the *Bacillus anthracis* surrogates, *Bacillus subtilis* and *Geobacillus stearothermophilus* spiked on building materials strongly depends on the type of material and time-temperature history of the simulants in the reactor.

INTRODUCTION

After a biological terrorist attack occurring in a public building, a significant amount of contaminated building material and waste may need to be disposed of. Some of the materials to be disposed of may have already been externally decontaminated. This material could include a porous material such as contaminated wall board, ceiling tile, and carpet. It is possible that much of this material could be disposed of in high temperature thermal incineration facilities, including medical/pathological waste incinerators, municipal waste combustors, or hazardous waste combustors. Portable incineration units might be field erected to dispose of these materials on-site in order to minimize exposure. The disposal of building materials contaminated with harmful biological agents by thermal incineration is complicated by matrix effects, such as the heat transfer rate associated with the material on which the agent is bound. It is important to know the relative difficulty of destroying these biological agents bound on different materials. Selection of appropriate disposal facilities requires fundamental knowledge of the behavior of the matrix-bound

contaminants in various thermal environments. A review of existing data showed viable spores were detected in some of the air emissions and residual ash samples obtained from microbiological survivability tests conducted at several medical waste incinerators (MWIs).[1] Very little is known about the behavior of the likely contaminants bound in these various matrices within incineration facilities, and complete destruction of the contaminants without releasing air emissions of contaminants and contaminated combustion residues from the disposal of these materials is very important.

This project examined the destruction of surrogate biological agents bound on several common building materials including ceiling tiles and wallboard. A laboratory-scale reactor was used in this project to examine building material, heating temperature and residence time affecting the destruction of surrogate biological contaminants including *Bacillus subtilis* and *Geobacillus stearothermophilus*, surrogates for *Bacillus anthracis*. The results from these studies can be used to evaluate incineration technologies for appropriateness for disposal of contaminated building materials.

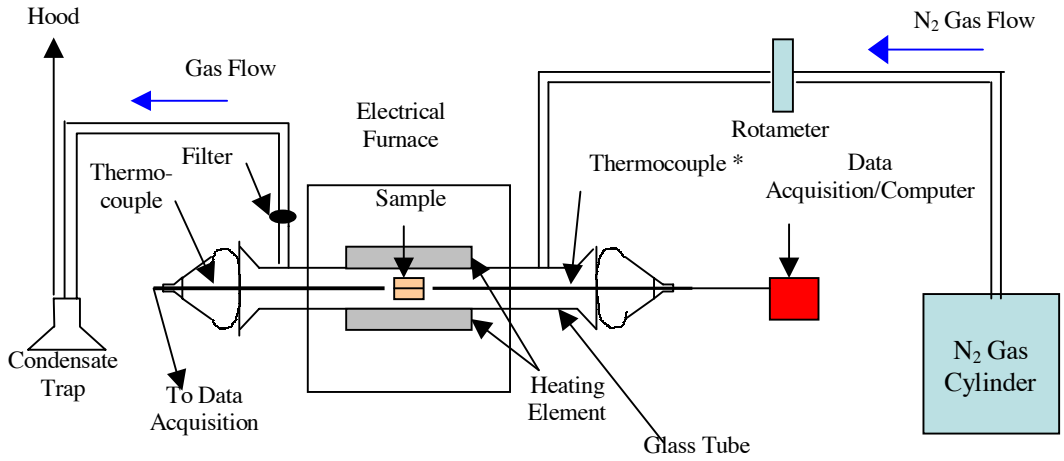
EXPERIMENTAL

The primary objective of this project was to heat different building materials in a bench-scale tube furnace at specified gas temperatures and time periods. The building materials were inoculated with bacteriological spores (surrogates for *Bacillus anthrax*) at a level of $10^4 – 10^6$ spores/cm^2 prior to heating in the furnace, and after heating, the materials were subsequently analyzed to determine survivability of the spores. The overall purpose of the project was to determine the effect that different building materials have on the heat resistance of *Bacillus anthrax* surrogates. A secondary objective of the project is to obtain internal temperature profiles (temperature versus time) of the building materials when subjected to the specified gas temperatures. These temperature profile tests of the building materials were conducted prior to the microbial survivability tests.

Thermal Reactor System

A bench-scale reactor was constructed for studying the destruction of anthrax surrogates bound on different building materials. A schematic of the reactor system is shown in Figure 1. The heating source of the reactor was a Thermcraft electrical furnace, which is 46 cm long and has a 30 cm long heating element. A borosilicate glass tube of 5 cm outside diameter (4 cm inside diameter), and 71 cm long is placed in the tube furnace for heating of the samples. At each end of the glass tube is a ball and socket joint with a 0.6 cm nipple to allow for insertion of thermocouples (T/C). The glass tube also has a 5 cm long, 0.6 cm diameter nipple at each end (perpendicular to the tube) to allow for connection of 0.6 cm Teflon tubing for nitrogen gas flow. All tubing connections in the apparatus use compression type fittings.

Figure 1. Schematic of Experimental Apparatus to Investigate Thermal Destruction of *Bacillus anthracis* Surrogates on Various Building Materials



* For Temperature Profile Test Only

Test Procedures

The building material samples tested included ceiling tile and wallboard (drywall). Each set of the two building material samples was cut from the same source. Each sample consisted of two layers, each of which is 7.6 cm long by 3.8 cm wide by 0.6 cm thick, and stacked together. The building material samples were placed in rectangular aluminum sample pans. Nitrogen (N_2) gas regulated with a rotameter at 500 cm^3/min flowed through the glass tube to prevent combustion of the sample placed inside the tube. A filter (0.22 micron pore size) was placed at the outlet of the glass tube to capture any spores entrained in the nitrogen carrier gas.

For the tests to determine the internal temperature profiles of the materials, a hole was drilled between the two layers of material to allow for insertion of the T/C. The tip of a 0.16 cm diameter T/C was placed between the two layers of building material. The layers were fastened together and the sample was placed in the center of the tube furnace. Temperatures were measured using Omega brand Type E T/C. A 0.3 cm diameter T/C was used to measure the gas temperature within the glass tube. The tip of the T/C was placed in the center of the tube (in terms of height), and just left of the sample. The furnace has its own thermocouple placed at the center of the top heating element and its temperature reading is displayed on the temperature control box. This temperature was also recorded (manually) during the experiments. Once the gas temperature within the glass tube reached the desired temperature, the sample pan with the building material was inserted into the glass tube to the center of the oven and gas and material temperature data were recorded every 5 seconds with an Iotech data acquisition system (DAS).

For the spore survivability tests, no internal material temperatures were measured, although the same materials, gas temperatures and heating procedure were used for the

corresponding temperature profile tests. The spores were placed between the two layers of the material and then fastened together prior to a survivability test. When the specified heating period for a test had been reached, the sample was removed from the furnace and analyzed for the spore survivability.

Prior to each spore survivability test all the components of the furnace glass tube except T/Cs were steam sterilized for 60 minutes at 121 °C and 15 lb/in^2. Due to the heat sensitivity of the T/Cs, they were sanitized with 95% (v/v) ethanol.

Materials and Analytical Methods

Bacterial Strains, Growth Conditions and Preparation of Spore Suspensions

Bacillus anthracis surrogates were used in this study to prepare spore suspensions. These were *Bacillus subtilis* (ATCC 19659) and *Geobacillus stearothermophilus* (ATCC 7953). *Bacillus subtilis* spores were harvested from 72 hr cultures grown on 10% Columbia Broth with 0.1 mM Mn^{+2} (ASTM E 2197) at 35 °C ± 2 °C. After the third centrifugation, the pellet was resuspended in sterile deionized water (1/10 volume of the original culture medium) and placed in a water bath at 80 °C for 10 min to heat-kill vegetative cells.[2] The final spore concentration was approximately 10^8 spores/mL. *Geobacillus stearothermophilus* spores were harvested from 72 hr cultures grown on sporulation broth[3] at 55 °C ± 2 °C using the procedure as previously described. The final spore concentration was approximately 10^6 spores/mL.

Building Materials Preparation and Inoculation

The bulk building materials (BBM) used in this study included ceiling tile and wallboard. Ceiling tiles that had been in use for approximately 1 year in a laboratory were made available due to construction changes. These were Class A, standard-white, fire-retardant, textured-faced ceiling tiles composed of wood fiber (0 – 60%) and fibrous glass (0 – 13%) as specified by the manufacturer. New drywall was used for these tests which consisted of gypsum core (5 mg/m^3) wrapped with a paper lining (cellulose - 5 mg/m^3) as specified by the manufacturer.

All the BBM were cut into sample sizes measuring 7.62 x 3.81 cm, weighed, individually wrapped in aluminum foil and steam sterilized by autoclaving. The sterile BBM were inoculated with either 1.0 ml of *Bacillus subtilis* spores for a final concentration of 10^8 spores/ml or 1.0 ml of *Geobacillus stearothermophilus* spores for a final concentration of 10^6 spores/ml. Once inoculated the BBM pieces were placed on a sterile tray and allowed to dry overnight within a biological safety cabinet. Once dried, the samples were prepared by stacking two (7.62 x 3.81 cm) pieces with the inoculated sides facing each other and then tested in thermal destruction experiments.

Spore Recovery after the Thermal Destruction Tests

Immediately after the thermal destruction tests, the samples were aseptically placed in a sterile jar with cold water for quenching. To recover the spores the sample was transferred to a sterile polyethylene bag and if necessary more sterile water was added to prepare a 1/10 (weight of sample to volume of water, w/v) dilution of the sample. The bag with sample was inserted in a masticator-blender (Nasco Sampling Products, Modesto, CA) and homogenized for 15 sec at 10 beats per sec.[4] The homogenate was then diluted as needed and plated in triplicate on Trypticase Soy Agar (TSA). Unheated, inoculated BBM samples were homogenized at the same time to determine the spore concentration prior to the thermal destruction tests.

For recovery of *Bacillus subtilis* spores, TSA plates were incubated at 35 °C ± 2 °C for 24 hr. For recovery of *Geobacillus stearothermophilus* spores, TSA plates were incubated at 55 °C ± 2 °C for 24 hr. Spore concentration before and after the heating was determined by colony-forming units (CFU, CFU = number of colonies x dilution factor).[5]

Analysis of Heated BBM Samples

Spore survivability was determined by Log10 reduction (LR) using the CFU prior to the heating and the CFU after the heating as in the equation shown below.[6]

$LR = \log10 (c) - \log10 (t)$

Where:
c = CFU of inoculated building material prior to heating
t = CFU of inoculated building materials after heating

RESULTS AND DISCUSSION

Temperature Profiles

The internal temperature profiles of the ceiling tile and wallboard were measured at several gas temperatures (148, 204, 260, and 315 °C) in a constant N_2 flow (500 cc/min). The two building materials showed significantly different temperature profiles under the same heating conditions. Examples of the temperature profiles for the ceiling tile and wallboard heated at 204 °C gas temperature are shown in Figures 2 and 3, respectively. Ceiling tile approached close to the gas temperature of 204 °C in about 6 min (360 sec). However, the wallboard only reached 125 °C after it was heated at the same gas temperature for 15 min (900 sec). It appears that wallboard which is made of gypsum ($CaSO_4 \cdot 2H_2O$), contained high moisture content (20 wt. %), and evaporation of water was observed at the same time when the internal temperature profile of wallboard flattened out. It is likely that the heat provided by the furnace was used to evaporate the moisture contained in the wallboard, not to raise the temperature of the material.

Figure 2. Internal Temperature Profile of Ceiling Tile Measured at 204 °C Initial Gas Temperature

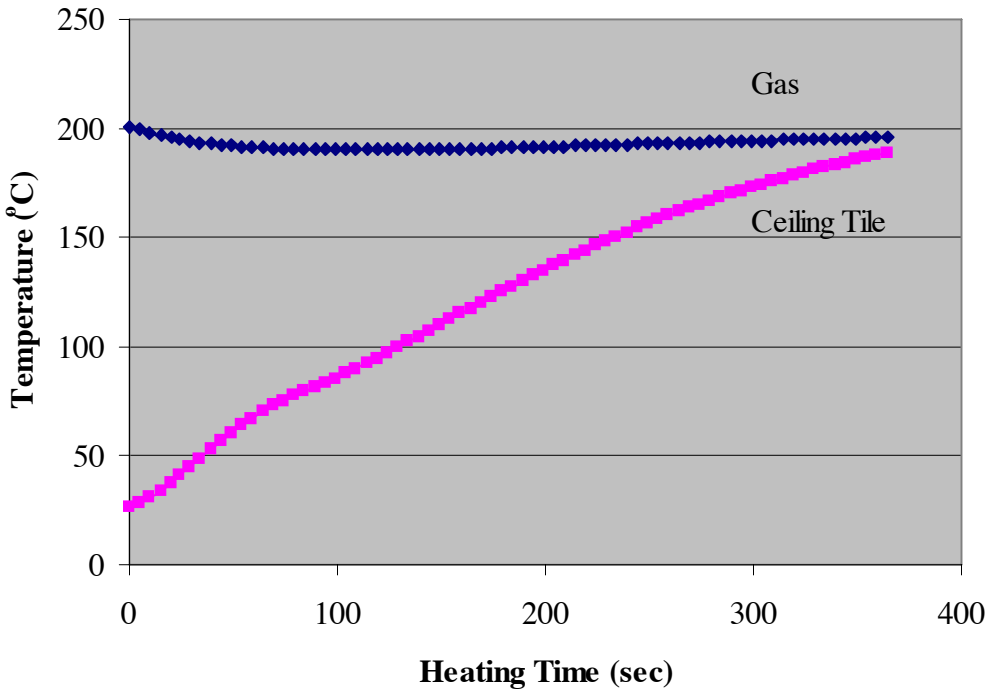
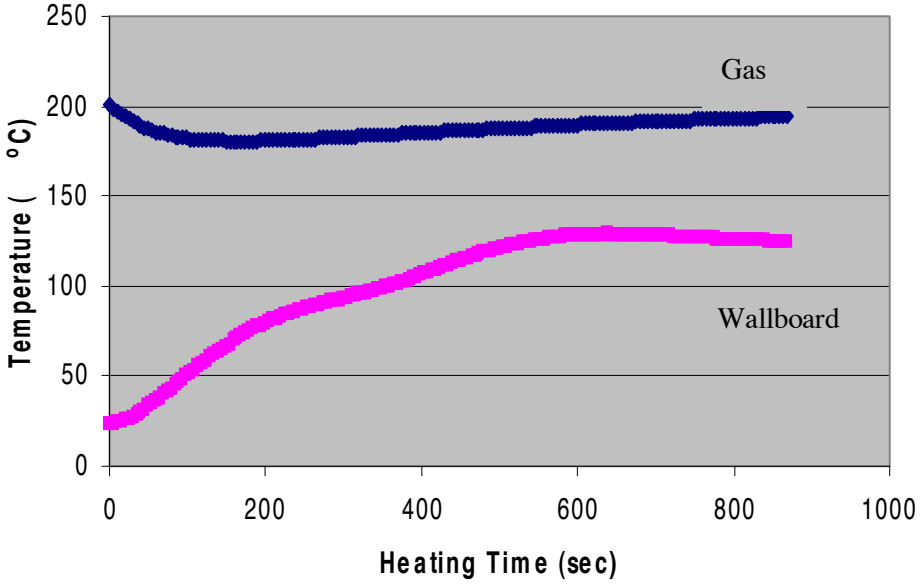


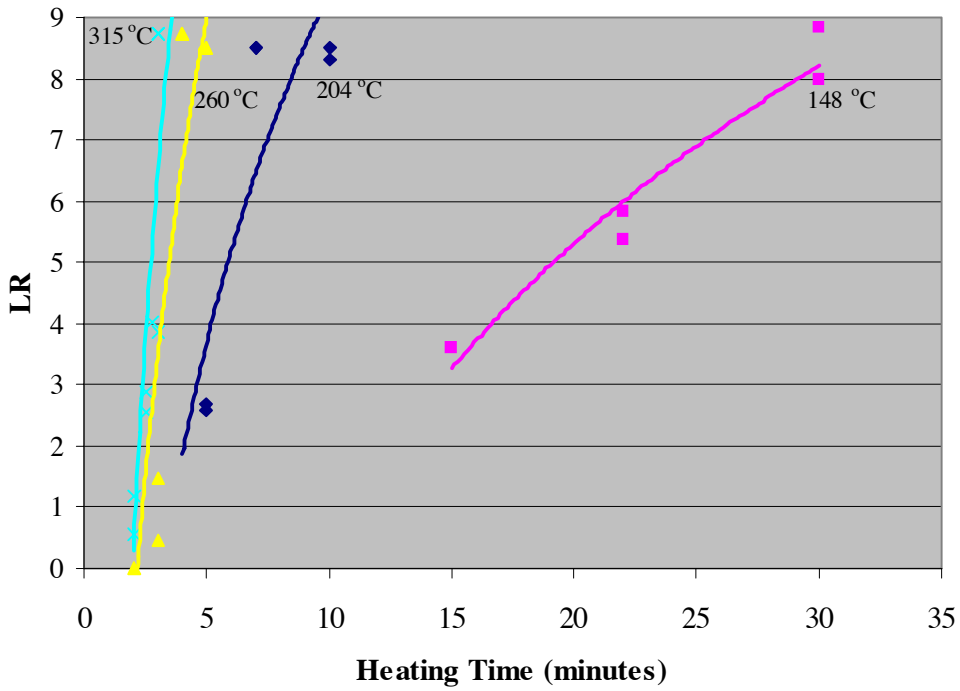
Figure 3. Internal Temperature Profile of Wallboard Measured at 204 °C Initial Gas Temperature



Bacillus anthracis Surrogate Spores Survivability Tests

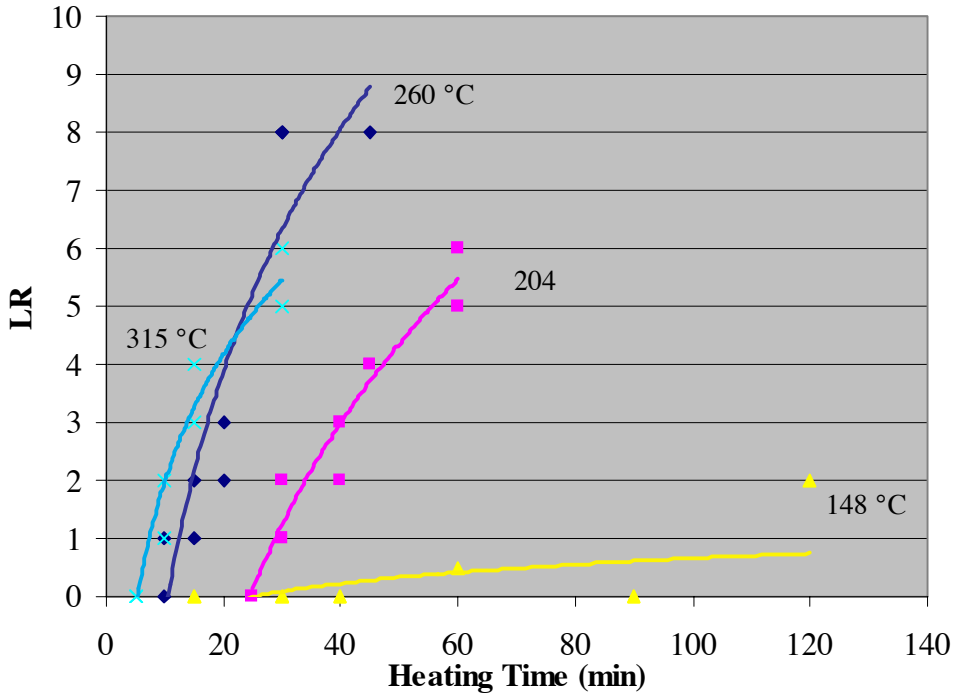
The survivability of *Bacillus subtilis* bound on ceiling tile and wallboard was tested at gas heating temperatures of 148, 204, 260, and 315 °C. Results of the survivability tests for the ceiling tile and wallboard are shown in Figures 4, and 5, respectively. Figure 4 indicates that the destruction (expressed in Log10 reduction, LR) of *Bacillus subtilis* spiked on ceiling tile occurs rapidly when they are heated at gas temperatures above 200 °C. Almost complete destruction (with a LR value of greater than 8) of the ceiling tile bound spores occurs within 2 min of heating at 315 °C. Similar level of destruction occurs within 5 and 10 min when the gas heating temperature is reduced to 260 and 204 °C, respectively. The rate of destruction decreases significantly when the heating temperature is reduced further to 148 °C, and reduction of a LR value of 6 is achieved in about 25 min.

Figure 4. Effect of Heating Temperature and Time on Reduction of *Bacillus subtilis* Spiked on Ceiling Tile



Results shown in Figure 5 indicate that the *Bacillus subtilis* spiked on wallboard have significantly slower destruction rates than those for the ceiling tile bound spores (Figure 4) under the similar gas heating temperatures. Destruction of the wallboard bound spores with the LR value of 6 occurs in about 30 min when the sample is heated at 315 °C gas temperature. Similar high level of destruction occurs at about 60 min of heating when the gas heating temperature is reduced to 204 °C. Very little spore destruction is observed when the heating temperature is reduced further to 146 °C even at extended heating period of over 100 min.

Figure 5. Effects of Heating Temperature and Time on Reduction of *Bacillus subtilis* Spiked on Wallboard



The survivability of *Geobacillus stearothermophilus* spiked on ceiling tile and wallboard was also tested at the similar temperature range for the *Bacillus subtilis*. Results of the survivability tests for the *Geobacillus stearothermophilus* spiked on ceiling tile and wallboard are shown in Figures 6 and 7, respectively. Similar to the *Bacillus subtilis*, the destruction of the *Geobacillus stearothermophilus* spiked on ceiling tile (shown in Figure 6) occurs very fast, and the destruction rate decreases with decreasing heating temperature. Complete destruction of the spores occurs in less than 5 min at heating temperatures of 260 and 315 °C, which increases to about 7 min when the heating temperature is reduced to 204 °C. The similarly fast destruction rates observed for the two different spores spiked on ceiling tile (Figures 4 and 6) suggest that the thermal destruction of the *Bacillus anthracis* surrogate spores bound on a building material depends strongly on the heat transfer characteristics of the material. The high spore destruction rates observed for the two different surrogate spores bound on ceiling tile are consistent with the fast heat transfer rates measured for this building material in the internal temperature profile tests (see Figure 2).

Results shown in Figure 7 indicate that the destruction rates of *Geobacillus stearothermophilus* bound on wallboard are much slower than those for the same organism bound on ceiling tile. For example, complete destruction of the wallboard bound spores occurs within 10 min at 315 °C, which increases to over 20 min when the gas heating temperature is reduced to 260 °C. Spore destruction occurs at much slower rates when the temperature is reduced further to 204 and 148 °C. The slow rates of destruction

Figure 6. Effects of Heating Temperature and Time on Reduction of *Geobacillus Stearothermophilus* Spiked on Ceiling Tile

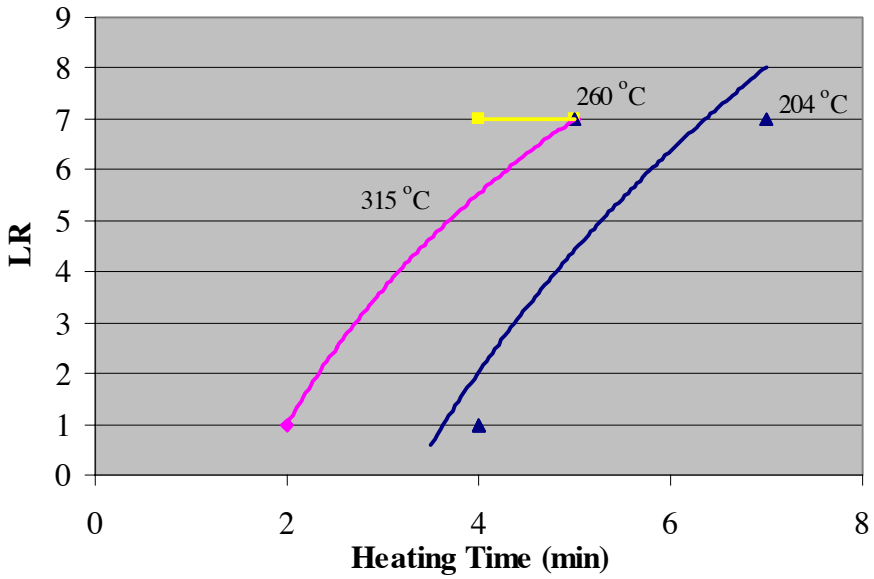
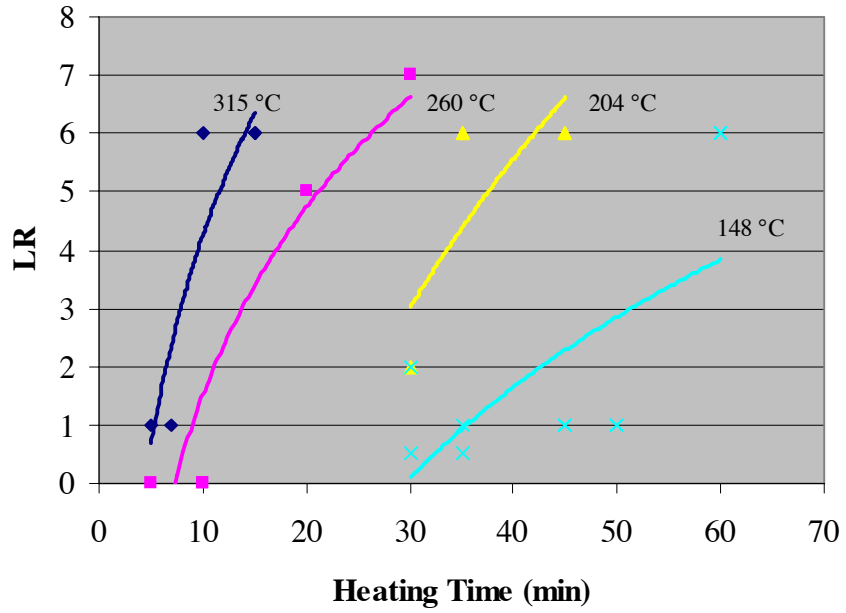


Figure 7. Effect of Heating Temperature and Time on Reduction of *Geobacillus stearothermophilus* Spikbed on Wallboard



observed for wallboard bound *Geobacillus stearothermophilus* are similar to those observed for *Bacillus subtilis* bound on the same building material (Figure 5). The slow destruction rates are consistent with the slow heat transfer rates measured for this material

(Figure 3), which provide further support that the thermal destruction of *Bacillus anthracis* surrogate spores bound on a building material depends strongly on the heat transfer rate of the material.

CONCLUSIONS

A laboratory-scale thermal reactor was constructed to evaluate the effect of building material, heating temperature, and time on the destruction of *Bacillus anthracis'* surrogates. The building materials tested included ceiling tile and wallboard, and tests for another building material, carpet, are ongoing. Results of the initial tests conducted for measuring the internal temperature profiles of the ceiling tile and wallboard indicate that ceiling tile has much faster heat transfer rate than the wallboard. The survivability of two different *Bacillus anthracis* surrogates, *Bacillus subtilis* and *Geobacillus stearothermophilus* spiked on these two building materials was also tested. Results of the tests showed that the destruction of the two *Bacillus anthracis* surrogates spiked on the building materials strongly depends on the heat transfer characteristics of the material. The destruction of the two surrogates spiked on ceiling tile occurs at much faster rates than those of the surrogates spiked on wallboard. The different anthrax thermal destruction behaviors observed for these two building materials are consistent with their different heat transfer characteristics.

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